MORPHOMETRIC VARIATIONS OF STINGLESS BEES IN SOUTHERN KERALA AND ASSESSMENT OF HONEY QUALITY

DIVYA K. K.

(2013-11-135)

DEPARTMENT OF AGRICULTURAL ENTOMOLOGY

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM – 695 522

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by

DIVYA K. K.

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VELLAYANI, THIRUVANANTHAPURAM – 695 522

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2016

DECLARATION

I, hereby declare that the thesis entitled "MORPHOMETRIC VARIATIONS OF STINGLESS BEES IN SOUTHERN KERALA AND ASSESSMENT OF HONEY QUALITY" is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

Vellayani, **Divya K. K.**

Date: **(2013-11-135)**

CERTIFICATE

Certified that this thesis entitled "MORPHOMETRIC VARIATIONS OF STINGLESS BEES IN SOUTHERN KERALA AND ASSESSMENT OF HONEY QUALITY" is a record of research work done independently by Miss. Divya K. K. under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

Vellayani,
Date:

Dr. Amritha, V. S.
(Major Advisor, Advisory Committee)
Assistant Professor
AICRP on Honeybees &Pollinators
Department of Agricultural Entomology
College of Agriculture, Vellayani

CERTIFICATE

We, the undersigned members of the advisory committee of Miss. Divya, K. K. (2013-11-135), a candidate for the degree of **Master of Science in Agriculture** with major in Agricultural Entomology, agree that thesis entitled "MORPHOMETRIC VARIATIONS OF STINGLESS BEES IN SOUTHERN KERALA AND ASSESSMENT OF HONEY QUALITY" may be submitted by Miss. Divya, K. K. (2013-11-135) in partial fulfilment of the requirement for the degree.

Dr. Amritha V. S.
(Chairman, Advisory committee)
Assistant Professor and Major Advisor
AICRP on Honey Bees and Pollinators
Department of Agricultural Entomology
College of Agriculture, Vellayani
Thiruvananthapuram 695 522

Dr. K. Sudharma
(Member, Advisory committee)
Professor and Head
Department of Agricultural Entomology
College of Agriculture, Vellayani
Thiruvananthapuram 695 522

Dr. S. Devanesan
(Member, Advisory committee)
Dean i/c
Professor and Principal Investigator
AICRP on Honey Bees and Pollinators
Department of Agricultural Entomology
College of Agriculture, Vellayani
Thiruvananthapuram 695 522

Dr. Aparna B.
(Member, Advisory committee)
Assistant Professor
Department of Soil Science and Agricultural Chemistry
College of Agriculture, Vellayani
Thiruvananthapuram 695 522

EXTERNAL EXAMINER

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DIVYA K. K.

INDEX

LIST OF TABLES

13	Antioxidant content in stingless bee honey samples from midland and upland physiographic regions of southern Kerala	
14.	Microbial load in stingless bee honey samples from midland and upland physiographic regions of southern Kerala	

LIST OF PLATES

LIST OF FIGURES

LIST OF ABREVIATIONS

MSL	-	Mean Sea Level
LH	-	Head Length
WH	-	Head Width
DDO	-	Distance Between Ocelli
DOOD	-	Dorsal Ocello Ocular Distance,
LA	-	Antennae Length
PL	-	Proboscis Length
FL	-	Femur Length
TL	-	Tibia Length
LM	-	Metatarsus Length
WM	-	Metatarsus Width
FWL	-	Forewing Length
FWW	-	Forewing Width
HAM	-	Number of hamuli
3TL	-	Third Tergite Length
3SL	-	Third Sternite Length
3SW	-	Third Sternite Width
LWT	-	Lateral Width of Fourth Tergite Tomentum
ML	-	Midland
UL	-	Upland
EC	-	Electrical Conductivity

µS cm^{-1}	-	micro Siemens per centimetre
TDS	-	Total Dissolved Solids
ppm	-	parts per million
%	-	per cent
g	-	gram
>	-	greater than
<	-	lesser than
w/v	-	Weight per volume
^{o}C	-	Degree Celsius
nm	-	nano metre
mAU	-	milli Absorbance Unit
ml	-	milli litre
HCl	-	Hydrochloric acid
NaOH	-	Sodium hydroxide
$NaNO_2$	-	Sodium nitrite
$AlCl_3$	-	Aluminium chloride
M	-	molar
Na_2CO_3	-	Sodium carbonate
CEQ	-	catechin equivalents
A	-	Absorbance

DN	-	Diastase number
pNPG	-	p-nitrophenyl-α-D-glucopyranoside
KH_2PO_4	-	Potassium hydrogen phosphate
$Na_2HPO_4.2H_2O$	-	disodium hydrogen phosphate
L	-	litre
min	-	minutes
IN	-	Invertase number
FRAP	-	Ferric Reducing Ability of Plasma
Fe (III)-TPTZ	-	Ferric tripyridyltriazine
Fe (II)-TPTZ	-	Ferrous tripyridyltriazine
mM	-	milli molar
rpm	-	revolutions per minute
$FeCl_3$	-	Ferric chloride
µl	-	micro litre
SDA	-	Sabouraud dextrose agar
NA	-	Nutrient agar
CFU	-	colony forming units
CRD	-	Complete randomized design
CD	-	Critical difference
cm	-	centimeter
m	-	metre

et al.	-	And others
Fig.	-	Figure
mg kg^{-1}	-	milli gram per kilogram
viz.	-	Namely
NS	-	Not significant
S	-	Significant
A.O.A.C.	-	Association of the Official Agricultural Chemists
sp.	-	Species (singular)

LIST OF APPENDICES

Sl. No.	Title	Appendix No.
1	Data-Sheet	I
2	Composition of different media	II

Introduction

1. INTRODUCTION

Stingless bees are world's largest and most diverse group of social insects and they are considered as a key factor for pollination in tropical flora (Roubik, 1989) that has gained an increasing attention recently. They live in perennial colony which comprises of different castes (queen, workers and drones), of which the workers exhibit division of labour and task specialization. Stingless bees belong to the family Apidae and the subfamily Meliponinae, which consists of two tribes, Trigonini and Meliponini. Like honey bees, they also produce honey which is appreciated from ancient times onwards. Their colony strength varies from species to species which ranges from a few hundred to more than thousand. Morphological adaptations, particularly the proboscis length and hind tibiae, where proboscis is an elongated tongue for nectar collection and the hind tibiae are modified as pollen basket for collecting and transporting pollen and resins from the foraging site to the hive.

Stingless bees are confined to tropical and subtropical areas and most of their genera have a capacity to vary their nest habit, architecture and defense. They usually inhabit in concealed places, exposed or partially exposed nests and some of them build their nest inside termitophile or ant nest. Majority of the nest architectural modifications is focused on defense mechanisms, which starts from the construction of nest entrance thereby they can protect food reserves and colony members present inside the hive.

Stingless bee species which were commonly seen in India is *Trigona* (*Tetragonula*) *iridipennis* (Smith) (Raakhee and Devanesan, 2000; Swaminathan, 2000 and Danaraddi, 2007). Apart from *T. iridipennis*, another seven stingless bee species were also reported from India. Though stingless bees are abundant in southern part of India (Rasmussen, 2013), only limited studies were

conducted on the morphometric variations of stingless bees in different physiographic regions of Kerala.

Stingless beekeeping (Meliponiculture) is an emerging trend in the backyards of Kerala, even though it was not extended in the crop field for pollination purpose. In economic terms, the ecosystem service that pollinators provide is worth of an estimated $ 220 billion annually world-wide (Gallai *et al*., 2009). In the case of stingless bees, because of their smaller body size they can visit different types of flowers with varied size and shape; contribute more in ecosystem stability through their pollination services than other pollinators.

Stingless bee honey is a precious bee product which has been consumed since ancient times. Their honey have got medicinal property and is used for controlling digestive, respiratory, female fertility, skin and visual disorders (Souza *et al*., 2006). Due to the limited knowledge about the product, it is not included in the international standards for honey (CAC, 2001) and is also not controlled by any of the food quality control authorities from India and abroad. Thus, there is no assurance to consumers in terms of the quality and safety of the product.

The morphometric variations of stingless bees in different physiographic regions as well as the physicochemical, antioxidant and microbial properties of stingless bee honey (*T. iridipennis*) remain still unexplored. Hence, the present study is mainly focussed on

- Nest architecture and defense mechanism in stingless bee colonies
- Foraging flora visited by stingless bees
- Morphometric variations of stingless bees in midland and upland physiographic regions
- Physico chemical and antioxidant properties of stingless bee honey
- Microbial load in stingless bee honey

Review of literature

2. REVIEW OF LITERATURE

2.1. ORIGIN AND DISTRIBUTION OF STINGLESS BEES

Stingless bees constitute a diverse group of eusocial bees which belong to Meliponinae, one of the three subfamilies of the family Apidae (Winston and Michner, 1977). The subfamily Meliponinae is divided into two tribes Meliponini and Trigonini, based on their nest architecture, the dorsal vessel, ventral nerve cord and alimentary canal (Willie, 1979). In general, stingless bees are easily distinguished from the other bees by the characters: reduction and weakness of wing venation, presence of penicillium and reduction of sting (Willie, 1983).

Stingless bees were apparently originated in and dispersed from Africa. The major three supporting significant factors for their origin and dispersion from Africa was the wide acceptance of plate tectonic and continental drift, discovery of European fossils from the early tertiary (late Eocene) and a better developed sting in African Meliponinae. The presence of stingless bees in Europe in the early tertiary indicated that the Meliponinae migrated first to the North, possibly during the Eocene, when tropical moist climate had wide development. Since Europe was is in contact with North America, these bees dispersed to that continent, as well as into Asia and Australia (Wille, 1979). The tribe Trigonini which was distributed in Asia and Africa includes various genera *Trigona*, *Plebeia*, *Tetragona* and *Nanotrigona* (Camargo *et al.*, 1988).

All the neotropical stingless bees belong to the tribe Meliponini, which is comprised of forty species. The greatest diversity of stingless bees is found in the Neotropical regions of South America with 412 described species (Camargo and Pedro, 2012).

2.2. STINGLESS BEE SPECIES IN INDIAN SUBCONTINENT

The most common stingless bee species found in India is *Melipona iridipennis* Smith. The scientific genus-group names for the different stingless bees from the Indian subcontinent have long been *Trigona* Jurine and *Lisotrigona* Moure by Michener (2007). Rasmussen and Cameron (2010) revealed that *Trigona* was not evolved from single evolutionary lineage and it encompasses a Neotropical clade and a distantly related Indo-Malayan/Australasian clade. Recently Michner (2013) restricted the usage of genus name *Trigona* in the Neotropical lineage and the bees present in the Indo-Malayan/Australasian taxa were under genus *Lepidotrigona* Schwarz and *Tetragonula* Moure. Thus the name of *Trigona iridipennis* Smith present in the Indo-Malayan/Australasian taxa was renamed into *Tetragonula iridipennis* (Smith).

Globally, more than 400 species of stingless bees were reported by Velthuis (1997) and Costa *et al.* (2005), out of which eight species (*Lepidotrigona arcifera* (Cockerell), *Lisotrigona mohandasi* Jobiraj and Narendran, *Tetragonula* aff. *laeviceps* (Smith), *Tetragonula iridipennis* (Smith), *Tetragonula bengalensis* (Cameron), *Tetragonula ruficornis* (Smith), *Tetragonula praeterita* (Walker), *Lisotrigona cacciae* (Nurse) and *Tetragonula gressitti* (Sakagami)) are reported from India (Rasmussen, 2013). Though these stingless bee species are abundantly seen in South India, their diversity in India is unknown hitherto.

From Kerala, two stingless bees are mainly reported - *Trigona* (*Tetragonula*) *iridipennis* Smith by Raakhee and Devanesan (2000) and *Lisotrigona mohandasi* by Jobiraj and Narendran (2004).

2.3. NEST ARCHITECTURE AND DEFENSE MECHANISM

Stingless bee nest being the central place of bee activity, nesting biology forms a highly visible aspect of stingless bee behavior (Michener, 1974).

Stingless bee nests contain elaborate structures made from cerumen, a mixture of collected plant resins and wax produced by the bees (Wille and Michener, 1973). According to Sakagami (1982), these wax flakes were secreted from the wax glands which were located on the dorsal side of the abdomen in stingless bees, whereas they are ventrally located in *Apis* sp. A typical nest of stingless bees comprises of entrance, brood cells, involucrum, honey and pollen storage pots, batumen, waste and resin dumps (Raakhee and Devanesan, 2000). The stingless bee species varied the nest architecture by means of internal and external structure, they built these nests in hollow trees, on the ground, or occasionally in active colonies of social insects like termites, ants, wasps or other stingless bee colonies (Rasmussen, 2004). According to Rasmussen and Camargo (2008), the nest characteristics and nesting habits are useful in taxonomic studies where the individual species are recognizable from nest entrances and often from their particular site.

Majority of nest characteristics were similar between the stingless bee genera and these similarities were incorporated by the bees in terms of a small number of apparently uniform nest construction materials, functions and designs (Michener, 2000). Franck *et al.* (2004) stated that the nest architectural modifications were not the key factor in the speciation process of stingless bees. Whereas nest architectural innovations may occur in a taxon after its divergence from ancestors and at the same time, unrelated species may converge due to the similarity of nesting materials or sites (Roubik, 2006).

In stingless bee colony the different tasks are performed by workers (Bassindale, 1955) where polytheism forms the phenomenal ecological success of the

eusocial insects. Willie (1983) reported that stingless bees live in social, perennial colonies which comprised of a single queen (except in *Melipona bicolor* Lepeletier with more number of queens), a variable number of males (drones) and hundreds to thousands of female workers. According to O'Donnell *et al*. (2000) specialized foragers bring food to the nest while others carry out majority of the tasks within the nests. Dollin (2001) reported that colonies led by virgin queen have specific task sequence identified as self-grooming, incubation, repair of brood chamber, construction and provisioning of cells, feeding of young ones, reconstruction of involucrum, guard duty and collection of nectar and pollen.

In stingless bee colonies, defensive strategies were categorized into two types - protective building and defensive reactions. Protective building was done by modifying the internal or external nest architecture whereas the defensive reactions were done by guard bees positioned at the mouth of the entrance tube through biting and spitting attack (Wittmann, 1985). Though the most common defense strategy was to make the stingless bee nests and their entrance invisible to intruder (Bruijin *et al*., 1997), Ayasse and Paxton (2002) reported this protective building as a preliminary defense mechanism. Closure of the nest entrance and placing of sticky resin around the entrance tube exhibited by stingless bees are also considered as protective building behavior (Roubik, 2006).

The modification of hive entrance and internal parts with sticky resinous matter by bees (Lindauer, 1957; Devanesan *et al.*, 2009) prevents the invasion of ants. Similarly the entrance tubes of *Lepidotrigona terminate* Smith, *Tetrigona apicalis* (Smith) and *Tetragonula collin* (Smith) were also employed with diverse, viscid and adhesive resins, which constitute a generally effective first-line of defense against depredations by weaver ant, *Oecophylla smaragdina* (Fab.) (Duangphakdee *et al.*, 2009). The stingless bees were also found to use resins, mud and wax as nest enclosure materials (Pavithra *et al*., 2013).

2.3.1. Number of Guard Bees

Unlike honey bees, stingless bees have a vestigial sting and their defensive reaction is mainly through the mandibles which have comparatively larger muscles than *Apis* sp. (Sakagami, 1982). Based on their efficacy of attack, some of them are aggressive while others are mild. *O. tataira,* the stingless bees and its relatives have a unique system of defense mechanism where they have enlarged mandibular glands capable of producing enough caustic liquid to cause blisters when they bite (Willie, 1983). Downs and Ratnieks (1999) reported that the guard bees standing at the nest entrance used to check incomers and reject conspecific and allospecific intruders. According to Couvillon *et al.* (2008) based on necessity of guarding, the number of guard bees in a nest varies from a single soldier bee to several bees and they usually stand on, near or at the nest entrance. When intruders disturb the colony, the guard bees become alert and they start attacking and congregating more defenders by using alarm pheromones. Studies conducted by Nunes *et al*. (2008) in the colonies of *Frieseomelitta varia* (Lepeletier) at Brazil revealed that the guard bees are able to distinguish between nest mates and non-nest mates by chemical cues present on their cuticle.

Roubik *et al*. (1986) noted that the absolute size of a nest entrance need not be associated with intensive forager traffic whereas a positive correlation of foraging activity and external nest entrance size was observed by Biesmeijer *et al*. (2007). Jayalekshmi (2015) reported that the mean number of guard bees in *T. iridipennis* colonies ranged from 6.00 to 9.00.

2.3.2. Design of Nest Entrance

In stingless bee colonies, the form of the nest entrance varied from one species to another and it is useful for the orientation of the bees and nest defense (Sakagami and Inove, 1989). Melo (1996) observed variation in nest entrance

characteristics within *Melipona capixaba* (Moure and Camargo), which varied from simple to a very elaborate structure whereas Camargo and Pedro (2004) reported pronounced differences in the nest entrances of Amazonian *Ptilotrigona lurida* Smith with respect to the geographical locations. Thus the architecture of stingless bee nest entrance is species-specific (Franck *et al.*, 2004) and the individual species can be recognized from the nest entrances and often from their nesting site (Roubik, 2006). According to Biesmeijer *et al.* (2007) the variations of nest entrance in the same stingless bee species was due to the pressure of natural selection in stingless bee colonies for foraging requirements, adequate defense and maintenance in terms of nest entrance architectural parameters including size, number, shape and conspicuousness.

Roubik (2006) observed multiple nest entrance designs in the colonies of stingless bee belonging to the genus *Lepidotrigona, Plebeia, Scaptotrigona* and *Tetragona*. Similarly the results of AICRP (2013) also revealed the presence of long tubular, short tubular, round and cryptic nest entrances based on their length. Though different types of entrance tubes *viz.*, round, elliptical, irregular, short tube entrance, long tube entrance, cryptic entrance and toad mouth were recorded by Lima *et al*. (2013) from the feral colonies of six stingless bees (*Plebeia* sp., *Tetragonisca fiebrigi* (Schwarz), *Scaptotrigona depilis* Moure, *Tetragona clavipes* Fabricius, *Partamona cupira* Smith and *Oxytrigona tataira* (Smith)). *T. iridipennis* preferred oval shape (elliptical) followed by circular and irregular opening (Pavithra *et al*., 2013)

2.3.3. Length and Width of Entrance Tube

The entrance tube of stingless bee colonies, made up of cerumen, is either a simple hole or extended from the nest as an external tube. The length of the entrance tube varies with the nest site (Sakagami *et al.*, 1989), where he reported that hive entrance length of *Tetragonula hockingsi* (Cockerell) extended up to one cm in tree

trunk whereas it was simple in the case of nest in cracks. In the case of *Plebia poecilochora* Dumeril, Drumond *et al.* (1995) reported a simple and small circular entrance without any external extension and the nest opening was surrounded by dark resin with no outer tube.

In Philippines, the length and width of hive entrance tube in *Trigona fuscobalteata* Cameron, was in between 0.00 - 0.31 cm and 0.05 - 0.15 cm (Starr and Sakagami, 1987) while it varied from 0.56 to 1.45 cm and 0.02 to 0.06 cm in *T. iridipennis* nest, India (Danaraddi, 2007) in India. According to Pavithra *et al*. (2013), nest entrance width in most of the stingless bee (*T. iridipennis*) colonies were in between 0.80 - 1.40 cm. Jayalekshmi (2015) reported that the length and width of hive entrance tube of *T. iridipennis* was between 1.56 to 5.48 cm and 1.10 to 2.08 cm respectively in southern Kerala.

2.3.4. Height from the Ground Level

In one of the study of over 200 nests in Uganda, nest predators were found mostly attacking the stingless bee colonies in trees under seven metres height (Kajobe and Roubik, 2006). According to Khan and Srivastava (2013) the most preferred height by the stingless bees *Tetragonula laeviceps* Smith was less than one metre from the ground level. Whereas the preferred height for keeping domesticated hives of *T. iridipennis* was in between 3.0 - 4.5 m (Jayalekshmi, 2015). Thus the nest site selection purely depends on the safer strategies such as availability of flora, protection from predators etc. for better and safe survival at the nesting sites.

2.3.5. Materials Used for Nest Entrance Construction

Generally, the nest construction materials observed in the colonies of *Trigona* sp. were sticky resin, faecal material, pollen accumulation or scutellum, wax deposit, wood fiber (paper) and trash (Roubik, 2006), whereas the entrance tubes of *T. fiebrigi* and *T. clavipes* were constructed only with pure wax and propolis

respectively (Lima *et al*., 2013). According to Pavithra *et al.* (2013) the hive entrance of *T. iridipennis* was made up of sand, mud, grease, resin, wax, wooden pieces, tar, blue paint, pollen, stones, cow dung and animal faeces. In addition to the common nest construction materials, Jayalekshmi (2015) reported saw dust, bee cadavers, leaves and vegetative parts and dried bark of trees from the stingless bee nest. She also revealed that resins collected from native trees *viz.*, *Mangifera indica* L. (Mango), *Artocarpus heterophyllus* Lam. (Jack fruit tree), *Cocos nucifera* L. (Coconut), *Anacardium occidentale* Linn. (Cashew), *Terminalia* spp. (Maruthu), *Calophyllum inophyllum* L. (Punna tree), *Garcinia gummi-gutta* L. (Kudampuli), *Artocarpus hirsutus* Lam. (Anjili) were also present in the stingless bee nest.

2.4. FORAGING BEHAVIOUR OF STINGLESS BEES

The pollen collecting insects such as bees contribute to the highest quality pollination services in terms of cross-pollination, yield, quality produce, uniform crops pollination as well as earlier produce (McGregor, 1976). Pollination carried out by stingless bees allows the production of fruits and seeds of several native plants and without the help of these pollinators, several plant species would be threatened of extinction (Campos, 1983). Stingless bees are true generalists, collecting nectar and pollen from a vast array of plants (Roubik, 1989). Michener and Houston (1991) reported that bees are dependent on nectar from flowers as their source of carbohydrates and on pollen as their protein source. Forager activities of bees were highest in the vicinity of the nest and more than 75 per cent of the foraging activity normally occurs within 50 per cent of the maximum foraging distance (Kuhn-Neto *et al.*, 2009).

Studies conducted by Premila *et al.* (2007) on foraging flora of stingless bees in Kerala, recorded 77 foraging plants including medicinal plants, plantation crops, condiments and spices, vegetable crops, ornamental plants, field crops, fibre crops, fruit crops, tuber crops, green manure crops and forest trees. The bees visited 21

plants for collecting nectar and pollen, 37 plants for nectar alone and 19 plants for pollen alone. Raju *et al.* (2013) identified 87 families of foraging plants visited by stingless bees and Indian honey bees from Kerala, Tamil Nadu, Andhra Pradesh, Karnataka and Jammu Kashmir.

Foraging activities in social insects are influenced by unpredictable environmental variables in terms of timing and location of food (Biesmeijer and Ermers, 1999). In the case of stingless bee workers foraging activities are governed by internal factors, such as individual memory and threshold response towards the foraging stimuli, and external factors, such as environmental and colony conditions which determine the level of exposure to stimuli associated with the decision. Saavendra *et al.* (2003) revealed that the elevated temperature have a capacity to influence the physiology of flowering plants in terms of altered production of flower, nectar and pollen.

According to Devanesan *et al.* (2002), the foraging activity of stingless bees (*T. iridipennis*) started at 0007 hr and ceased at 1800 hr. Peak foraging activity of bees was observed at 1200 hr and 1500 hr and their activity was maximum in the month of July while it was lower during December and January.

2.5. MORPHOMETRIC STUDIES OF STINGLESS BEES

A major part of the morphological variation in Meliponini occurs independently of phylogeny due to the fact that, for social bees, worker body size has been generally considered as an adaptation to foraging activity and floral resource exploitation (Baumgartner and Roubik, 1989). About 75.50 per cent of body size variation in Meliponini corresponds to adaptive factors associated with resource exploitation (Pignata and Diniz-Filho, 1996). The literature pertaining to morphometric studies reveals a strong relationship between morphometric structures and climatic data which leads to the adaptation and natural selection. Though a

handful of work has already been conducted in stingless bee morphometry, it is very much limited in our state.

Schwarz (1948) suggested that bees with greater number of wing hamuli were associated with wing stability and flight capacity. This was reported by Araujo *et al.* (2004) in six species of stingless bees *viz*., *Plebeia droryana* Friese, *Cephalotrigona capitata* Smith, *Melipona quadrifasciata* Lepeletier, *M. compressipes* Fabricius, *M. marginata* Lepeletier and *Trigona spinipes* Fabricius where the flight distance is highly correlated to their wing size. According to Kuberappa *et al.* (2005) the stingless bee workers, *T. iridipennis* had five hamuli irrespective of the zones but the extent of hamuli changed with the width of the wing. He also recorded that the body size of the stingless bees collected from the hilly zone was wider and broader than stingless bees at plains.

Results of morphometric studies on stingless bee workers (*T. iridipennis*) in Kerala were: length of proboscis (1.38 mm) and antennae (2.57 mm), size of mandible (0.60 mm), length of forewing (3.60 mm) and hind wing (2.47 mm), width of forewing (1.36 mm) and hind wing (0.63 mm), number of hamuli (5 nos.), extent of hamuli (0.22 mm), length of coxa (0.35 mm), trochanter (0.29 mm), femur (0.75 mm), tibia (0.91 mm) and tarsus (1.25 mm) in foreleg, length of coxa (0. 53 mm), trochanter (0.32 mm), femur (0.86 mm), tibia (0.91 mm) and tarsus (1.12 mm) in mid leg, length of coxa (0.53 mm), trochanter (0.36 mm), femur (1.03 mm), tibia (1.43 mm) and tarsus (1.20 mm) in hind leg and total length of body length (4.068 mm) (Raakhee, 2000) .

According to Kuberappa *et al*. (2005) biometric parameters of stingless bee samples collected from six agro climatic zones of Karnataka varied based on altitude, where the body length of bees was maximum in hilly zone (5.051 mm) followed by northern transitional zone (4.992 mm) and eastern zone (4.903 mm) while lowest value was recorded from central dry zone (4.621 mm). They also reported that the

bees from hilly zone had longest head (1.986 ± 0.041 mm) whereas the shortest head (1.886 mm ± 0.040) was recorded from central dry zone.

Studies conducted by Danaraddi (2007) on *T. iridipennis* samples, which were collected from Bangalore, Dharwad, Sirsi, Bijapur, Shimoga, Chitradurga, Raichur and Gulbarga of Karnataka revealed the measurement of parameters like head width (1.52 to 1.61 mm), proboscis length (1.30 to 1.41 mm), forewing length (3.54 to 3.78 mm), forewing width (1.17 to 1.37 mm), length and width of femur (0.86 to 0.93 mm and 0.23 to 0.26 mm), length and width of tibia (1.32 to 1.39 mm and 0.47 to 0.50), length and width of metatarsus (0.46 to 0.52 mm and 0.27 to 0.31mm and inter ocellar distance (0.32 to 0.39 mm).

Akum *et al*. (2012) studied the morphometric parameters of stingless bees domesticated at Medziphema and Mima in Nagaland. They identified two types of stingless bees based on the number of wing hooks, length and breadth of metatarsus, length and breadth of forewing and hind wing, abdomen colour and nest entrance. They also reported that the strain variation was evident externally in both the samples of Medziphema and Mima in the colour of abdomen and was classified as *Trigona conifrons* Smith (black colour abdomen) and *Trigona lutea* Bingham (yellow colour abdomen) according to the Fauna of British India.

Rasmussen (2013) conducted morphometric studies of *T. iridipennis* and was expressed in terms of body length (3.55 mm), width of head (1.60 mm), length of head (1.30 mm), length and width of compound eye (1.10 and 0.40 mm), upper, lower and maximum inter orbital distance (1.00, 0.82 and 1.13 mm), diameter of median ocelli (0.15 mm), inter ocellar distance (0.33 mm), ocello orbital distance (0.23 mm), inter alveolar distance (0.15 mm), alveoli orbital distance (0.30 mm), alveoli ocellar distance (0.71 mm), alveolar diameter (0.14 mm), length of clypeus (0.33 mm), inter tentorial distance (0.48 mm), clypeo ocellar distance (0.96 mm), length of malar space (0.03 mm), length of scape (0.57 mm), diameter of scape and

third flagellomere (0.10 and 0.12 mm), length of pedicel + flagellomere (1.24 mm), length of 1st, 2nd and 3rdflagellomere (0.09, 0.07 and 0.10 mm), length and width of mandible (0.62 and 0.19 mm), length of forewing excluding and including tegula (3.44 and 3.80 mm), width of forewing (1.32 mm), length and width of pterostigma (0.48 and 0.11 mm), length and width of marginal cell (1.21 and 0.30 mm), length of 1st abscissa of M (0.58 mm), length of Cu (0.71 mm), length of wing diagonal (1.01 mm), hamuli (5 nos.), length and width of mesoscutum (0.87 and 1.01 mm), length and width of scutellum (0.33 and 0.57 mm), length and width of tibia III (1.55 and 0.54 mm), length and width of basitarsus III (0.50 and 0.29 mm), width of tergum (1.36 mm), length of hairs on clypeus, frons, vertex and scutellum apex (<0.01, <0.01, 0.11 and 0.12 mm).

2.6. QUALITY ANALYSIS OF STINGLESS BEE HONEY

Honey is a natural sweet viscous fluid produced by honey bees from the nectar of plants, which they collect and transform by combining with their salivary secretions, and deposit, dehydrate and store in the honey comb to ripen (CAC, 2009).

Crane (1992) reported that stingless bees honey is stored in irregularly built honey pots which were made up of plant resin and bee wax (cerumen). According to Ramanujam *et al.* (1993), *Trigona* bees produce dense, darkish, sour honey with a maximum quantity of 100 ml colony^{-1} while Nisha (2002) reported it as 120-350 ml hive^{-1} and Sureshkumar *et al.* (2012) as 600-700 g of honey year^{-1} which was reared by Kani tribes.

Kamal and Pulak (1994) asserted that the medicinal quality of honey was contributed by small foraging plants which contribute the acidic character whereas Boon (2002) suggested that it is due to the leaching of resinous chemicals from the storage pots to honey.

Honey consists of different sugars, predominantly fructose and glucose and other substances such as organic acids, enzymes and solid particles derived from the nectar collected from different plants (Saxena *et al*., 2010).

CAC (2001) reported that Meliponine (stingless bee) honey is a valuable bee product with a long consumption tradition with several medical uses; because of the limited knowledge about the product it is not included in the international standards for honey. Researchers from different parts of the world have found various types of honey that differ substantially in their physico-biochemical composition. However, the physico-chemical parameters which constitute the quality indicators characterizing the different honey types are strictly defined (Juszczak *et al*., 2009).

2.6.1. Physico-chemical Properties of Honey

2.6.1.1. pH

Generally pH content in honey can be used as an index of possible microbial growth (Conti *et al.,* 1998) where he suggested that the favourable climate for bacterial growth is neutral and mildly alkaline environment, while that of yeast and mould is acidic medium (4.0 - 4.5). The major factors which influence the pH is nature of honey and presence of gluconic acid (Khalil *et al.*, 2001), honey texture, stability against spoilage and shelf life (Kumar *et al.*, 2013).

Studies conducted by Raakhee (2000) observed that the pH of stingless bee honey (*T. iridipennis*) was 3.98. According to Souza *et al.* (2006), pH content in stingless bee honey samples from different entomological origin (species level) were recorded as 3.27 (*Melipona mandacaia* Smith) 3.27 (*Melipona asilvasi* Lepeltier), 3.72 (*M. compressipes*), and 3.93 (*Tetragonisca angustula* Latreille). Devanesan *et al.* (2009) reported that pH of stingless bee honey (*T. iridipennis*) was 4.13 while Jayalekshmi (2015) in her studies at Thiruvananthapuram, Kollam,

Pathanamthitta, Kottayam and Idukki districts of Kerala reported the pH of honeys as 3.77, 3.74, 4.03, 3.66 and 3.62 respectively.

2.6.1.2. Moisture Content

The amount of water present in honey determines its stability against fermentation and granulation (Dyce, 1975) and their shelf-life during storage (Perez-Arquilluve *et al*., 1994).

Though the moisture content of stingless bee, *Trigona* is high (Vit *et al.*, 1994), the spoilage problem is much reduced because of the incorporation of enzymes and other substances which could lead to antibiotic or preservative activity in honey. Maximum limit of moisture content in *Apis mellifera* honey is 20 per cent (CAC, 1994) whereas in stingless bee's honey it is extended up to 30 per cent (Vit *et al*., 2006). Raakhee (2000) asserted that the moisture content in *T. iridipennis* honey was 20.70 per cent while Bijilshma *et al.* (2006) reported that moisture content of stingless bee honey samples collected from colonies of *Plebeia tobagoensis* Melo, *Trigona nigra* Cresson, *Melipona trinitatis* Evans and *Melipona favosa* Fabricius as 42, 36.20, 32.20 and 35 per cent respectively.

2.6.1.3. Electrical Conductivity

Electrical conductivity is considered as the most important parameter to discriminate honey and is included recently in the new international standards for honey (CAC, 2001). It is a good criterion for determining the botanical origin (different floral origin) of the honey and is determined in all honey quality analysis instead of ash content (Sahindler and Gul, 2004 and Malika *et al*., 2005). Apart from these, EC also measures all ionisable organic and inorganic substances present in honey and it is closely related to the concentration of mineral salts, organic acids and proteins (Kebede-Nigussie *et al*., 2012).

The honey is categorized into honey dew honey (> 800 µScm^{-1}) and blossom honey (< 800 µScm^{-1}) based on their electrical conductivity (CAC, 2001). Electrical conductivity of stingless bee honey samples collected from Ecuador was reported as 480 µScm^{-1} (Guerrini *et al.*, 2009) while that from Nigeria ranged from 50 to 410 µS cm^{-1} (Buba *et al*., 2013). According to Jayalekshmi (2015) electrical conductivity of *T. iridipennis* honey of southern Kerala ranged from 28.46 to 41.76 µS cm^{-1}.

2.6.1.4. Total Dissolved Solids (TDS)

TDS is a measure of the combined content of all inorganic and organic substances in honey such as molecular, ionized or micro-granular (colloidal solution) suspended forms and it act as a good indicator for honey purity (Gomes *et al*., 2010).

2.6.1.5. Colour Intensity

According to White and Doner (1980), the colour of honey forms a continuous range from very pale yellow through amber to a darkish red amber to nearly black. The major factor which contributes the colour variation is plant source and climatic conditions rather than through the darkening action of heat. Frankel (1998) reported that colour intensity in honey was related to the presence of pigments, such as carotenoids and flavanoids. According to Khalil *et al.* (2012) colour intensity in Algerian honey samples varied from 724 - 1188 mAU while that of honeys from different regions of India ranged from 106.60 to 1592 mAU (Kumar *et al.,* 2013).

2.6.1.6. Total Sugar (Reducing and Non-reducing)

Monosaccharides-hexoses like fructose and glucose act as the main reducing sugars in honey, which were produced by the hydrolysis of disaccharide sucrose (Doner, 1977). White and Doner (1980) asserted that in addition to glucose

(dextrose) and fructose (levulose) honey also had small amounts of at least 22 other complex sugars and all these sugars were the major reason for the principal physical and behavioural characteristics in honey. According to Elazeu *et al.* (2013) amount of sugar is higher in lighter honeys than darker honeys.

Studies conducted by Nisha (2002) in *T. iridipennis* honeys obtained from Kerala revealed that the total reducing sugar was 73.57 per cent and sucrose content was 1.48 per cent. According to Vit *et al.* (2006) the allowable limit of reducing and non-reducing sugar in honey is > 65 per cent and < 6 per cent respectively.

2.6.1.7. Protein Content

Generally, in Indian honeys, the protein content is normally less than 0.05 per cent (Anklam, 1998). Analysis of protein content in the honey is a new tool for evaluation of its quality and it helps to identify the authenticity of honey in a given geographical region (Mohammed and Babiker, 2009).

Phadke (1968) observed that protein content in stingless bee honey was 0.78 per cent whereas total protein content in *T. iridipennis* honey was 1.49 per cent (Raakhee, 2000).

2.6.1.8. Proline Content

Louveaux (1985) reported that majority of proline in honey comes from bee salivary secretions. According to CAC (2001), minimum limit of proline in honey is 180 mg kg^{-1}. Proline was used as an indication of honey ripeness and it also act as an indicator of honey adulteration when it falls below a certain limit (Muli *et al.*, 2007).

2.6.1.9. Ascorbic Acid

AICRP (2011) reported that ascorbic acid content in stingless bee (*T. iridipennis*) honey ranged between 1.04 to 2.80 mg kg^{-1}. USDA (2012) suggested

the minimum amount of ascorbic acid in honey as 0.5 mg kg^{-1}. According to Sulieman *et al.* (2013), ascorbic acid content in mountain honey was 2500 mg kg^{-1} while Khalil *et al.* (2012) reported that ascorbic acid content in Algerian honey was 159.70 mg kg^{-1}.

2.6.1.10. Flavanoids

Flavanoids behave as antioxidants in a variety of ways, including direct trapping of reactive oxygen species, inhibition of enzymes responsible for producing superoxide anions, chelation of transition metals involved in processes forming radicals and prevention of the peroxidation process by reducing alkoxyl and peroxyl radicals (Rice-Evans *et al.,* 1996). Khalil *et al*. (2012) reported that flavanoids are low-molecular-weight phenolic compounds that affect the aroma and antioxidant properties of honey.

According to Amiot *et al.* (1989), flavanoids were less in dark coloured honey whereas it contains more phenolic acid derivatives than light coloured honey. The flavonoid content of Turkish and Malaysian honey ranged from 4.80 to 22.80 mg kg^{-1} and 11.52 - 25.31 mg kg^{-1} respectively (Ozkok *et al.,* 2010). Similar value of flavanoid content (54.23 ± 0.62 mg kg^{-1}) was also reported from Algeria (Khalil *et al.*, 2012) whereas higher value of flavanoid content (975.50 ± 0.24 mg kg^{-1}) was reported from India (Kumar *et al.*, 2013).

2.6.1.11. Total Phenols

Donar (1977) reported that phenolic compounds observed in honey were derived from secondary metabolism of plants and it consists of flavanoids, flavonols and proanthocyanidins. Abell (1996) revealed that phenolic compounds present in the honey can act as potent antioxidants compared to other constituents like vitamin C and E. Antioxidant properties in honey play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing

peroxides (Zheng and Wang, 2001). Beretta (2005) reported that dark coloured honey had higher amount of total phenolic content and consequently higher antioxidant capacities. Alvarez-Suarez *et al.* (2012) suggested that phenolic compounds like phenolic acids and flavanoids can be considered as potential markers for the botanical origin of honeys.

Studies on total phenolic content in honey produced by *Melipona seminigramerrillae* Cockerell collected from the central and southern region of Amazonas state in Brazil revealed that total phenolic content ranged from 1700 to 6600 mg kg^{-1} of extract. He also added that the antioxidant activity was higher in the samples that had higher quantities of phenolic compounds (Silva *et al.*, 2013).

2.6.1.12. Diastase Activity

White (1978) reported that diastase or ∞ - amylase found in nectar is added by bees during the collection and ripening of nectar whereas he also suggested during 1992 that diastase content can be used as an indicator of honey quality and the principle behind this is the sensitive behaviour of diastase against heat, excessive heat during processing will tend to reduce the diastase number. According to Babacan *et al.* (2002), the floral origin of honey also influences its diastase content. They also reported that in addition to floral origin, pH, nectar flow and foraging patterns of the bees were also influencing the diastase activity and he proved that diastase levels do not correlate with honey quality.

Oddo *et al*. (1990) reported that diastase activity in *Trigona carbonaria* Smith honey was 0.40 ± 0.50 whereas according to Patricia and Patrizio (1996) diastase activity of *Trigona* sp. honey samples (g of starch hydrolysed per 100 g honey per hive) was observed in a range of 6.60 to 35.60. According to Carvalho *et al.* (2006) the diastase value of *Melipona scutellaris* Latreille and

M. quadrifasciata honey ranged from 1.73 to 3.01 and 1.34 to 2.14 respectively. Vit *et al*. (2006) recommended quality standards for stingless bee honey in terms of diastase number specific for different genera; *Melipona, Scaptotrigona* and *Trigona* having 3, 3 and 7 Diastase Number (DN) respectively. Jayalekshmi (2015) reported that the diastase number in stingless bee (*T. iridipennis*) honey was 1.35-2.40.

2.6.1.13 Invertase Activity

Belitz and Grosch, (1992) reported that invertase or ∞ glucosidase was one of the important glycoproteinaceous enzyme in honey, which was secreted by hypopharyngeal glands of bees. This enzyme was useful for honey ripening through the hydrolysation of sucrose and maltose to glucose and fructose. The amount of invertase in the honey depends on many aspects such as the age, physiological stage of stingless bee worker, food, condition of bee colony, temperature and intensity or type of honey flow (Lipp, 1994).

Invertase was more sensitive than diastase and loses activity during storage at a faster rate compared to amylases. As freshness indicator invertase was used in honey standards of the bee keepers association in Germany, Belgium and Spain and it was also used as an additional honey quality criterion in Italy and Switzerland (Bogdanov *et al.*, 1997)

Oddo *et al*. (1999) reported that the invertase activity (µ moles p-nitrophenyl glucopyranoside per kg honey per minute) of *T. carbonaria* was 5.70 ± 1.50 invertase number (IN). The invertase value of stingless bee honey samples in Brazil ranged from 19.80 to 90.10 (Souza *et al*., 2006). Jayalekshmi (2015) studied the invertase activity of stingless bee honey (*T. iridipennis*) and was recorded as 65.53 – 98.00 IN

2.6.2. Antioxidant Properties of Honey

Antioxidant property in honey was contributed by enzymatic (catalase and glucose oxidase, peroxidase) and non-enzymatic (organic acids, maillard reaction products, amino acids, proteins, flavanoids, phenolics, α-tocopherol, ascorbic acid and carotenoids) substances (NHB, 2003). Botanical origin of honey has the greatest influence on its antioxidant activity, whereas processing, handling and storage can affect the antioxidant activity of honey only to a minor extent (Beretta *et al*., 2005).

2.6.3. Microbial Load in Honey

The principal sources of microorganisms in honey were contributed by the nectar of the flowers as well as from intestinal contents of the honey bee (Frazier and Westhoff, 1994). The presence of these micro-organisms in honey, gives an idea of its sanitary or commercial quality (Snowden and Cliver, 1996).

The high sugar concentration in honey ties up water molecules so that bacteria have insufficient water to support their growth (Efem, 1988) and thus prevent the growth of microorganisms (Bogdanov, 2009). In addition to sugar content, the low pH as well as the phenolic compounds also prevents the growth of microorganisms (Lurlina *et al.,* 2009).

The standard plate count of *Bacillus* in the honey collected from Ibdan ranged from 10 cfu g^{-1} to 3 x 10^2 cfu g^{-1} (Adenekan, 2010). Studies conducted by Elazeu *et al.* (2013) in the honey samples, revealed that there was a positive correlation between colour versus pH, phenol, antioxidant and flavonoid contents whereas a negative correlation was observed between the bacterial load as well as fungal load in honey samples versus pH, phenol, reducing sugar and flavanoids.

Materials and methods

3. MATERIALS AND METHODS

The present study on 'Morphometric variations of stingless bees in southern Kerala and assessment of honey quality' was carried out in the All India Co-ordinated Research Project (AICRP) on Honey bees and Pollinators, Department of Agricultural Entomology, College of Agriculture, Vellayani. The objective was to generate information on the morphometric variations among the stingless bee population, their documentation and to evaluate the physico-chemical and antioxidant properties of their honey.

3.1. SELECTION OF STINGLESS BEE COLONIES

The stingless bee colonies were selected from two districts of southern Kerala*viz.*, Thiruvananthapuram andKollam (Fig. 1 and 2). A purposive sampling was conducted in beekeeping pocketsof midland (7.5 - 75 m above MSL) and upland (> 75 m above MSL) physiographic regions of Thiruvananthapuram and Kollam (Sakthimurukan, 2005). Fifteen domesticated feral colonies of stingless bees were selected from each physiographic region (Table 1).

3.2.NEST ARCHITECTURE AND DEFENSE MECHANISM

To determine the nest architecture and defense mechanism in stingless bee hive, the following architectural features were recorded.

3.2.1. Number of Guard Bees

The number of bees present in the nest entrance was recorded without disturbing the hive and it is expressed as muber of guard bees (Jayalekshmi, 2015)

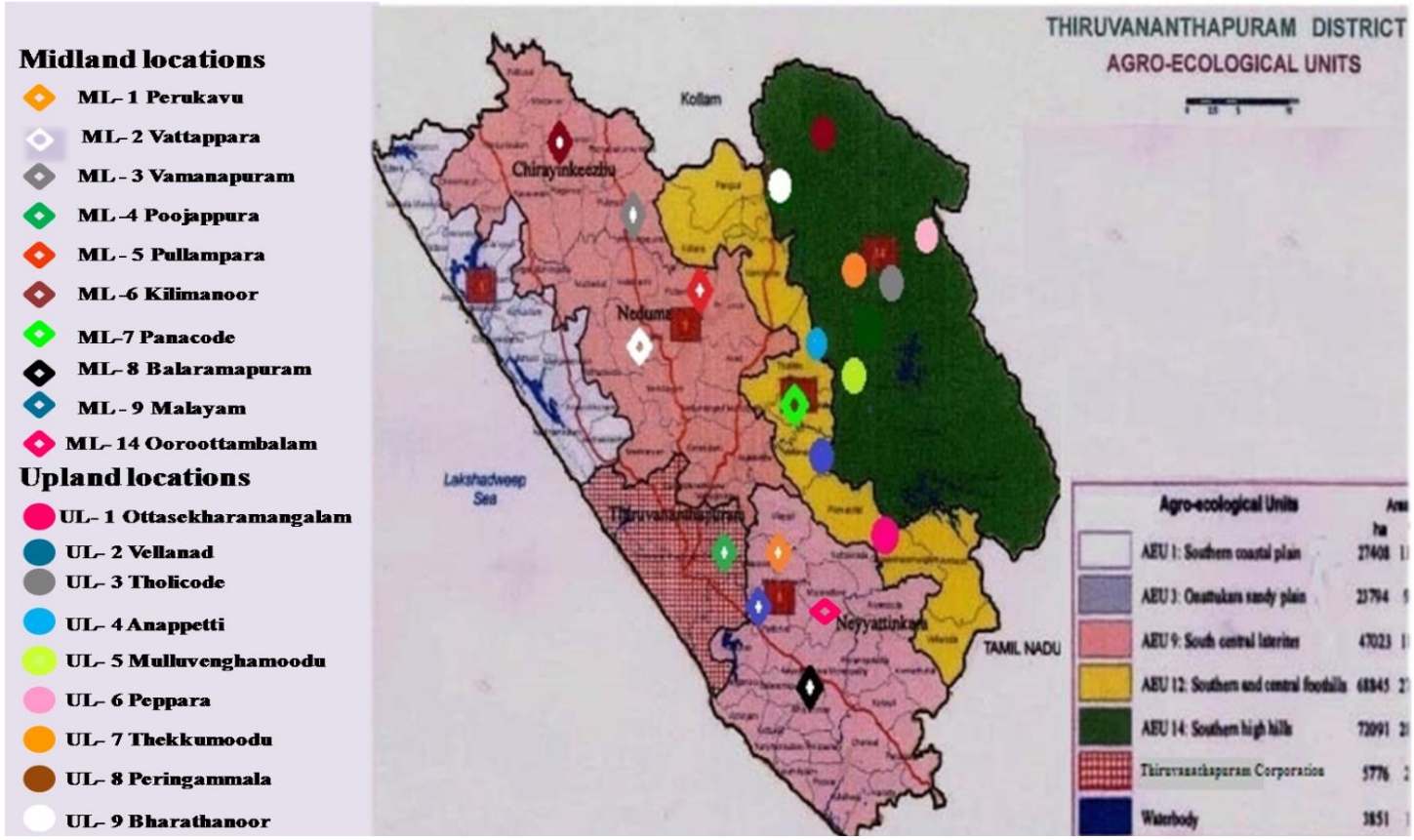

Fig 1.Physiographic details of the locations of stingless bee colonies in Thiruvananthapuram distr

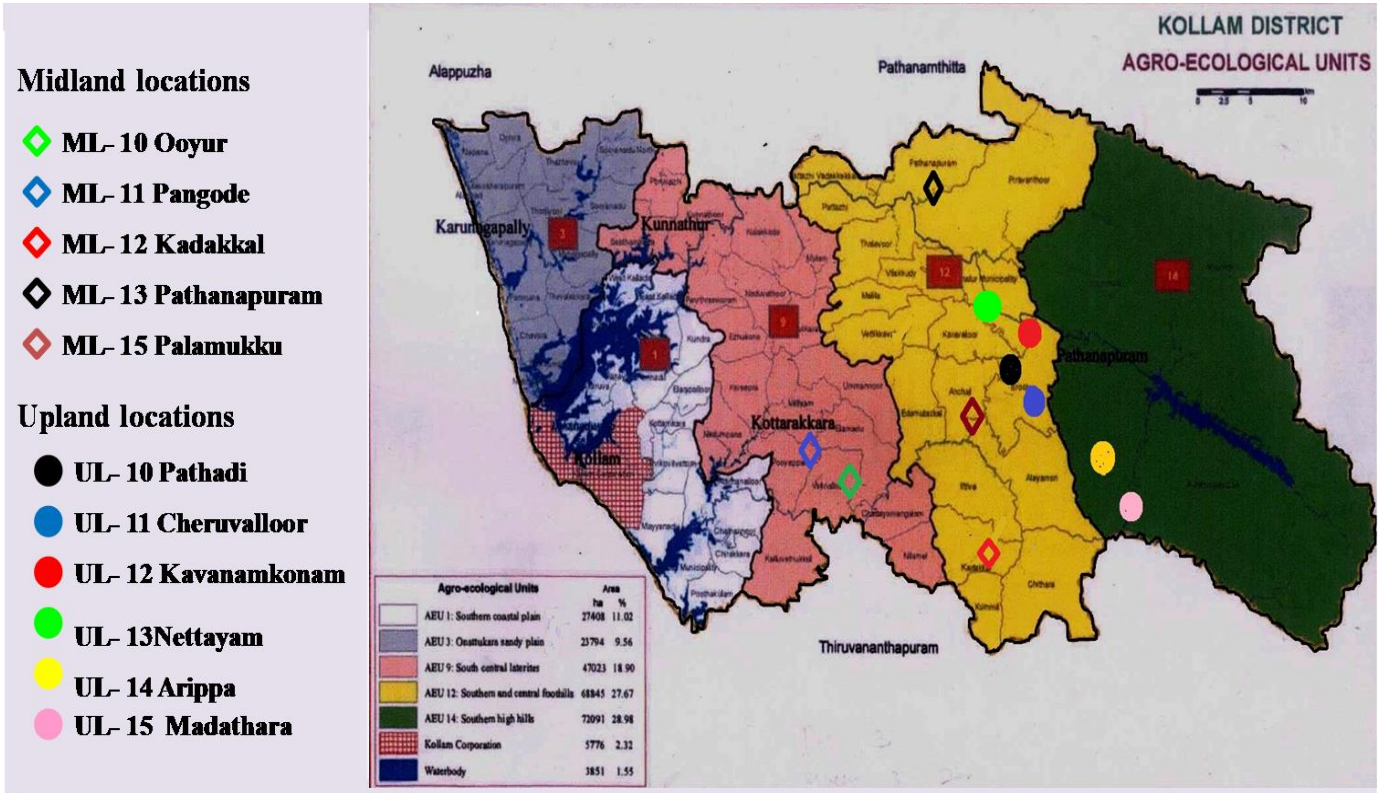

Fig. 2. Physiographic details of the locations of stingless bee colonies in Kollam district

Table 1. Physiographic details of the locations of stingless bee colonies in midland and upland regions

Sample No.	Locations	Latitude	Longitude
ML-1	Perukavu	8°49 N	77°01 E
ML-2	Vattappara	8°59 N	76°95 E
ML-3	Vamanapuram	8°72 N	76°89 E
ML-4	Poojappura	8°48 N	76°98 E
ML-5	Pullampara	8°41 N	76°57 E
ML-6	Kilimanoor	8°46 N	76°53 E
ML-7	Panacode	8°60 N	77°03 E
ML-8	Balaramapuram	8°38 N	77°08 E
ML-9	Malayam	8°28 N	77°10 E
ML-10	Ooyur	8°86 N	76°78 E
ML-11	Pangode	9°05 N	76°70 E
ML-12	Kadakkal	8°49 N	76°55 E
ML-13	Pathanapuram	9°50 N	76°51 E
ML-14	Ooroottambalam	8°45 N	77°05 E
ML-15	Palamukku	8°89 N	76°61 E
UL-1	Ottasekharamangalam	8°47 N	77°13 E
UL-2	Vellanad	8°56 N	77°05 E
UL-3	Tholicode	8°38 N	77°30 E
UL-4	Anappetti	8°65 N	77°05 E
UL-5	Mulluvenghamoodu	8°52 N	76°93 E
UL-6	Peppara	8°63 N	77°10 E
UL-7	Thekkumoodu	8°68 N	77°10 E
UL-8	Peringammala	8°72 N	77°04 E
UL-9	Bharathanoor	8°76 N	76°98 E
UL-10	Pathadi	8°93 N	76°96 E
UL-11	Cheruvalloor	8°89 N	76°61 E
UL-12	Kavanakonam	8°88 N	76°60 E
UL-13	Nettayam	8°94 N	76°93 E
UL-14	Arippa	8°83 N	77°02 E
UL-15	Madathara	8°64 N	77°02 E

3.2.2. Design of Hive Entrance

The design of the hive entrance was classified in to slit (elliptical), round, and multiple entrances (Roubik, 2006; Couvillion *et al.*, 2008; Pavithra *et al*., 2013) and the number of hive entrances under each design was recorded.

3.2.3. Length and Width of Hive Entrance

The length and width of the hive entrance was measured using a centimetre scale. The length of hive entrance was measured as the maximum length from the base to the apex of the hive entrance and it was categorized into<1 cm,1-3 cm and >3cm. The width of the hive entrance was taken from the mouth of the entrance tube by measuring maximum horizontal length and it was categorized into <1 cm, 1-2 cm and>2 cm.

3.2.4. Height from Ground Level

The height of stingless bee colonies from ground level was measured using a metre scale and was categorised into < 1m, 1-2 m and >2 m**.**

3.2.5. Materials Used for Constructing Hive Entrance

The materials used for the nest entrance construction were collected from each location and was observed under the microscope for identifying each and every material.

3.3. SAMPLE COLLECTION

Ten stingless bee workers and one honey sample each were collected from fifteen locations of each physiographic region.

3.3.1.Collection of Stingless Bee Workers

A polythene cover was placed with its mouth in front of the bee hive then the upper hive body was tapped so that the disturbed stingless bees were trapped inside the polythene cover. The collected stingless bees were killed by using

chloroform. The bees were wet preserved in ethyl alcohol (70%) in glass vials for morphometric studies (Muthuraman*et al*.,2013).

3.3.2.Collection of Stingless Bee Honey

The colony was subjected to honey extraction, once the stingless bee worker population was emptied up to 80-90 per cent from the hive. The honey was harvested from the honey pots by following the procedure ofAICRP(2004). All the honey samples were labelled and stored at room temperature in air tight containers for the analysis.

3.4.FORAGING PLANTS

The flora visited by the bees which serves as food source around the sampling locations ofstingless bee hive were documented and classified in to nectar providers, pollen providers and both nectar and pollen providers(Premila*et al.,* 2007 and Raju *et al*., 2013). The observations were taken at the peak foraging time (11 AM) from each physiographic region (Premila *et al.,* 2007).

3.5. MORPHOMETRIC VARIATIONS OF STINGLESS BEES IN SOUTHERN KERALA

The stingless bee workers preserved in ethyl alcohol (70%) were dissected out to separate the tagmata and the dissected body parts *viz.*, head, wing, hind leg, tergites and sternites;were subjected to morphometric measurements. The abdominal segments and proboscis were stretched with a drop of alcohol in a clean slide and fixed with a cover slip for getting an accurate measurement.

All the measurements were taken by stereomicroscope provided with high speed digital fire wire live camera, LAS measurement module and data transfer. The images were analyzed and the measurements of the following parameters were recorded.

3.5.1. Head

In head region, length and width of head (LH and WH) , distance between dorsal ocelli (DDO), dorsal ocello ocular distance (DOOD), proboscis length (PL) and antennal length (LA) were measured (Plate 1). The length of head was measured along the median line from the vertex of head to the apex of clypeus in a single vertical plane having same focal length whereas the width of head as the maximum distance across the eyes. The length:width ratio of head was also assessed. The distance between ocelli was measured as the shortest space between the lateral ocelli on dorsal surface of head and dorsal ocello ocular distance was measured as the shortest distance between the rim of lateral ocelli and compound eye (Muthuraman*et al*.,2013).

The proboscis length was measured along the median lines from the postmentum to labellum of glossa (Ruttner, 1988).

3.5.2. Thorax

In thorax, the parts of hind leg including length of femur (FL), length of tibia (TL) and length and width of metatarsus (LM and WM) were measured, the length and width of fore wings (FWL and FWW) and number of hamuli(HAM) (Plate 2) were also taken. The length of forewings was measured from the base of the coastal sclerite to the apical point and width was measured as the maximum width of the wing. The length: width ratio of metatarsus and forewing was also recorded.

3.5.3.Abdomen

The abdominal segments (tergites and sternites) were focussed (Snodgrass, 1956) and dissected in to individual segments. The segments measured in the abdominal region were length of third tergite (3TL), length and width of third sternite (3SL and 3SW) and the lateral width of fourth tergite tomentum (LWT)

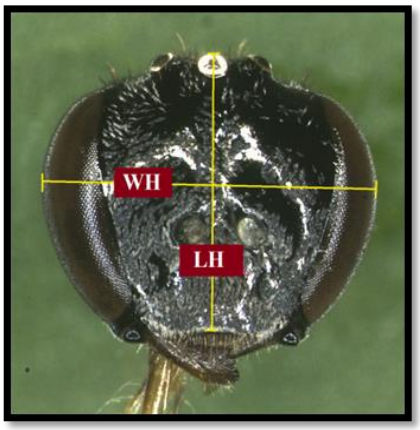

Plate 1a. Length and width of head (LH and WH)

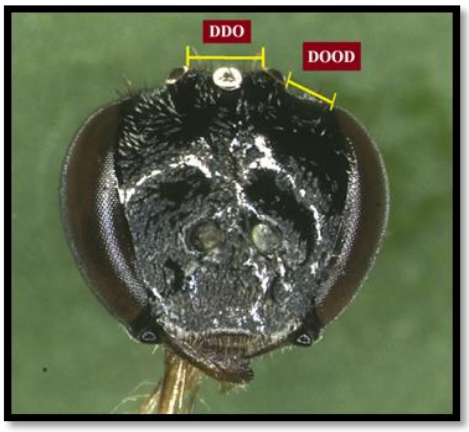

Plate 1b. Distance between dorsal ocelli (DDO), Dorsalocello ocular distance (DOOD)

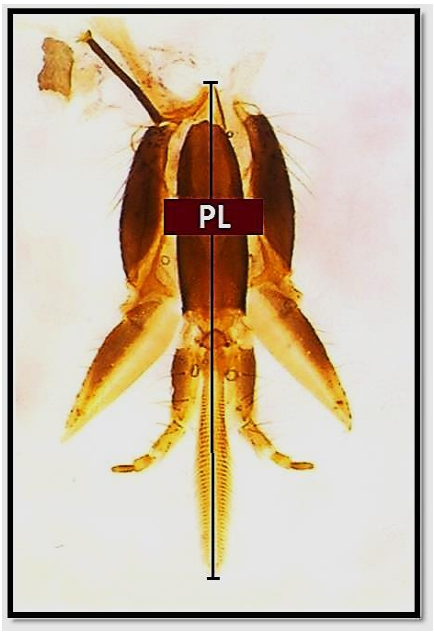

Plate 1c. Length of Proboscis (PL)

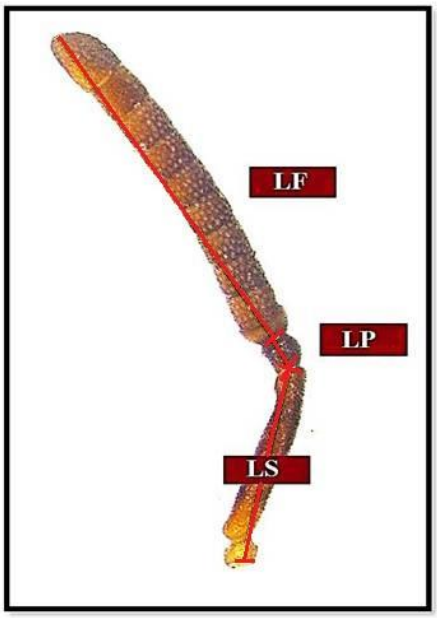

Plate 1d. Length of Antennae (LA) = Length of (Scape (LS) + Pedicel (LP) +Flagellum (LF))

Plate 1. Morphometric parameters in stingless bee head

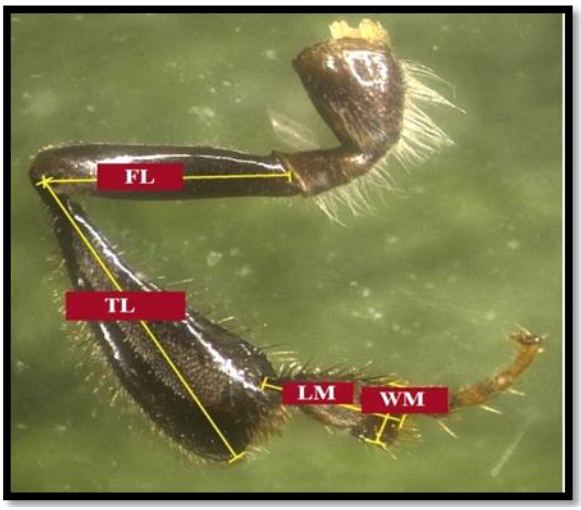

Plate 2a. FL – Femur Length, TL – Tibial Length, LM – Length of Metatarsus, WM – Width of Metatarsus

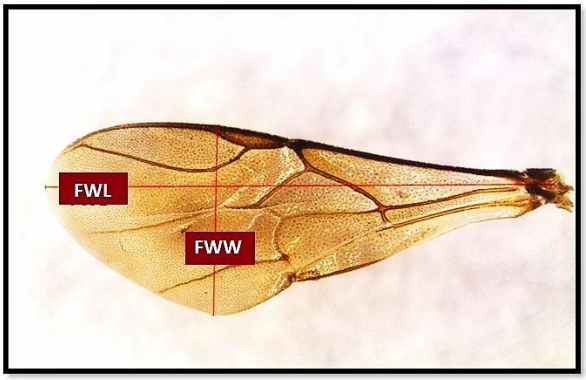

Plate 2b. Length and Width of Forewing (FWL and FWW)

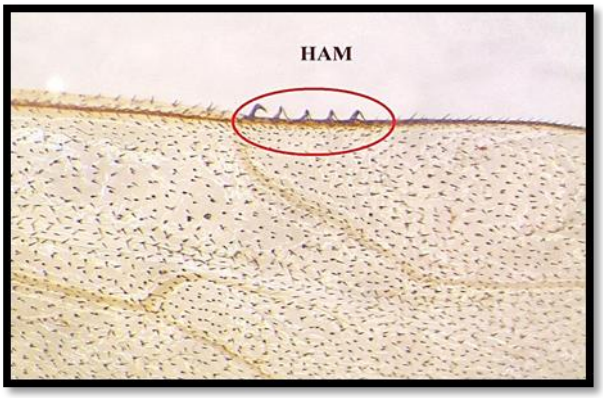

Plate 2c. Number of Hamuli (HAM)

Plate 2. Morphometric parameters in stingless bee thorax

(Plate 3). The length of tergites was measured across the midline. The length: width ratio of third sternite was also recorded.

3.6. QUALITY ANALYSIS OF STINGLESS BEE HONEY

3.6.1.Physicochemical Properties of Honey

The physical properties (pH, moisture content, electrical conductivity, total dissolved solids, colour intensity and total sugars (reducing and non reducing sugar)of stingless bee honey were estimated.

3.6.1.1. pH

Ten gram each of honey samples were dissolved in 100 ml CO_2 free distilled water to make 10 per cent honey solution. The pH was measured using a pH meter which was calibrated at room temperature using buffer solutions at pH 4 and 7 (Bogdanov*et al.*, 1997).

3.6.1.2. Moisture Content

Five gram of honey samples were dried for eight hours in a hot air oven at 105^{o}C in pre-weighed crucibles. The crucibles were transferred immediately to desiccators, cooled and weighed. The heating, cooling and weighing was continued until the honey attained a constant weight. The loss in weight represented the moisture content of the samples (AOAC, 1990).

$$\text{MoistureContent}(\%) = \frac{\text{Lossinweight}(g)}{\text{Weightofthesample}(g)} \text{x } 100$$

3.6.1.3. Electrical Conductivity (EC) and Total Dissolved Solids (TDS)

The EC and TDS were measured using a conductivity meter. The honey samples were prepared at a concentration of 20 per cent (w/v) by using distilled water. The conductivity meter was first calibrated with water and then conductance cell was dipped into honey (20%) solution and reading was noted. The EC was expressed in µS cm^{-1} and TDS in ppm (Khalil et al., 2012).

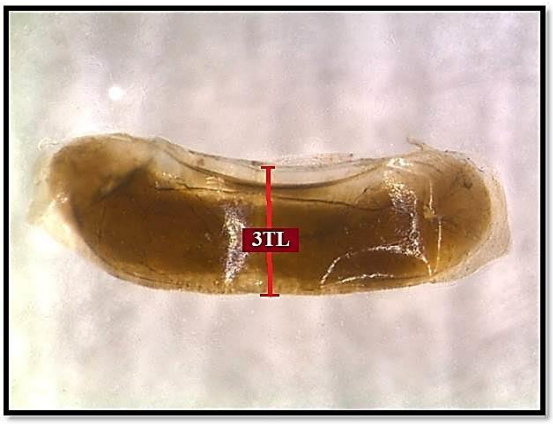

Plate 3a. Length of 3rd tergite

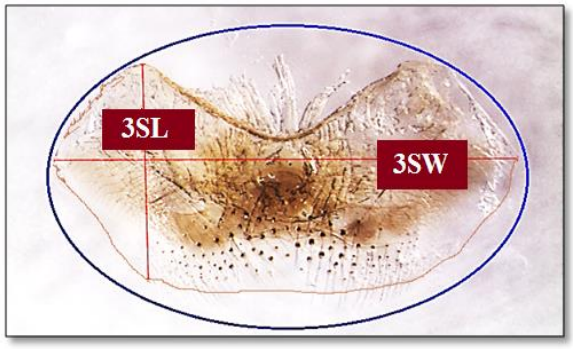

Plate 3b. Length (3SL) and Width (3SW) of 3rdSternite

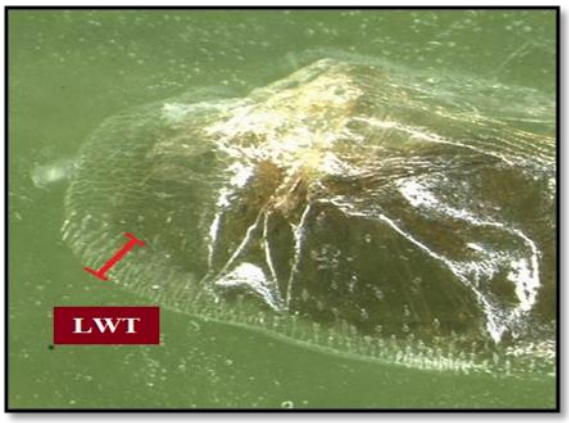

Plate 3c. Lateral Width of Fourth Tergite Tomentum (LWT)

Plate 3. Morphometric parameters in stingless bee abdomen

3.6.1.4. Colour Intensity

Honey colour intensity was determined using a spectrophotometer. The honey samples were diluted up to 50 per cent (w/v) with warm water (45-50°C) and the resulting solution was filtered to eliminate large particles. The net absorbance was defined as the difference between the spectrophotometric absorbance at 450 and 720 nm and it is expressed as mAU (Beretta *et al.*, 2005).

3.6.1.5.Total Sugar (Reducing and Non Reducing Sugars)

Five ml each of Fehling solution A and B was pipette out in to 250 ml conical flask. To this, 10 ml of water was added and boiled subsequently. The diluted honey solution was taken in the burette and titrated against boiling Fehling's solution. Few drops of methylene blue indicator were added to the boiling solution in conical flask. The appearance of brick red colour represented the end point of the titration (AOAC,1990).

$$\text{Reducing sugars (\%)} = \frac{0.05 \text{ x } 250}{\text{v x w}} \text{ x } 100$$

V = Titre value

W = wt. of sample (g)

A measured amount (50 ml) of the already prepared honey solution was taken and boiled by adding five ml of concentrated HCl for hydrolysis. The hydrolysed solution was cooled and neutralized with saturated NaOH solution followed by a drop of phenolphthalein; finally the volume was made up to 100 ml with distilled water. This solution was then titrated against boiling Fehling's solution with methylene blue indicator. Titre value was used to calculate the total sugars (%) using the formula.

$$\text{Nonreducingsugar} = \frac{100 \text{ x } 0.05 \text{ x } 0.95}{\text{v}}$$

V= Titre value after inversion

The chemical parameters (protein content, proline content, ascorbic acids, flavanoids, total phenols, diastase and invertase activity) of stingless bee honey were estimated.

3.6.1.6. Protein Content

Protein content of honey samples was estimated by using the biuret method. One ml of honey sample was made up to four ml with water followed by six ml of biuret reagent. This mixture was mixed well and incubated at 37°C for 10 min. This solution was further cooled and the absorbance was read at 520 nm against a reagent blank. Bovine serum albumin was used as standard for developing calibration curve. The protein content was calculated and expressed as percentage of protein in honey.

3.6.1.7.Proline Content

Honey sample (0.2 ml) was taken into a test tube and two ml of alkaline copper sulphate reagent was transferred into this solution and was thoroughly mixed. The resulting solution was incubated at room temperature for 10 min. Then 0.2 ml of Folin Ciocalteau was added to each tube and was incubated for 30 min. Then the absorbance was measured at 660 nm. The amount of proline was calculated from the standard graph prepared using bovine serum albumin and the proline content was calculated and expressed as mg of bovine serum albumin $100g^{-1}$ of honey (Khalil*et al*., 2012).

3.6.1.8. Ascorbic Acid

Ascorbic acid content in honey samples were determined by using redox indicator dye 2, 6 - dichlorophenol indophenol (Sadasivam and Manikam, 2008). The honey sample (1g) was made up to 100 ml by using oxalic acid (4%). From this, five ml of honey solution wastaken and mixed with 10 ml of oxalic acid

(4%) and this mixture was titrated against the dye solution(2, 6 - dichlorophenol indophenol). The dye was reduced to a colourless solution in the presence of ascorbic acid and the excess unreduced dye appeared as pink colour which indicated the end point and the colour persists for a few minutes. The amount of dye consumed is equivalent to the amount of ascorbic acid.

$$\text{Ascorbic acid (mg } 100^{-1}) = \frac{0.05}{v_1} \text{ x} \frac{v_2}{5} \text{ x } \frac{100}{w}$$

V_1= Amount of ascorbic acid titrated against dye

V_2 = Amount of honey solution titrated against dye

W = Weight of sample

3.6.1.9. Flavanoids

Flavanoid content in each honey sample was measured using the colorimetric assay developed by Bergner *et al.*(1972). One ml of honey was mixed with four ml of distilled water and subsequently with 0.3 ml of five per cent $NaNO_2$ (w/v). After five minutes of incubation, 0.3 ml of ten per cent $AlCl_3$was added. This was allowed to stand for five minutes followed by adding two ml of 1M NaOH to the mixture. The mixture was vigorously shaken to ensure adequate mixing after bringing the final volume to 10 ml with distilled water. The absorbance of the mixture was determined at a wavelength of 510 nm. A calibration curve was created using a standard solution of catechin. The results were expressed as mgcatechin equivalents (CEQ) kg^{-1} of honey.

3.6.1.10. Total Phenols

The concentration of phenols in honey samples was estimated using spectrophotometric Folin-Ciocalteu method (Malik and Singh., 1980). Honey sample (0.2ml) was pipetted out in to a 10 ml glass tube and was made up to a volume of three ml with distilled water. Then 0.5 ml of Folin ciocalteau reagent (1:1 with water) and two ml Na_2CO_3 (20 %) were added sequentially in each

tube. The test solutions were warmed for one minute and then cooled. Molybdenum blue colour was developed in each tube by the complex redox reaction of phenols with phosphomolybdic acid in alkaline medium and absorbance was measured at 650 nm against the reagent used as a blank. The amount of phenol content was determined from the calibration curve of catechol standard and was expressed as catechol equivalent of phenol kg $^{-1}$ of sample.

3.6.1.11. Diastase Activity

Sample solutions of each honey were preparedby dissolving 10 g of honey with water (15 ml) and of acetate buffer (5 ml) without heating. Subsequently the volume was made up to 50 ml with distilled water after the addition of three ml of sodium chloride solution. The standard water dilution was determined by reading the absorbance at a range of 0.770 to 0.745 A of iodine and starch solution with different water dilution at 660nm.

Ten ml each of honey and starch solution were pipetted out separately into two flasks(50 ml) and the flasks were placed in a 40°C water bath. After 15 minutes incubation, five ml of starch solution was pipetted out in to the honey solution, mixed very well. Thereafter, 0.5 ml aliquot of this mixture was removed periodically and five ml of iodine solution was rapidly added. This mixture was diluted by water (as determined in calibration of starch solution) mixed it well and absorbance of each separate solution was read immediately at 660 nm. The diastase value was calculated using the time taken for the absorbance to reach 0.235 and the results were expressed in diastase number (Bogdanov, 2002).

$$\text{Diastase Number (DN)} = \frac{60 \text{ min}}{tx} \text{x} \frac{0.10}{0.01} \text{x} \frac{1.0}{2.0} = \frac{300}{tx}$$

tx = reaction time in minutes

3.6.1.12. Invertase Activity

The substrate,p-nitrophenyl-α-D-glucopyranoside (pNPG) was used for the determination of invertase number in honey. The sample solution of each honey was prepared by adding honey (5 g) with buffer solution and the volume was made up to 25 ml with distilled water. The buffer solution was prepared by dissolving 11.66 g of potassium hydrogen phosphate (KH_2PO_4) and 2.56 g of disodium hydrogen phosphate ($Na_2HPO_4.2H_2O$) in water and the volume was made up to 1L in a volumetric flask. Five ml of pNPG substrate solution was kept in water bath at 40°C for five minutes and subsequently 0.5ml of honey solution was added into this mixture. The mixture was vigorously shaken by a vortex mixer and incubated at 40°C for 20 min. Then 0.5ml of reaction terminating mixture was added and the content of this mixture was shaken briefly for adequate mixing. The solutions were cooled at room temperature and absorbance of each honey samples were measured immediately with separate blank at 400 nm. The honey invertase activity was calculated from the absorbance measured at 400 nm and is indicated as invertase number (IN). The IN indicates the amount of sucrose g^{-1} hydrolysed in one hour by the enzymes contained in 100 g of honey under test conditions(Bogdanov, 2002).

3.6.2. Antioxidant Property of Stingless Bee Honey

Antioxidant content in honey was determined by FRAP Method (Ferric Reducing Ability of Plasma) developed by Benzie and Strain (1996). The principle behind this method was reduction of ferric tripyridyltriazine (Fe(III)-TPTZ) complex to the ferrous tripyridyltriazine (Fe(II)-TPTZ) by a reductant at low pH. (Fe(II)-TPTZ has an intensive blue colour and was measured at 593 nm.

One gram of honey was mashed with a cool mortar and pestle using quartz sand and nine ml cool 0.1 M phosphate buffer (pH 7.6, containing 0.1mM EDTA) was added. This mixture was filtered through a filter paper and centrifuged at 15000 rpm for 10 min. The supernatant was then used for the measurements.FRAP reagent was prepared by adding 200 ml of acetate buffer,

20 ml of TPTZ (2,4,6-tripyridyl-s-triazine)solution, 20 ml of $FeCl_3$ and 24ml distilled water and this solutionwas kept in water bath at 37°C . Thirty µl of distilled water was pipetted out to each cuvette and vigorously mixed it with one ml of FRAP reagent and the absorbance was read at 593 nm.

3.6.3. Microbial Load in Stingless Bee Honey Samples

The media used for microbial load assessment was nutrient agar (NA) and Sabouraud dextrose agar (SDA). Hundred ml of media was poured in 250 ml conical flasks and was sterilized by autoclaving at 1.1 kg cm^{-2} for 20 min.

Colony forming units (cfu) of bacteria were estimated by spread plate method using NA medium. Hundredµl of the honey suspension was poured over the solidified NA medium in a petri dish and the suspension was carefully spread over the media. Honey samples were prepared as 10^{-1}, 10^{-2}, 10^{-3} dilutions. Three replications were maintained for each treatment. The cfu were estimated at 24 hrs interval up to five days.

The cfu of fungus was estimated by pour plate method using 10 per cent fructose amended SDA medium. Sabouraud dextrose agar is a selective medium that is formulated to allow fungal growth whereas it inhibits the bacterial growth. The media was amended with ten per cent fructose for more sugar source. Honey samples were prepared with honey alone and also of dilutions viz., 10^{-1}, 10^{-2}and10^{-3}. One ml of the honey suspension was poured into each petri dish and 15 ml molten media was added and gently rotated for uniform spreading of suspension and incubated at room temperature. Three replications were maintained for each treatment. Colony forming units were estimated at three days interval upto15 days as follows.

3.7. STATISTICAL ANALYSIS

The data regarding the morphometric parameters of stingless bee and honey quality parameters were subjected to statistical analysis by using CRD after performing the required transformations. The mean data of stingless bee morphometric parameters as well as honey quality parameters from different locations were subjected to hierarchial cluster analysis and respective dendrograms were constructed by using L2 Euclidean distances and the wards linkage between groups of different locations.

Results

4. RESULTS

The results on the morphometric variations of stingless bees in southern Kerala and the quality assessment of their honey are summarized in this chapter.

4.1. NEST ARCHITECTURE AND DEFENSE MECHANISM

Nest architecture and defense mechanism at stingless bee nest entrance were documented as number of guard bees, design of hive entrance, length and width of hive entrance and height of colonies from ground level and the results are presented in Table 2.

4.1.1. Number of Guard Bees

Studies on the number of guard bees at hive entrance of midland locations revealed that maximum number of guard bees were observed at the hive entrance of Pullampara (20) followed by Malayam (15). While minimum number (4) was recorded at the hive entrance of Kilimanoor and Panacode. In the case of upland locations, maximum number of guard bees (10) was recorded from the colonies of Tholicode and Bharathanoor and minimum number from Ottasekhramangalam (3). The mean number of guard bees was found to be more in midland locations (7.6) than upland locations (6.4).

4.1.2. Design of Hive Entrance

The results revealed that out of the thirty locations, slit like hive entrance (22 nos.) was dominant followed by round (6 nos.) and multiple entrance (2 nos.) (Plate 4.).

Considering the midland, stingless bee colonies of four locations (Perukavu, Panacode, Malayam and Ooroottambalam) had round type entrance. All the other midland locations had slit type entrance except Poojappura which had multiple type entrance.

Plate 4a. Round type nest entrance at Malayam

Plate 4b. Round type nest entrance at Peppara;

Plate 4c**.** Slit like nest entrance at Ottasekharamangalam

Plate 4d. Slit like nest entrance at Pullampara

Plate 4e. Multiple nest entrance at Tholicode

Plate 4f. Multiple nest entrance at Poojappura

Plate 4. Design of nest entrance in stingless bee colonies

Table 2. Nest architectural variations of stingless bees in midland and upland physiographic regions

Midland locations	Number of guard bees	Design of hive entrance			Hive entrance tube						Height from ground level (m)		
					Length (cm)			Width (cm)					
		Round	Slit	Multiple entrance	<1	1-3	>3	<1	1-2	> 2	<1	1-2	>2
Perukavu	6	+					+		+			+	
Vattappara	6		+			+		+					+
Vamanapuram	6		+			+			+				+
Poojappura	5			+			+		+				+
Pullampara	20		+			+				+			+
Kilimanoor	4		+		+				+				+
Panacode	4	+				+		+				+	
Balaramapuram	8		+				+		+		+		
Malayam	15	+				+			+		+		
Ooyur	6		+		+				+				+
Pangode	6		+			+			+			+	
Kadakkal	6		+			+			+			+	
Pathanapuram	8		+		+				+			+	
Ooroottambalam	8	+				+			+			+	
Palamukku	6		+				+		+			+	
Midland (Mean/Total)	7.6#	4*	10*	1*	3*	8*	4*	2*	12*	1*	2*	7*	6*

- Mean value, * - Total (Cont.)

Table 2. Nest architectural variations of stingless bees in midland and upland physiographic regions

Upland Locations	Number of guard bees	Design of hive entrance			Hive entrance tube						Height from ground level (m)		
					Length (cm)			Width (cm)					
		Round	Slit	Multiple entrance	<1	1-3	>3	<1	1-2	>2	<1	1-2	>2
Ottasekharamangalam	3		+		+			+					+
Vellanad	6		+		+			+					+
Tholicode	10			+			+		+				+
Anappetti	8		+		+				+		+		
Mulluvenghamoodu	5	+			+			+					+
Peppara	6	+				+			+				+
Thekkumoodu	4		+			+		+					+
Peringammala	6		+			+			+		+		
Bharathanoor	10		+			+			+			+	
Pathadi	6		+		+				+			+	
Cheruvalloor	6		+		+			+				+	
Kavanakonam	6		+			+			+			+	
Nettayam	8		+				+		+				+
Arippa	7		+		+			+					+
Madathara	5		+			+			+			+	
upland(Mean/Total)	6.4#	2*	12*	1*	7*	6*	2*	6*	9*	-	2*	5*	8*
Midland and Upland (Total)	7#	6*	22*	2*	10*	14*	6*	8*	10*	1*	4*	12*	14*

- Mean value, * - Total

Regarding the upland, stingless bee colonies of two

locations (Mulluvenghamoodu and Peppara) had round type hive entrance, whereas all her locations had slit like entrance except Tholicode which had multiple nest entrance. Thus a similar trend in nest entrance design was observed in both midland and upland locations.

4.1.3. Length and Width of Hive Entrance

4.1.3.1. Length of Hive Entrance

The length of stingless bee hive entrance was categorized in to < 1 cm, 1-3 cm and > 3 cm and the results of the study showed that among the thirty locations, the length of hive entrance in stingless bee colonies from 10 locations were categorized under less than 1 cm, 14 locations under 1-3 cm and six locations had greater than 3cm hive entrance length.

Out of the fifteen midland locations, the length of hive entrance was < 1 cm in colonies of three locations *viz*., Kilimanoor, Ooyur and Pathanapuram whereas it ranged from 1-3 cm in eight locations *viz*., Vattappara, Vamanapuram, Pullampara, Panacode, Malayam, Pangode, Kadakkal and Ooroottambalam and the colonies from remaining four locations (Perukavu, Poojappura, Balaramapuram and Palamukku) recorded > 3cm hive entrance length.

Regarding the fifteen upland locations, the length of hive entrance was < 1 cm in colonies of seven locations (Ottasekharamangalam, Vellanad, Anappetti, Mulluvenghamoodu, Pathadi, Cheruvalloor and Arippa) whereas it ranged from 1-3 cm in five locations *viz*., Peppara, Thekkumoodu, Bharathanoor, Kavanakonam and Madathara and the remaining two locations (Tholicode and Nettayam) had > 3 cm hive entrance length.

While comparing the length of hive entrance tube between midland and upland locations, maximum colonies (8 nos.) were in 1-3 cm in the midland whereas in upland maximum colonies recorded < 1 cm hive entrance length.

4.1.3.2. Width of Hive Entrance

The width of hive entrance was classified into three groups (> 1, 1 - 2 and > 2 cm) and among the thirty locations, the width of hive entrance in stingless bee colonies from 8 locations was observed under the category < 1 cm, 21 locations under 1-2 cm, while only one location (Pullampara) had hive entrance width > 2 cm.

Among the fifteen midland locations, the width of hive entrance was < 1 cm in colonies of two locations *viz*., Vattappara and Panacode whereas it ranged from 1- 2 cm in all other locations except one location (Pullampara) which were under the category > 2 cm.

Out of fifteen upland locations, the width of hive entrance was < 1cm in stingless bee colonies of six locations *viz*., Ottasekharamangalam, Vellanad, Mulluvenghamoodu, Thekkumoodu, Cheruvalloor and Arippa whereas all the other locations had hive entrance width of 1 - 2 cm.

While comparing the width of hive entrance tube between midland and upland locations, maximum colonies from both the midland and upland locations had 1-2 cm which was followed by less than 1cm (8 nos.). It was also observed that the hive entrance width of stingless bee colonies from midland location lied under three categories while in upland locations, only two categories (< 1 cm and 1-2 cm) were recorded.

4.1.4. Height of Stingless Bee Colonies from the Ground Level

The number of stingless bee colonies maintained at different heights from the ground level (< 1m, 1-2 m, and > 2 m) in the midland and upland of southern Kerala are presented in Table 2.

Out of the thirty locations, maximum number of colonies (14) was maintained at a height of > 2 m, which was followed by 12 colonies maintained at a height of 1-2 m and < 1m (4 colonies).

Observations recorded from the midland locations revealed that two stingless bee colonies (Balaramapuram and Malayam) were maintained at a height of < 1 m from the ground level, while seven stingless bee colonies (Perukavu, Panacode, Pangode, Kadakkal, Pathanapuram, Ooroottambalam and Palamukku) were kept at a height of 1-2 m. The remaining five stingless bee colonies at the locations - Pullampara, Kilimanoor, Vattappara, Ooyur and Vamanapuram were kept at a height of > 2m.

In the case of stingless bee colonies at upland, two colonies (Anappetti and Peringammala) were maintained at a height of < 1 m from the ground level, while five stingless bee colonies (Bharathanoor, Pathadi, Cheruvalloor, Kavanakonam and Madathara) were kept in between 1 to 2 m from the ground level. The remaining eight stingless bee colonies at the locations Ottasekharamangalam, Vellanad, Tholicode, Mulluvenghamoodu, Peppara, Thekkumoodu, Nettayam and Arippa were maintained at a height of > 2 m.

While comparing the height of stingless bee colonies from ground level, the trend observed in both midland and upland locations was different. In the case of midland locations maximum number of colonies was maintained at a height of 1-2 m whereas in upland it was at > 2 m.

4.1.5. Materials Used for Constructing the Hive Entrance

The materials used for construction of hive entrances in fifteen locations each of upland and midland of southern Kerala are presented in Table 3. The results revealed that the hive entrances of all stingless bee colonies except the midland location Malayam was constructed with bee wax, plant resin, dry leaves and vegetative parts, wood particles, bee cadavers and sand and soil particles (Plate 5). The hive entrance of stingless bee colony at Malayam was constructed only with beewax, plant resin and sand and soil particles.

Table 3. Materials used for nest entrance construction in stingless bee colonies

Locations	Bee wax	Plant resin	Dry leaves and vegetative parts	Wood particles	Bee cadavers /Body parts	Sand and soil particles
Perukavu (ML-1)	+	+	+	+	+	+
Vattappara (ML-2)	+	+	+	+	+	+
Vamanapuram (ML-3)	+	+	+	+	+	+
Poojappura (ML-4)	+	+	+	+	+	+
Pullampara (ML-5)	+	+	+	+	+	+
Kilimanoor (ML-6)	+	+	+	+	+	+
Panacode (ML-7)	+	+	+	+	+	+
Balaramapuram (ML-8)	+	+	+	+	+	+
Malayam (ML-9)	+	+				+
Ooyur (ML-10)	+	+	+	+	+	+
Pangode (ML-11)	+	+	+	+	+	+
Kadakkal (ML-12)	+	+	+	+	+	+
Pathanapuram (ML-13)	+	+	+	+	+	+
Ooroottambalam (ML-14)	+	+	+	+	+	+
Palamukku (ML-15)	+	+	+	+	+	+
Ottasekharamangalam (UL-1)	+	+	+	+	+	+
Vellanad (UL-2)	+	+	+	+	+	+
Tholicode (UL-3)	+	+	+	+	+	+
Anappetti (UL- 4)	+	+	+	+	+	+
Mulluvenghamoodu (UL-5)	+	+	+	+	+	+
Peppara (UL-6)	+	+	+	+	+	+
Thekkumoodu (UL-7)	+	+	+	+	+	+
Peringammala (UL-8)	+	+	+	+	+	+
Bharathanoor (UL-9)	+	+	+	+	+	+
Pathadi (UL-10)	+	+	+	+	+	+
Cheruvalloor (UL-11)	+	+	+	+	+	+
Kavanakonam (UL-12)	+	+	+	+	+	+
Nettayam (UL-13)	+	+	+	+	+	+
Arippa (UL-14)	+	+	+	+	+	+
Madathara (UL-15)	+	+	+	+	+	+

Plate 5a. Bee wax and plant resin mixture

Plate 5b. Plant resin globules

Plate 5c. Sand and soil particles

Plate 5d. Chewed vegetative materials

Plate 5. Materials used for nest entrance construction

4.2. FORAGING PLANTS OF STINGLESS BEES IN MIDLAND AND UPLAND PHYSIOGRAPHIC REGIONS

Studies on foraging plants of stingless bees based on food sources (nectar as carbohydrate and pollen as protein) and locations (Table 4) revealed that they visited fifty plants throughout the midland and upland physiographic regions. They included medicinal plants (7), plantation crops (5), condiments and spices (1), fruit crops (9), vegetable crops (7), ornamental plants (13), tuber crops (2), weeds (1) and forest trees (5) (Plate 6.). It was observed that out of the fifty foraging plants, fourteen were nectar providers; fourteen were pollen providers while 22 plants were both nectar and pollen providers. It was also revealed that maximum number of nectar (4) and pollen (4) providing plants were recorded from the group of ornamental plants.

A total of 36 foraging plants were found to be visited by stingless bees in midland physiographic region (Table 5) of which rubber and coconut were mostly visited by stingless bees from nine out of fifteen locations. Maximum number of foraging plants (8) was recorded from Perukavu which includes *Carica papaya* L., *Zinnia elegans* L., *Clematis vittalba* L., *Cymbidium* sp., *Begonia semperflorens* L., *Rosa* sp., *Euphorbia milii* Des Moul. and *Thumbergia grandiflora* Roxb. Whereas the lowest number of foraging plants were recorded from Palamukku (*Heavea brasiliensis* and *Cocos nucifera*).

Foraging plants visited by stingless bees in upland locations were recorded as thirty (Table 6), of which rubber was the most prominently observed foraging plant (10 locations). Maximum number of foraging plants was recorded from Vellanad, which included *Mimosa pudica* L., *Flacourtia inermis* (Burm f.)., *Lowsonia inermis* L., *Cocos nucifera* L., *Musa* sp., *Portulaca grandiflora* H., *Thumbergia grandiflora, Euginea jambosa* L., *Duranta plumier* L., *Manihot esculenta* L, *Azadirachta indica* L., *Carica papaya* and Santalum album.L. Among the fifteen uplands locations, only one foraging plant (Heavea brasiliensis L.) was recorded from peppara.

Table 4. Foraging flora of stingless bees based on their food sources

Sl. No.	Common name	Scientific name	Source		
			Nectar	Pollen	Nectar + Pollen
MEDICINAL PLANTS					
1.	Touch- me-not	*Mimosa pudica* L.			+
2.	Sida	*Sida acuta* Burm. f.			+
3.	Holy basil	*Ocimum sanctum* L.	+		
4.	Henna	*Lowsonia inermis* L.		+	
5.	Kacholam	*Kaempferia galanga* L.			+
6.	Ixora	*Ixora coccinea* L.		+	
7.	Malabar nut	*Adhatoda vasica* (L.)		+	
			1	3	3
PLANTATION CROPS					
8.	Rubber	*Hevea brasiliensis* L.	+		
9.	Coconut	*Cocos nucifera* L.			+
10.	Coffee	*Coffea arabica* L.	+		
11.	Coffee	*Coffea robusta* L.	+		
12.	Cashew	*Anacardium occidentale* L.			+
			3		2
CONDIMENTS AND SPICES					
13.	Tamarind	*Tamarindus indica* L.			+
					1
FRUIT CROPS					
14.	Mango	*Mangifera indica* L.		+	
15.	Banana	*Musa* sp.	+		
16.	Guava	*Psidium guajava* L.			+
17.	Papaya	*Carica papaya* L.		+	
18.	Rose apple	*Euginea jambosa* L.			+
19.	Lovi lovi	*Flacourtia inermis* (Burm. f.)			+
20.	Ramboottan	*Nephelium lappaceum* L.	+		

21.	Anjili	*Artocarpus hirsuta* Lam.			+
22.	Passion fruit	*Passiflora grandiflorum* L.		+	
			2	3	4
VEGETABLE CROPS					
23.	Cowpea	*Vigna unguiculata* (L.)	+		
24.	Snake gourd	*Trichosanthes cucumerina* L.		+	
25.	Bitter gourd	*Momordica charantia* L.		+	
26.	Drumstick	*Moringa oleifera* Lam.			+
27.	Bilimbi	*Averrhoa bilimbi* L.		+	
28.	Amaranthus	*Amaranthus* sp.			+
29.	Brinjal	*Solanum melongena* L.			+
ORNAMENTAL PLANTS			1	3	3
30.	Coral creeper	*Antigonon leptopus* L.			+
31.	Golden dew drop	*Duranta plumieri* L.			+
32.	Adenium	*Adenium obesum* (F.)	+		
33.	Hibiscus	*Hibiscus rosasinensis* L.		+	
34.	Ten O clock plant	*Portulaca grandiflora* H.		+	
35.	Euphorbia	*Euphorbia milii* Des Moul.	+		
36.	Clock wine	*Thumbergia grandiflora* Roxb.	+		
37.	Zinnia	*Zinnia elegans* L.		+	
38.	Wedding bouquet	*Clematis vittalba* L.		+	
39.	Begonia	*Begonia semperflorens* L.			+
40.	Rose	*Rosa* sp.			+
41.	Cymbidium	*Cymbidium* sp.	+		
42.	Hamelia	*Hamelia patens* Jacq.	+		
			4	4	4
TUBER CROPS					
43.	Tapioca	*Manihot esculenta* L.			+
44.	Wild Tapioca	*Manihot glaziovi* M.	+		
			1		1

WEED PLANT					
45.	Clerodendrum	*Clerodendrum infortunatum* L.	+		
			1		
FOREST TREES					
46	Acacia	*Acacia mangium* W.		+	
47	Teak	*Tectona grandis* L.			+
48	Neem	*Azadirachta indica* L.			+
49	Sandal	*Santalum album* L.			+
50	Copper pod tree	*Peltophorum ferrugineum* B.			+
				1	4
	TOTAL		14	14	22

Table 5. Flora visited by stingless bees in the midland locations

Sl. No.	Common name	Scientific name	ML 1	ML 2	ML 3	ML 4	ML 5	ML 6	ML 7	ML 8	ML 9	ML 10	ML 11	ML 12	ML 13	ML 14	ML 15
1	Touch- me-not	*Mimosa pudica*					+									+	
2	Sida	*Sida acuta*														+	
3	Holy basil	*Ocimum sanctum*						+					+				
4	Rubber	*Heavea brasiliensis*		+			+	+	+			+		+	+	+	+
5	Coconut	*Cocos nucifera*		+	+			+	+	+			+	+		+	+
6	Cashew	*Anacardium occidentale*										+					
7	Coffee	*Coffea robusta*											+				
8	Tamarind	*Tamarindus indica*											+				
9	Mango	*Mangifera indica*				+		+		+	+	+					
10	Banana	*Musa* sp.					+										
11	Guava	*Psidium guvajava*			+	+										+	
12	Papaya	*Carica papaya*	+														
13	Passion fruit	*Passiflora grandiflorum*				+		+									
14	Rose apple	*Euginea jambosa*															
15	Cowpea	*Vigna unguiculata*			+					+							
16	Snake gourd	*Trichosanthes cucumerina*								+							
17	Bitter gourd	*Momordica charantia*								+							
18	Drumstick	*Moringa oleifera*															
19	Bilimbi	*Averhoa bilimbi*				+							+				
20	Amaranthus	*Amaranthus viridis*															
21	Brinjal	*Solanum melongena*															
22	Coral creeper	*Antigonon leptopus*													+		
23	Golden dew drop	*Duranta plumieri*						+					+				

(contd.)

Table 5. Flora visited by stingless bees in the midland locations

24	Adenium	*Adenium obesum*														+	
25	Zinnia	*Zinnia elegans*	+														
26	Wedding bouquet	*Clematis vittalba*	+														
27	Begonia	*Begonia semperflorens*	+														
28	Rosa	*Rosa* sp.	+														
29	Cymbidium	*Cymbidium* sp.	+							+	+						
30	Euphorbia	*Euphorbia milii*	+	+	+					+	+				+		
31	Clock wine	*Thumbergia grandiflora*	+								+						
32	Ten O clock plant	*Portulaca grandiflora*															
33	Tapioca	*Manihot esculenta*							+								
34	Wild Tapioca	*Manihot glaziovi*					+			+	+						
35	Cleodendron	*Cleodendron infortunatum*															
36	Acacia	*Acacia mangium*														+	

ML1 - Perukavu, ML2 - Vattappara, ML3 - Vamanapuram, ML4 - Poojappura, ML5 - Pullampara, ML6 - Kilimanoor, ML7 - Panacode, ML8 - Balaramapuram, ML9 - Malayam, ML10 - Ooyur, ML11 - Pangode, ML12- Kadakkal, ML13 - Pathanapuram, ML14- Ooroottambalam, ML15- Palamukku.

Table 6. Flora visited by stingless bees in the upland locations

Sl. No.	Common name	Scientific name	UL 1	UL 2	UL 3	UL 4	UL 5	UL 6	UL 7	UL 8	UL 9	UL 10	UL 11	UL 12	UL 13	UL 14	UL 15
1	Touch- me-not	*Mimosa pudica*	+	+													
2	Malabar nut	*Adhatoda vasica*								+							
3	Holy basil	*Ocimum sanctum*		+							+						+
4	Henna	*Lowsonia inermis*	+	+							+						
5	Kacholam	*Kaemferia galanga*								+							
6	Ixora	*Ixora coccinea*									+						
7	Rubber	*Heavea brasiliensis*				+	+	+		+	+	+	+	+	+	+	
8	Coconut	*Cocos nucifera*	+	+								+			+		+
9	Coffee	*Coffea arabica*									+						
10	Coffee	*Coffea robusta*												+			
11	Tamarind	*Tamarindus indica*										+	+				
12	Banana	*Musa* sp.		+						+							
13	Guava	*Psidium guvajava*	+									+					
14	Papaya	*Carica papaya*		+													
15	Rose apple	*Euginea jambosa*	+	+													
16	Lovi lovi	*Flacourtia inermis*		+													
17	Ramboottan	*Nephelium lappaceum*			+												
18	Anjili	*Artocarpuus hirsuta*			+				+	+							
19	Bilimbi	*Averhoa bilimbi*			+						+				+	+	
20	Amaranthus	*Amaranthus* sp.														+	+
21	Coral creepar	*Antigonon leptopus*													+		
22	Golden dew drop	*Duranta plumieri*		+													

(contd.)

Table 6. Flora visited by stingless bees in the upland locations

23	Ten O clock plant	*Portulaca grandiflora*		+	+	+											
24	Euphorbia	*Euphorbia mlilii*															+
25	Clock wine	*Thumbergia grandiflora*		+													
26	Tapioca	*Manihot esculenta*		+													
27	Copper pod tree	*Peltophorum ferrugineum*							+								
28	Teak	*Tectona grandis*															+
29	Neem	*Azadirachta indica*		+													
30	Sandal	*Santalum album*		+						+							

UL1 - Ottasekharamangalam, UL2 - Vellanad, UL3 - Tholicode , UL4 - Anappetti, UL5 - Mulluvenghamoodu, UL6 - Peppara,

UL7 - Thekkumoodu, UL8 - Peringammala, UL 9 - Bharathanoor, UL 10 - Pathadi, UL11 - Cheruvalloor, UL12 - Kavanakonam,

UL 13- Nettayam, UL14- Arippa, UL15 - Madathara.

Plate 6. Foraging plants of stingless bee: 6a. - *Portulaca grandiflora*; 6b- *Ixora coccinea*; 6c. - *Carica papaya*; 6d.- *Moringa oleifera*; 6e. - *Averrhoa bilimbi;* 6f. - *Peltophorum ferrugineum*

4. 3. MORPHOMETRIC VARIATIONS IN STINGLESS BEES

The results on the morphometric parameters of stingless bee head, thorax and abdomen are presented below.

4.3.1. Head

The results on variations of morphometric parameters in stingless bee head (head length (LH), head width (WH), proboscis length (PL), antennal length (LA), distance between dorsal ocelli (DDO) and dorsal ocello occular distance (DOOD)) are presented in Table 7. Among the various morphometric parameters observed, significant variations between midland and upland samples was observed only in the proboscis length.

4.3.1.1. Head Length

Maximum head length was observed in the stingless bee from the midland location, Malayam (1.086 mm) which was on par with that of samples collected from the upland location, Peppara (1.073 mm) and midland location, Vattappara (1.063 mm). Minimum head length was recorded in the samples from midland location Ooyur (0.957 mm), which was found to be on par with that of samples from upland location Pathadi (0.979 mm) and midland location Ooroottambalam (0.969 mm). The head length of remaining samples ranged from 0.991 to 1.055 mm.

4.3.1.2. Head Width

The results of head width in stingless bee samples revealed that the maximum width was observed in the samples from upland location, Peppara (1.342 mm), while it was on par with that of samples collected from midland locations, Perukavu (1.325mm), Malayalam (1.319 mm) and Kilomanoor (1.314 mm). Lowest head width was recorded in the stingless from midland

Table 7. Variations of morphometric parameters of stingless bee head in midland and upland physiographic regions of southern Kerala

Locations	LH (mm)	WH (mm)	DDO (mm)	DOOD (mm)	LA (mm)	PL (mm)
Perukavu (ML-1)	1.021#	1.325	0.312	0.183	1.530	1.136
Vattappara (ML-2)	1.063	1.242	0.307	0.199	1.466	0.970
Vamanapuram (ML-3)	1.003	1.285	0.298	0.190	1.475	0.956
Poojappura (ML-4)	1.029	1.214	0.285	0.182	1.488	1.130
Pullampara (ML-5)	0.999	1.262	0.296	0.196	1.460	1.068
Kilimanoor (ML-6)	0.994	1.314	0.292	0.174	1.494	0.898
Panacode (ML-7)	1.004	1.163	0.292	0.189	1.434	0.957
Balaramapuram (ML-8)	1.012	1.237	0.299	0.176	1.494	1.171
Malayam (ML-9)	1.086	1.319	0.315	0.196	1.633	1.502
Ooyur (ML-10)	0.957	1.171	0.287	0.202	1.458	0.893
Pangode (ML-11)	1.037	1.273	0.299	0.191	1.563	1.043
Kadakkal (ML-12)	1.020	1.292	0.286	0.184	1.474	0.883
Pathanapuram (ML-13)	1.017	1.215	0.309	0.201	1.427	1.048
Ooroottambalam (ML-14)	0.969	1.173	0.288	0.203	1.495	0.926
Palamukku (ML-15)	1.033	1.243	0.296	0.189	1.576	1.157
Mean (Midland)	1.016	1.248	0.298	0.178	1.431	1.049
Ottasekharamangalam (UL-1)	1.041	1.229	0.290	0.184	1.467	0.949
Vellanad (UL-2)	1.002	1.219	0.285	0.186	1.467	0.985
Tholicode (UL-3)	1.029	1.243	0.310	0.186	1.489	1.149
Anappetti (UL-4)	1.002	1.212	0.295	0.191	1.494	0.977
Mulluvenghamoodu (UL-5)	1.005	1.167	0.287	0.194	1.460	1.010
Peppara (UL-6)	1.073	1.342	0.309	0.201	1.471	0.928
Thekkumoodu (UL-7)	1.006	1.254	0.278	0.172	1.471	0.933
Peringammala (UL-8)	0.991	1.252	0.288	0.184	1.460	1.158
Bharathanoor (UL-9)	1.029	1.255	0.307	0.194	1.547	1.215
Pathadi (UL-10)	0.979	1.228	0.288	0.185	1.450	1.000
Cheruvalloor (UL-11)	1.004	1.190	0.294	0.202	1.455	0.993
Kavanakonam (UL-12)	1.008	1.207	0.312	0.207	1.489	0.982
Nettayam (UL-13)	1.004	1.213	0.298	0.192	1.503	0.993
Arippa (UL-14)	1.055	1.252	0.316	0.178	1.533	1.055
Madathara (UL-15)	1.000	1.247	0.296	0.272	1.493	1.125
Mean (Upland)	1.015	1.234	0.297	0.195	1.483	1.030
Midland Vs upland	NS	NS	NS	NS	NS	S**
CD (0.05 level)	0.030	0.035	0.010	NS	0.048	0.104

#Average of 10 replications; ** - Significant at 1 per cent level, NS - Non significant
LH - Head Length, WH - Head Width, DDO - Distance Between Ocelli, DOOD - Dorsal Ocello Ocular Distance, LA - Antennae Length, PL - Proboscis Length, ML - Midland, UL - Upland

location, Panacode (1.163 mm) which was found to be on par with that of samples collected from other midland locations, Mulluvenghamoodu (1.167 mm), Ooyur (1.171 mm), Ooroottambalam (1.173 mm) and Cheruvalloor (1.190 mm). The head width of bees observed from the remaining locations ranged from 1.207 to 1.292 mm.

4.3.1.3. Distance Between Ocelli

The maximum distance between ocelli in the head of stingless bee workers was observed as 0.316 mm in the samples collected from upland location, Arippa followed by Malayam (0.315 mm), Perukavu (0.312 mm), Kavanakonam (0.312 mm), Tholicode (0.310 mm), Peppara (0.309 mm), Pathanapuram (0.309 mm), Vattappara (0.307 mm) and Bharathanoor (0.307 mm) and were found to be on par. The stingless bee samples from the upland location, Thekkumoodu recorded the lowest distance between ocelli (0.278 mm) which was on par with that of samples collected from Poojappura (0.285 mm), Vellanad (0.285 mm), Kadakkal (0.286 mm), Mulluvenghamoodu (0.287 mm) and 0.287 mm (Ooyur). The distance between ocelli of the remaining samples ranged between 0.288 and 0.299 mm.

4.3.1.4. Dorsal Ocello Ocular Distance

The highest dorsal ocello ocular distance was observed in stingless bee samples from the upland location, Madathara (0.272 mm) and the lowest one was that from Thekkumoodu (0.172 mm). The dorsal ocello ocular distance of the remaining samples was in between 0.174 and 0.207 mm.

4.3.1.5. Antennae Length

The antennae length was observed to be maximum in stingless bee samples from midland location, Malayam (1.633 mm) which was on par with that of samples collected from upland location, Palamukku (1.576 mm). The stingless

4.3.1.6. Proboscis Length

Regarding the proboscis length of stingless bees, the midland location Malayam had significantly higher proboscis length (1.502 mm). It was followed by the proboscis length of stingless bee samples from Bharathanoor (1.215 mm), Balaramapuram (1.171 mm), Peringammala (1.158 mm), Palamukku (1.157 mm), Tholicode (1.149 mm), Perukavu (1.136 mm), Poojappura (1.130 mm) and Madathara (1.125 mm) which were statistically on par. The lowest value was recorded from midland location, Kadakkal (0.883). Proboscis length recorded from the rest of the samples ranged from 0.893 to 1.068 mm.

4.3.2. Thorax

The morphometric parameters of stingless bee thorax (length of femur (FL), length of tibia (TL), length and width of metatarsus (LM and WM), length and width of fore wing (FWL and FWW) and number of hamuli (HAM) are shown in Table 8. The stingless bee thorax parameters *viz.*, femur length, metatarsus length and length and width of forewing of the samples collected from midland location were found to be statistically superior over the upland samples.

4.3.2.1. Femur Length

Maximum femur length was observed in the stingless bee samples collected from midland location, Malayam (0.918 mm) which was significantly superior over the remaining samples. The lowest femur length was recorded in the stingless bees from Ottasekharamangalam (0.721) which was on par with that of the samples from Kadakkal (0.739 mm). The femur length of stingless bee samples from remaining locations was observed in between 0.745 and 0.801 mm.

4.3.2.2. Tibial Length

Stingless bee samples collected from midland location, Malayam (1.260 mm) had maximum tibial length which was on par with the samples from upland location, Tholicode (1.209 mm). Lowest tibial length was recorded in the

Table 8. Variations of morphometric parameters of stingless bee thorax in midland and upland physiographic regions of southern Kerala

Locations	FL (mm)	TL (mm)	LM (mm)	WM (mm)	FWL (mm)	FWW (mm)	HAM
Perukavu (ML-1)	0.797#	1.192	0.443	0.239	2.949	1.059	5.00
Vattappara (ML-2)	0.759	1.073	0.392	0.192	2.865	1.047	5.00
Vamanapuram (ML-3)	0.773	1.172	0.414	0.210	2.853	1.079	5.00
Poojappura (ML-4)	0.791	1.154	0.406	0.198	2.823	1.035	5.00
Pullampara (ML-5)	0.787	1.206	0.372	0.228	2.844	1.014	5.00
Kilimanoor (ML-6)	0.756	1.165	0.399	0.175	2.886	1.050	5.00
Panacode (ML-7)	0.774	1.087	0.390	0.187	2.738	1.017	5.00
Balaramapuram (ML-8)	0.775	1.131	0.370	0.189	2.937	1.097	5.00
Malayam (ML-9)	0.918	1.260	0.480	0.257	3.284	1.183	5.00
Ooyur (ML-10)	0.760	1.080	0.382	0.212	2.746	1.076	5.00
Pangode (ML-11)	0.790	1.078	0.399	0.194	2.809	1.055	5.00
Kadakkal (ML-12)	0.739	1.065	0.423	0.200	2.813	1.024	5.00
Pathanapuram (ML-13)	0.776	1.084	0.397	0.194	2.689	1.045	5.10
Ooroottambalam (ML-14)	0.749	1.037	0.383	0.178	2.763	1.007	5.00
Palamukku (ML-15)	0.781	1.079	0.410	0.202	2.806	1.054	5.00
Midland (Mean)	0.782	1.124	0.404	0.204	2.854	1.056	5.01
Ottasekharamangalam (UL-1)	0.721	1.127	0.422	0.183	2.936	1.077	5.00
Vellanad (UL-2)	0.757	1.127	0.399	0.222	2.648	1.011	5.00
Tholicode (UL-3)	0.807	1.209	0.402	0.204	2.779	1.030	5.00
Anappetti (UL-4)	0.761	1.074	0.397	0.202	2.778	1.000	5.00
Mulluvenghamoodu (UL-5)	0.779	1.110	0.414	0.186	2.782	1.027	5.00
Peppara (UL-6)	0.801	1.122	0.422	0.204	2.805	1.020	5.00
Thekkumoodu (UL-7)	0.745	1.113	0.383	0.187	2.796	1.019	5.00
Peringammala (UL-8)	0.765	1.157	0.365	0.209	2.773	1.045	4.80
Bharathanoor (UL-9)	0.773	1.123	0.375	0.208	2.846	1.059	4.90
Pathadi (UL-10)	0.762	1.088	0.419	0.195	2.791	1.044	5.00
Cheruvalloor (UL-11)	0.783	1.085	0.355	0.195	2.782	1.055	5.00
Kavanakonam (UL-12)	0.788	1.089	0.372	0.215	2.817	1.036	5.00
Nettayam (UL-13)	0.774	1.087	0.376	0.194	2.784	1.026	5.00
Arippa (UL-14)	0.847	1.184	0.437	0.207	2.884	1.064	5.00
Madathara (UL-15)	0.775	1.085	0.405	0.208	2.849	1.007	5.10
Upland (Mean)	0.776	1.119	0.396	0.201	2.803	1.035	4.97
Midland Vs upland	S**	NS	S*	NS	S**	S**	NS
CD(0.05 level)	0.019	0.052	0.022	0.012	0.063	0.023	NS

#Average of 10 replications; * - Significant at 5 per cent level ; ** - Significant at 1 per cent level, NS - Non significant

FL - Femur Length, TL - Tibia Length, LM - Metatarsus Length, WM - Metatarsus Width, FWL - Forewing Length, FWW - Forewing Width, HAM – Number of hamuli, ML - Midland, UL - Upland

4.3.2.2. Tibial Length

Stingless bee samples collected from midland location, Malayam (1.260 mm) had maximum tibial length which was on par with the samples from upland location, Tholicode (1.209 mm). Lowest tibial length was recorded in the

samples from Ooroottambalam (1.037) and was on par with that of the samples collected from Kadakkal (1.065 mm), Vattappara (1.073 mm), Anappetti (1.074 mm), Pangode (1.078 mm), Palamukku (1.079), Ooyur (1.080), Pathanapuram (1.084 mm), Madathara (1.085 mm), Cheruvalloor (1.085 mm), Nettayam (1.087 mm), Panacode (1.087 mm), Pathadi (1.088) and Kavanakonam (1.089 mm). The tibial length of remaining stingless bee samples collected from other locations ranged from 1.110 to 1.206 mm.

4.3.2.3. Length of Metatarsus

The results showed that the stingless bees from midland location, Malayam (0.480 mm) had significantly superior metatarsus length compared to other locations. The upland location Cheruvalloor had the lowest metatarsus length (0.355 mm), which was on par with the samples collected from Peringammala (0.365 mm), Balaramapuram (0.370 mm), Kavanakonam (0.372 mm), Pullampara (0.372 mm), Bharathanoor (0.375 mm) and Nettayam (0.376 mm). The metatarsus length of stingless bees from the other locations ranged from 0.382 to 0.443 mm.

4.3.2.4. Width of Metatarsus

Among the thirty locations, metatarsus width observed in the stingless bee samples from the midland location, Malayam (0.257 mm) was found to be significantly different from all other treatments. Lowest metatarsus width was recorded in the stingless bees from Kilimanoor (0.175 mm) which was on par with that of samples collected from Ooroottambalam (0.178 mm), Ottasekharamangalam (0.183 mm), Mulluvenghamoodu (0.186 mm) and

Panacode (0.187 mm). The metatarsus width of the remaining samples varied from 0.192 to 0.239 mm.

4.3.2.5. Forewing Length

Regarding the forewing length, significantly superior value was recorded in the stingless bees from midland location, Malayam (3.284 mm). The lowest forewing length was observed in the stingless bees from Vellanad (2.648 mm), which was on par with that of samples collected from Panacode (2.738 mm) and Pathanapuram (2.689 mm). The forewing length of stingless bees of the remaining locations ranged from 2.746 to 2.949 mm.

4.3.2.6. Forewing Width

Considering the forewing width of stingless bees among thirty locations, significantly higher value was recorded from the midland location - Malayam (1.183 mm) while the lowest value was recorded from Anappetti (1.000 mm) and this was on par with that of samples collected from Ooroottambalam (1.007 mm), Madathara (1.007 mm), Vellanad (1.011 mm), Pullampara (1.014 mm), Panacode (1.017 mm), Thekkumoodu (1.019 mm) and Peppara (1.020 mm). The forewing width of the stingless bee samples from remaining locations varied from 1.054 to 1.097 mm.

4.3.2.7. Number of Hamuli

Results on the number of hamuli observed in the hind wings of stingless bee samples from all locations showed not much variation and it ranged from 4.8 to 5.1.

4.3.3. Abdomen

The results of morphometric variations in the parameters of stingless bee thorax (length of third tergite (3TL), length and width of third sternite (3SL and 3SW) and the lateral width of fourth tergite tomentum (LWT)) are given in

Table 9. Of these four parameters, third sternite width of stingless bees showed significant difference between midland and upland locations.

4.3.3.1. Third Tergite Length

Stingless bees from the midland location, Malayam had maximum third tergite length (0.527 mm) which was closely followed by upland locations, Pathadi (0.521 mm) and Tholicode (0.517 mm) and were on par. While the lowest tergite length was recorded in the samples from Vellanad (0.468 mm) which was on par with the samples from Anappetti (0.479 mm), Nettayam (0.478 mm) and Balaramapuram (0.477 mm). The tergite length of the stingless bee samples collected from remaining locations ranged from 0.483 to 0.511 mm.

4.3.3.2. Third Sternite Length

Interestingly, the maximum sternite length was observed in the samples collected from upland location, Tholicode (0.607 mm) followed by midland location, Malayam (0.601 mm) and upland location, Arippa (0.599 mm). They were significantly different from the remaining samples in the third sternite length. Third sternite length of stingless bees at Anappetti (0.519 mm) were the smallest among thirty locations and was found to be on par with the samples collected from Panacode (0.520 mm). Stingless bee samples of the remaining locations had their third sternite length which varied from 0.522 to 0.587 mm.

4.3.3.3. Third Sternite Width

Maximum third sternite width in stingless bee abdomen was recorded in the samples collected from midland location Malayam (1.294 mm) which was closely followed by five midland locations (Ooyur (1.259 mm), Vamanapuram (1.189 mm), Perukavu (1.188 mm), Balaramapuram (1.185 mm) and Palamukku (1.175 mm)) and three upland locations (Ottasekharamangalam (1.273 mm), Arippa (1.188 mm) and Tholicode (1.183 mm)). The smallest third sternite width was recorded as 1.045 mm from the midland location, Panacode which was found

Table 9. Variations of morphometric parameters of stingless bee abdomen in midland and upland physiographic regions of southern Kerala

#Average of 10 replications; * - Significant at 5 per cent level, NS - Non significant

Locations	3TL (mm)	3SL (mm)	3SW (mm)	LWT (mm)
Perukavu (ML-1)	0.507#	0.587	1.188	0.065
Vattappara (ML-2)	0.511	0.533	1.078	0.061
Vamanapuram (ML-3)	0.499	0.558	1.189	0.062
Poojappura (ML-4)	0.487	0.539	1.146	0.073
Pullampara (ML-5)	0.493	0.540	1.101	0.081
Kilimanoor (ML-6)	0.499	0.563	1.165	0.071
Panacode (ML-7)	0.484	0.520	1.045	0.061
Balaramapuram (ML-8)	0.477	0.536	1.185	0.064
Malayam (ML-9)	0.527	0.601	1.294	0.077
Ooyur (ML-10)	0.483	0.549	1.259	0.074
Pangode (ML-11)	0.483	0.566	1.129	0.074
Kadakkal (ML-12)	0.502	0.533	1.096	0.062
Pathanapuram (ML-13)	0.491	0.534	1.089	0.088
Ooroottambalam (ML-14)	0.487	0.522	1.088	0.066
Palamukku (ML-15)	0.492	0.554	1.175	0.074
Mean (Midland)	0.494	0.549	1.148	0.070
Ottasekharamangalam (UL-1)	0.507	0.554	1.273	0.073
Vellanad (UL-2)	0.468	0.546	1.094	0.066
Tholicode (UL-3)	0.517	0.607	1.183	0.070
Anappetti (UL-4)	0.479	0.519	1.060	0.062
Mulluvenghamoodu (UL-5)	0.490	0.538	1.125	0.071
Peppara (UL-6)	0.496	0.523	1.148	0.061
Thekkumoodu (UL-7)	0.509	0.539	1.173	0.076
Peringammala (UL-8)	0.502	0.532	1.078	0.075
Bharathanoor (UL-9)	0.494	0.557	1.104	0.083
Pathadi (UL-10)	0.521	0.559	1.112	0.068
Cheruvalloor (UL-11)	0.486	0.543	1.076	0.075
Kavanakonam (UL-12)	0.490	0.535	1.072	0.067
Nettayam (UL-13)	0.478	0.533	1.114	0.081
Arippa (UL-14)	0.508	0.599	1.188	0.059
Madathara (UL-15)	0.500	0.554	1.127	0.067
Mean (Upland)	0.496	0.549	1.128	0.070
Midland Vs upland	NS	NS	S*	NS
CD (0.05 level)	0.014	0.016	0.052	0.006

#Average of 10 replications; * - Significant at 5 per cent level, NS - Non significant

3TL -Third Tergite Length, 3SL - Third Sternite Length, 3SW -Third Sternite Width, LWT -Lateral Width of Fourth Tergite, ML - Midland, UL-upland

to be on par with Anappetti (1.060 mm), Kavanakonam (1.072 mm), Cheruvalloor (1.076 mm), Peringammala (1.078 mm), Vattappara (1.078 mm), Ooroottambalam (1.088 mm), Pathanapuram (1.089 mm) and Vellanad (1.094 mm). Stingless bee samples of remaining locations ranged from 1.096 to 1.173 mm.

4.3.3.4. Lateral Width of Fourth Tergite Tomentum

Regarding the lateral width of fourth tergite tomentum, highest value was recorded in the stingless bees from midland location Pathanapuram (0.088 mm) which was on par with upland location, Bharathanoor (0.083 mm). The smallest width of tergite tomentum was recorded as 0.059 mm in the stingless bee samples from upland location, Arippa which was on par with Panacode, Vattappara and Peppara (0.061), Kadakkal, Anappetti and Vattappara (0.062) and Balaramapuram (0.064). The lateral width of tergite tomentum of the stingless bee samples collected from remaining locations ranged from 0.065 to 0.081 mm.

4.3.4. Length /width Ratio of Morphometric Parameters in Stingless Bees

The length/width ratio of stingless bee body parameters were also estimated (Table 10). Studies on length and width ratio of morphometric parameters of stingless bees revealed that there was no significant variation between midland and upland samples in terms of head, metatarsus, forewing and third sternite.

4.3.4.1. Head Length/ width Ratio

Length and width ratio of stingless bees head was maximum at Panacode (0.863) samples which were closely followed by Mulluvenghamoodu (0.862), Vattappara (0.856), Poojappura (0.848), Ottasekharamangalam (0.848), Cheruvalloor (0.845) and Arippa (0.843). Whereas the length: width ratio was lowest for the stingless bee samples from Kilimanoor (0.757) which was also on par with Perukavu (0.770).

Table 10. Length/width ratio of stingless bee body parameters in upland and midland physiographic region of Southern Kerala

Locations	Head L/W Ratio	Metatarsus L/W ratio	Forewing L/W ratio	3rd sternite L/W ratio
Perukavu (ML-1)	0.770#	1.850	2.785	0.494
Vattappara (ML-2)	0.856	2.032	2.737	0.494
Vamanapuram (ML-3)	0.781	1.972	2.643	0.470
Poojappura (ML-4)	0.848	2.054	2.729	0.471
Pullampara (ML-5)	0.792	1.639	2.803	0.491
Kilimanoor (ML-6)	0.757	2.274	2.750	0.484
Panacode (ML-7)	0.863	2.088	2.690	0.498
Balaramapuram (ML-8)	0.819	1.961	2.675	0.455
Malayam (ML-9)	0.825	1.867	2.775	0.464
Ooyur (ML-10)	0.817	1.803	2.553	0.436
Pangode (ML-11)	0.815	2.047	2.664	0.502
Kadakkal (ML-12)	0.790	2.105	2.748	0.487
Pathanapuram (ML-13)	0.839	2.039	2.571	0.490
Ooroottambalam (ML-14)	0.826	2.152	2.745	0.480
Palamukku (ML-15)	0.832	2.030	2.663	0.472
Mean (Midland)	0.815	1.994	2.702	0.479
Ottasekharamangalam (UL-1)	0.848	2.304	2.727	0.450
Vellanad (UL-2)	0.823	1.805	2.620	0.501
Tholicode (UL-3)	0.828	1.971	2.697	0.513
Anappetti (UL-4)	0.828	1.963	2.777	0.491
Mulluvenghamoodu (UL-5)	0.862	2.216	2.709	0.479
Peppara (UL-6)	0.799	2.059	2.751	0.457
Thekkumoodu (UL-7)	0.802	2.048	2.743	0.460
Peringammala (UL-8)	0.792	1.743	2.652	0.494
Bharathanoor (UL-9)	0.820	1.799	2.686	0.505
Pathadi (UL-10)	0.797	2.130	2.675	0.503
Cheruvalloor (UL-11)	0.845	1.824	2.636	0.505
Kavanakonam (UL-12)	0.831	1.723	2.721	0.500
Nettayam (UL-13)	0.829	1.931	2.715	0.478
Arippa (UL-14)	0.843	2.101	2.712	0.505
Madathara (UL-15)	0.802	1.943	2.827	0.492
Mean (Upland)	0.823	1.971	2.710	0.489
Midland Vs upland	NS	NS	NS	NS
CD (0.05 level)	0.021	0.174	0.070	0.020

Average of 10 replications, L/W - Length/width ratio, ML - Midland, UL - Upland,

4.3.4.2. Metatarsus Length/ width Ratio

Studies on metatarsus length and width ratio revealed that the stingless bee samples collected from Kilimanoor (2.274), possessed the highest ratio which was on par with that of the samples from Ottasekharamangaalam (2.304) and Mulluvengamoodu (2.216). The lowest ratio was recorded in samples collected from Pullampara (1.639) which was on par with the samples collected from Ooyur (1.803), Bharathanoor (1.799), Peringammala (1.743) and Kavanakonam (1.723).

4.3.4.3. Forewing Length/ width Ratio

In the case of forewing length and width ratio, highest value was obtained in the stingless bee samples collected from Madathara (2.827) which was on par with the stingless bees from Pullampara (2.803), Perukavu (2.785), Anappetti (2.777) and Malayam (2.775). Ooyur had the lowest length and width ratio (2.553) which was on par with that of samples collected from Vellanad (2.620) and Pathanapuram (2.571).

4.3.4.4. Third Sternite Length/ width Ratio

Considering the length and width ratio of third sternite of stingless bee samples, maximum value was observed in samples collected from Tholicode (0.513) which was on par with that of samples collected from Bharathanoor, Arippa and Cheruvalloor (0.505), Pathadi (0.503), Pangode (0.502), Vellanad (0.501), Kavanakonam (0.500), Panacode (0.498), Vattappara, Perukavu and Peringammala (0.494). The stingless bee samples collected from Ooyur had the lowest ratio (0.436) which was on par with Thekkumoodu, Peppara, Balaramapuram and Ottasekharamangalam having a sternite length: width ratio of 0.460, 0.457, 0.455 and 0.450 respectively.

4.3.5. *Cluster Analysis of Morphometric Parameters of Stingless Bees*

Cluster analysis of morphometric parameters of stingless bee samples from different locations resulted in three major clusters (Fig 3.). The first cluster

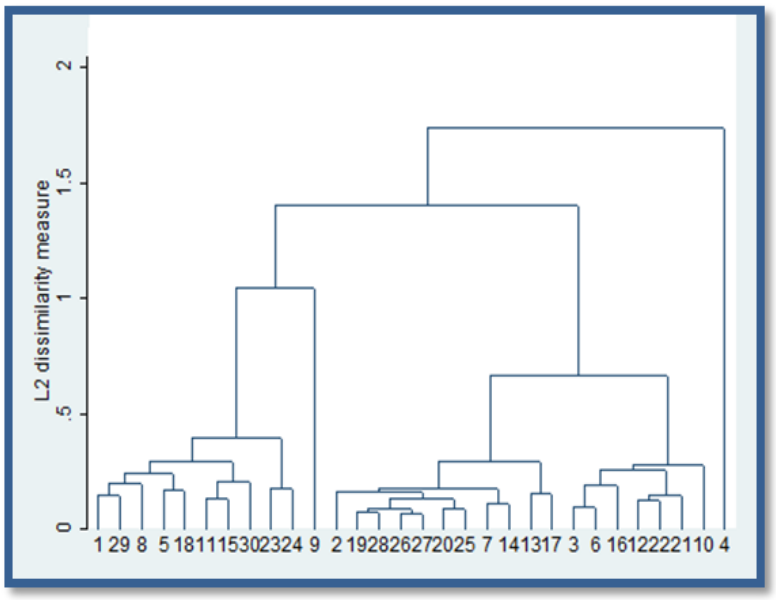

Fig. 3. Dendrogram showing cluster analysis of locations based morphometric parameters of stingless bees

Sl. No.	Midland ocations	Sl. No.	Upland locations
1	Perukavu	16	Ottasekharamangalam
2	Vattappara	17	Vellanad
3	Vamanapuram	18	Tholicode
4	Poojappura	19	Anappetti
5	Pullampara	20	Mulluvenghamoodu
6	Kilimanoor	21	Peppara
7	Panacode	22	Thekkumoodu
8	Balaramapuram	23	Peringammala
9	Malayam	24	Bharathanoor
10	Ooyur	25	Pathadi
11	Pangode	26	Cheruvalloor
12	Kadakkal	27	Kavanakonam
13	Pathanapuram	28	Nettayam
14	Ooroottambalam	29	Arippa
15	Palamukku	30	Madathara

was divided in to two sub clusters and the sub cluster 1 includes the stingless bee samples from ten locations, of which five from the midland locations (Perukavu, Balaramapuram, Pullampara, Pangode and Palamukku) and four from the upland locations (Arippa, Tholicode, Madathara, Peringammala and Bharathanoor) while the sub cluster 2 contains only one midland location, Malayam.

Cluster 2 comprises of two sub clusters with four midland locations (Vattappara, Panacode, Ooroottambalam and Pathanapuram) and seven upland locations (Anappetti, Nettayam, Cheruvalloor, Kavanakonam, Mulluvenghamoodu, Pathadi, and Vellanad) in sub cluster-1. While sub cluster 2 had four midland locations (Vamanapuram, Kilimanoor, Kadakkal and Ooyur) and three upland locations (Ottasekharamangalam, Thekkumoodu and Peppara). Stingless bee samples from midland location Poojappura was categorized alone in the cluster 3.

4.4. QUALITY ANALYSIS OF STINGLESS BEE HONEY

4.4.1. Physico - chemical Properties of Honey

Fifteen honey samples of stingless bees each collected from the midland and upland physiographic regions of southern Kerala were subjected to different physico-chemical analysis.

The physical properties (pH, moisture content, electrical conductivity, total dissolved solids, colour intensity, total reducing and non-reducing sugars) of stingless bee honey are shown in Table 11. Of the seven parameters only five parameters (pH, moisture content, total dissolved solids, colour intensity and total reducing sugars) exhibited significant variation between the midland and upland honey samples.

4.4.1.1. pH

The stingless bee honey collected from upland location, Kavanakonam had maximum pH (4.04) which was closely followed by the samples collected from

Table 11. Physical properties of stingless bee honey from midland and upland physiographic regions of southern Kerala

Locations	pH	Moisture content (%)	EC (µS cm^{-1})	TDS (ppm)	Colour intensity (mAU)	Total sugar	
						Reducing sugar (%)	Non reducing sugar (%)
Perukavu (ML-1)	3.77	20.40	18.32	24.38	708.33 (2.85)	63.04	3.70
Vattappara (ML-2)	3.27	19.70	21.64	42.30	191.67 (2.28)	68.33	3.03
Vamanapuram (ML-3)	3.16	22.00	18.19	29.83	1032.67 (3.01)	72.09	4.23
Poojappura (ML-4)	3.57	20.83	12.59	47.92	1877.67 (3.27)	56.80	2.64
Pullampara (ML-5)	3.41	22.07	15.32	18.93	526.67 (2.72)	71.53	3.31
Kilimanoor (ML-6)	3.48	20.57	16.96	21.69	562.67 (2.76)	70.78	4.02
Panacode (ML-7)	3.25	20.57	30.92	19.48	652.67 (2.81)	72.92	4.05
Balaramapuram (ML-8)	2.87	19.63	13.58	18.48	716.33 (2.86)	64.53	2.41
Malayam (ML-9)	2.95	19.74	17.38	19.17	760.00 (2.88)	72.68	2.56
Ooyur (ML-10)	3.27	18.67	8.76	22.94	877.00 (2.94)	67.83	1.96
Pangode (ML-11)	3.57	21.00	11.38	16.54	661.67 (2.82)	69.58	2.48
Kadakkal (ML-12)	3.40	21.57	25.51	16.34	446.00 (2.65)	66.01	2.31
Pathanapuram (ML-13)	3.19	21.83	21.73	17.00	349.33 (2.54)	68.32	3.13
Ooroottambalam (ML-14)	3.56	19.61	13.77	28.24	1081.00 (3.03)	66.60	2.31
Palamukku (ML-15)	3.19	18.50	24.12	23.62	492.33 (2.69)	70.73	1.79
Mean (Midland)	3.33	20.45	18.01	24.46	729.07	68.12	2.93
Ottasekharamangalam(UL-1)	3.03	18.60	19.66	39.71	1104.61(3.04)	71.80	2.13
Vellanad (UL-2)	3.77	20.47	18.07	26.96	131.00 (2.11)	70.78	3.03
Tholicode (UL-3)	3.44	20.47	23.10	18.89	790.00 (2.90)	71.45	2.67
Anappetti (UL-4)	3.40	18.03	23.59	19.59	657.00 (2.82)	57.92	2.69
Mulluvenghamoodu (UL-5)	3.36	19.47	14.95	24.30	458.33 (2.66)	67.10	2.96
Peppara (UL-6)	3.43	19.69	18.65	31.49	1129.30 (3.06)	69.58	3.24
Thekkumoodu (UL-7)	3.52	18.71	14.95	36.08	642.00 (2.81)	72.30	2.62
Peringammala (UL-8)	3.12	20.75	11.41	42.57	690.00 (2.84)	72.98	2.68
Bharathanoor (UL-9)	3.89	18.50	10.91	16.22	703.33 (2.85)	71.47	2.64
Pathadi (UL-10)	3.35	17.93	21.96	18.57	924.67 (2.97)	78.64	3.71
Cheruvalloor (UL-11)	3.21	20.33	18.53	26.96	1136.33 (3.06)	72.01	2.19
Kavanakonam (UL-12)	4.04	17.37	22.81	23.56	600.67 (2.78)	76.59	2.48
Nettayam (UL-13)	3.69	19.83	23.35	22.71	1186.33 (3.07)	62.91	2.83
Arippa (UL-14)	3.33	22.43	12.07	68.09	1437.33 (3.16)	74.35	2.83
Madathara (UL-15)	3.17	21.13	18.32	32.17	1617.67 (3.20)	59.50	1.50
Mean (Upland)	3.45	19.58	20.16	29.85	880.57	69.96	2.68
Midland Vs Upland	S**	S**	NS	S**	S**	S**	NS
CD (0.05 level)	0.158	1.555	2.350	2.215	0.021	4.617	NS

Figures in parenthesis are expressed in terms of Log10 X transformed values, ML - Midland, UL - Upland, EC - Electrical conductivity, TDS - Total dissolved solids, ** - Significant at 1 per cent level,* - Significant at 5 per cent level, NS - Non significant

Plate 7 a. Stingless bee honey samples collected from midland physiographic region

Plate 7 b. Stingless bee honey samples collected from upland physiographic region

Plate 7. Stingless bee honey samples collected from midland and upland physiographic region

upland location, Bharathanoor (3.89) and were on par. Highest acidic pH was recorded in the stingless bee honey samples collected from midland location Balaramapuram (2.87) and it was statistically on par with that of midland location, Malayam (2.95) and upland location Ottasekhramagalam (3.03). The pH of honey samples from remaining locations ranged from 3.12 to 3.77.

4.4.1.2. Moisture Content

Among the thirty honey samples, maximum moisture content was recorded in the stingless bee honey sample collected from the upland location, Arippa (22.43 %) which was on par with that of honey samples from Pullampara, Vamanapuram, Pathanapuram, Kadakkal, Madathara, Pangode and Perukavu having a moisture content of 22.07, 22.00, 21.83, 21.57, 21.13, 21.00 and 20.40 per cent respectively. Lowest moisture content was recorded in the honey samples collected from Kavanakonam (17.37 %) which was on par with the honey samples from Palamukku (18.50 %), Bharathanoor (18.50 %), Anappetti (18.03 %) and Pathadi (17.93 %). Moisture content observed in the remaining honey samples varied from 18.60 to 20.83 per cent.

4.4.1.3. Electrical Conductivity and Total Dissolved Solids

In the case of electrical conductivity in stingless bee honey, the highest value was recorded in the honey samples from midland location, Panacode (30.92 µS cm^{-1}) which was statistically superior to the remaining samples. The honey samples from midland location, Ooyur (8.76 µS cm^{-1}) had the lowest EC and it was on par with the samples from upland location Bharathanoor (10.91 µS cm^{-1}). The EC values of the honey samples from the rest of the locations were in between 11.38 and 25.51 µS cm^{-1}.

Regarding the total dissolved solids in stingless bee honey, maximum TDS was recorded in the honey sample from upland location, Arippa (68.09 ppm) which was statistically superior to the remaining samples. Lowest TDS was recorded in the honey samples collected from Bharathanoor (16.22 ppm) which

was on par with the honey samples from Kadakkal (16.34 ppm), Pangode (16.54 ppm) and Pathanapuram (17.00 ppm). TDS present in the honey samples from remaining locations varied from 18.48 to 47.92 ppm.

4.4.1.4. Colour Intensity

The stingless bee honey sample from the midland location, Poojappura recorded the highest colour intensity (1877.67 mAU) which was on par with the samples from Madathara (1617.67 mAU), Arippa (1437.33 mAU) and Nettayam (1186.33 mAU). The lowest colour intensity was recorded from Vellanad honey sample (131.00 mAU) which was on par with the samples from Vattappara (191.67 mAU). While the colour intensity of the remaining honey samples ranged from 349.33 to 1136.33 mAU.

4.4.1.5. Total sugar (reducing and non-reducing)

Analysis of the total sugars (reducing and non-reducing sugars) in stingless bee honey samples revealed that maximum amount of total reducing sugars (78.64 %) was observed from honey sample collected from Pathadi. The lowest value was reported in the honey samples collected from Poojappura (56.80 %) which was statistically on par with Madathara (59.50 %) and Anappetti (57.92 %). Total reducing sugar observed from the remaining samples ranged from 62.91 to 76.59 per cent.

Regarding the non-reducing sugar content in honey, highest value was observed in the honey samples collected from Vamanapuram (4.23 %) which was on par with all the remaining treatments. The lowest value was obtained from upland location Madathara (1.50 %).

The results of the chemical analysis (protein content, proline content, ascorbic acid, flavanoid content, total phenol content, diastase and invertase activity) of stingless bee honey are presented in Table 12. Studies on the biochemical parameters of stingless bee honey revealed that flavanoid content,

total phenol content, diastase and invertase activity were significantly different in midland and upland samples.

4.4.1.6. Protein Content

Considering the protein content in stingless bee honey samples among thirty locations of southern Kerala, highest protein content was recorded in the honey samples collected from Kilimanoor (0.92 %) which was significantly different from the remaining samples. Stingless bee honey collected from Ooroottambalam (0.17 %) recorded the lowest protein content which was on par with the samples collected from Pathanapuram (0.18 %), Bharathanoor (0.18 %) and Arippa (0.21 %). The protein content in the honey samples collected from the remaining locations ranged from 0.27 to 0.62 per cent.

4.4.1.7. Proline Content

Maximum proline content was recorded from the stingless bee honey sample of Kilimanoor (1513.42 mg kg^{-1}) which was closely followed by Pullampara (1502.78 mg kg^{-1}), Panacode (1502.82 mg kg^{-1}), Ooroottambalam (1499.70 mg kg^{-1}) and Cheruvalloor (1498.42 mg kg^{-1}) which were statistically on par. The lowest value was recorded from the stingless bee honey of Pathanapuram (995.16 mg kg^{-1}) which was statistically different from the honey samples of the remaining locations. The proline content in the remaining samples varied from 1130.73 to 1483.55 mg kg^{-1}.

4.4.1.8. Ascorbic Acid

Among the thirty locations, ascorbic acid content of stingless bee honey samples ranged from 1.6 to 5.2 mg kg^{-1}, with the highest amount from Peringammala which was on par with the remaining honey samples.

Table 12. Chemical properties of stingless bee honey from midland and upland physiographic regions of southern Kerala

Locations	Protein (%)	Proline (mg kg^{-1})	Ascorbic acid (mg kg^{-1})	Flavanoids (mg catechin kg^{-1})	Total phenols (mg catechol kg^{-1})	Diastase activity (DN)	Invertase activity (IN)
Perukavu (ML-1)	0.46	1249.89 (35.37)	3.2	172.20 (13.16)	562.41 (23.74)	21.33	155.33 (12.50)
Vattappara (ML-2)	0.53	1424.96 (37.76)	2.8	167.16 (12.97)	994.16 (31.54)	23.67	145.33 (12.10)
Vamanapuram (ML-3)	0.34	1130.73 (33.64)	4.0	283.96 (16.88)	890.12 (29.85)	35.00	131.76 (11.52)
Poojappura (ML-4)	0.29	1479.52 (38.47)	2.0	374.22 (19.37)	1145.83 (33.86)	24.33	182.77 (13.56)
Pullampara (ML-5)	0.57	1502.82 (38.78)	3.6	263.07 (16.25)	866.23 (29.45)	35.00	144.32 (12.05)
Kilimanoor (ML-6)	0.92	1513.42 (38.91)	3.6	248.50 (15.80)	600.40 (24.52)	29.67	77.00 (8.83)
Panacode (ML-7)	0.23	1502.78 (38.78)	2.4	102.22 (10.16)	890.12 (29.85)	18.00	124.63 (11.21)
Balaramapuram (ML-8)	0.58	1472.75 (38.39)	2.8	186.99 (13.71)	924.90 (30.43)	22.67	126.96 (11.31)
Malayam (ML-9)	0.34	1171.54 (34.24)	2.4	186.99 (13.71)	648.27 (25.48)	39.00	132.79 (11.57)
Ooyur (ML-10)	0.42	1468.00 (38.33)	2.4	92.85 (9.69)	634.48 (25.21)	19.33	126.96 (11.31)
Pangode (ML-11)	0.34	1444.17 (38.02)	2.0	176.50 (13.32)	621.87 (24.96)	19.33	116.35 (10.83)
Kadakkal (ML-12)	0.46	1483.55 (38.53)	3.6	232.46 (15.28)	1084.69 (32.96)	20.67	156.68 (12.56)
Pathanapuram (ML-13)	0.18	995.16 (31.56)	1.6	130.75 (11.48)	460.16 (21.47)	22.33	134.92 (11.66)
Ooroottambalam (ML-14)	0.17	1499.70 (38.74)	3.2	226.44 (15.08)	782.29 (27.99)	41.00	113.60 (10.71)
Palamukku (ML-15)	0.58	1338.76 (36.60)	1.6	104.60 (10.28)	519.00 (22.80)	25.00	145.99 (12.12)
Mean (Midland)	0.43	1378.52	2.7	196.6	775.00	26.42	117.05

Table 12. Chemical properties of stingless bee honey from midland and upland physiographic regions of southern kerala

Locations	Protein (%)	Proline (mg kg^{-1})	Ascorbic acid (mg kg^{-1})	Flavanoids (mg catechin kg^{-1})	Total phenols (mg catechol kg^{-1})	Diastase activity (DN)	Invertase activity (IN)
Ottasekharamangalam (UL-1)	0.40	1141.87 (33.80)	3.6	369.98 (19.26)	866.21 (29.45)	25.67	137.05 (11.75)
Vellanad (UL-2)	0.37	1349.47 (36.75)	3.2	171.17 (13.12)	1125.17 (33.56)	31.00	120.73 (11.03)
Tholicode (UL-3)	0.32	1476.81 (38.44)	2.4	180.35 (13.47)	769.85 (27.76)	29.00	114.85 (10.76)
Anappetti (UL-4)	0.53	1153.53 (33.98)	2.4	124.10 (11.18)	756.01 (27.51)	42.33	123.68 (11.17)
Mulluvenghamoodu (UL-5)	0.36	1375.41 (37.10)	3.6	249.83 (15.84)	863.75 (29.41)	30.33	139.39 (11.84)
Peppara (UL-6)	0.55	1211.28 (34.82)	3.2	174.18 (13.24)	937.79 (30.64)	40.67	109.03 (10.45)
Thekkumoodu (UL-7)	0.56	1350.57 (36.76)	2.4	255.76 (16.02)	787.79 (28.09)	46.00	98.48 (9.97)
Peringammala (UL-8)	0.27	1330.54 (36.49)	5.2	155.51 (12.51)	1289.27 (35.92)	40.00	134.39 (11.64)
Bharathanoor (UL-9)	0.18	1376.94 (37.12)	2.4	166.01 (12.92)	665.81 (25.82)	22.00	118.70 (10.94)
Pathadi (UL-10)	0.49	1179.99 (34.37)	1.6	125.38 (11.24)	516.95 (22.76)	28.33	109.31 (10.50)
Cheruvalloor (UL-11)	0.58	1498.42 (37.50)	3.6	216.26 (14.74)	888.78 (29.83)	37.67	67.82 (8.30)
Kavanakonam (UL-12)	0.57	1405.59 (37.50)	2.8	328.99 (18.17)	1318.20 (36.32)	22.33	117.05 (10.87)
Nettayam (UL-13)	0.42	1473.06 (38.39)	3.6	206.47 (14.40)	915.01 (30.27)	22.00	124.51 (11.20)
Arippa (UL-14)	0.21	1470.59 (38.36)	2.4	177.78 (13.37)	734.01 (27.11)	28.00	113.12 (10.68)
Madathara (UL-15)	0.62	1341.51 (36.64)	3.2	345.38 (18.61)	763.00 (27.64)	22.67	154.55 (12.47)
Mean (Upland)	0.43	1342.37	2.8	216.48	879.84	21.33	155.33 (12.50)
Midland Vs Upland	NS	NS	NS	S**	S**	S**	S**
CD (0.05 level)	0.041	0.359	NS	0.444	0.152	2.332	1.010

Figures in parenthesis are expressed $\sqrt{X+1}$ transformed values; ** - Significant at 1 per cent level, ML-Midland, UL- Upland
DN - Diastase number, IN - Invertase number

4.4.1.9. Flavanoids

The highest flavanoid content was recorded in the honey sample collected from the midland location Poojappura (374.22 mg catechin kg^{-1}) which was on par with the samples collected from upland location Ottasekharamangalam (369.98 mg catechin kg^{-1}). The lowest value was observed from the honey sample of Ooyur (92.85 mg catechin kg^{-1}) which was statistically different with the honey sample of all the other locations. Flavanoid content recorded in the remaining honey samples was in between 130.75 and 345.38 mg catechin kg^{-1}.

4.4.1.10. Total Phenols

Maximum amount of total phenol content was observed in the honey collected from Kavanakonam (1318.20 mg catechol kg^{-1}) followed by Peringammala, Poojappura, Vellanad, Kadakkal, Vattappara, Peppara, Balaramapuram and Nettayam having 1289.27, 1145.83, 1125.17, 1084.69, 994.16, 937.79, 924.90 and 915.01 mg catechol kg^{-1} respectively. Whereas the lowest amount of phenol was obtained from the sample collected from Pathanapuram (460.16 mg catechol kg^{-1}) which was statistically different from all the other honey samples. Phenol content of the remaining samples ranged from 516.95 to 890.12 mg catechol kg^{-1}.

4.4.1.11. Diastase Activity

Stingless bee honey samples collected from Thekkumoodu had maximum diastase activity (46 DN) which was statistically different from all the other samples. The diastase activity was lowest in the honey samples collected from Panacode (18 DN) which was statistically different to all other samples. Diastase activity of rest of the stingless bee honey samples lied between 19.33 and 42.33 Diastase Number.

4.4.1.12. Invertase Activity

Of the thirty stingless bee honey samples, maximum invertase activity was observed in the samples collected from Poojappura (182.77 IN) which was found to be on par with that of Kadakkal (156.68 IN). The minimum value of invertase activity was recorded in the honey sample collected from Cheruvalloor (67.82 IN) which was on par with the samples from Kilimanoor (77.00 IN) and Thekkumoodu (98.48 IN).

4.4.2. Antioxidant Properties of Stingless Bee Honey Samples

Significant difference in the antioxidant content of stingless bee honey samples was observed in between the midland and upland locations (Table 13). Stingless bee honey obtained from Peringammala recorded highest antioxidant content (647.52 μM Fe (II) $100g^{-1}$) which was significantly different from all other honey samples. This was followed by the honey samples collected from Peppara (607.09 μM Fe (II)$100g^{-1}$) and Thekkumoodu (547.48 μM Fe (II) 100 g^{-1}) which were statistically different from each other and superior to the remaining samples. Lowest antioxidant content was recorded in the honey sample from Bharathanoor (104.86 μM Fe (II) $100g^{-1}$) which was statistically different from all the other honey samples.

4.4.3 Microbial Load in Stingless Bee Honey Samples

The results on the microbial load (bacteria, fungi and actinomycetes) assessment in the stingless bee honey samples from thirty locations of southern Kerala (Table 14.) revealed that bacterial colonies were recorded in all honey samples except the honey collected from Peringammala while fungal colonies were recorded only from three honey samples (Pullampara, Peppara and Palamukku) (Plate 8). No actinomycetes could be isolated from any of the honey samples.

Table 13. Antioxidant content in stingless bee honey samples from midland and upland physiographic regions of southern Kerala

Midland locations	Antioxidant content (μM Fe (II) 100 g^{-1})	Upland locations	Antioxidant content (μM Fe (II) 100 g^{-1})
Perukavu (ML-1)	243.67 (15.64)	Ottasekharamangalam (UL-1)	419.29 (20.50)
Vattappara (ML-2)	257.30 (16.07)	Vellanad (UL-2)	480.24 (21.94)
Vamanapuram (ML-3)	275.63 (16.63)	Tholicode (UL-3)	504.05 (22.47)
Poojappura (ML-4)	457.27 (21.40)	Anappetti (UL-4)	513.21 (22.68)
Pullampara (ML-5)	232.88 (15.29)	Mulluvenghamoodu (UL-5)	512.14 (22.65)
Kilimanoor (ML-6)	436.60 (20.92)	Peppara (UL-6)	607.09 (24.66)
Panacode (ML-7)	298.47 (17.31)	Thekkumoodu (UL-7)	547.48 (23.42)
Balaramapuram (ML-8)	247.95 (15.78)	Peringammala (UL-8)	647.52 (23.42)
Malayam (ML-9)	364.59 (19.12)	Bharathanoor (UL-9)	104.86 (10.28)
Ooyur (ML-10)	114.95 (10.77)	Pathadi (UL-10)	213.49 (14.65)
Pangode (ML-11)	287.97 (17.00)	Cheruvalloor (UL-11)	114.39 (10.74)
Kadakkal (ML-12)	290.34 (17.07)	Kavanakonam (UL-12)	188.39 (13.76)
Pathanapuram (ML-13)	110.35 (10.55)	Nettayam (UL-13)	237.66 (15.45)
Ooroottambalam (ML-14)	219.11 (14.84)	Arippa (UL-14)	223.60 (14.99)
Palamukku (ML-15)	176.92 (13.34)	Madathara (UL-15)	309.22 (17.61)
Mean (Midland)	267.60	Mean (Upland)	374.84
Midland Vs Upland			S**
CD (0.05)			0.172

ML - Midland, UL - Upland, S** - Significant at 1 per cent level

Table 14. Microbial load in stingless bee honey samples from midland and upland physiographic regions of southern Kerala

Midland locations	Population of bacteria (cfu ml^{-1})	Population of fungus (cfu ml^{-1})	Upland locations	Population of bacteria (cfu ml^{-1})	Population of fungus (cfu ml^{-1})
Perukavu	1.25×10^2	-	Ottasekharamangalam	1.00×10^1	-
Vattappara	1.03×10^2	-	Vellanad	2.05×10^2	-
Vamanapuram	1.00×10^1	-	Tholicode	2.00×10^1	-
Poojappura	1.50×10^1	-	Anappetti	1.50×10^1	-
Pullampara	1.50×10^1	1.33	Mulluvenghamoodu	2.50×10^1	-
Kilimanoor	1.50×10^1	-	Peppara	1.47×10^2	1
Panacode	4.50×10^2	-	Thekkumoodu	1.60×10^1	-
Balaramapuram	1.60×10^1	-	Peringammala	0.00	-
Malayam	1.00×10^1	-	Bharathanoor	1.03×10^1	-
Ooyur	1.00×10^1	-	Pathadi	5.50×10^1	-
Pangode	2.17×10^1	-	Cheruvalloor	2.88×10^2	-
Kadakkal	1.56×10^2	-	Kavanakonam	1.00×10^1	-
Pathanapuram	2.50×10^1	-	Nettayam	7.58×10^1	-
Ooroottambalam	1.50×10^1	-	Arippa	2.15×10^2	-
Palamukku	1.03×10^2	3.67	Madathara	4.50×10^1	-

cfu - Colony forming unit

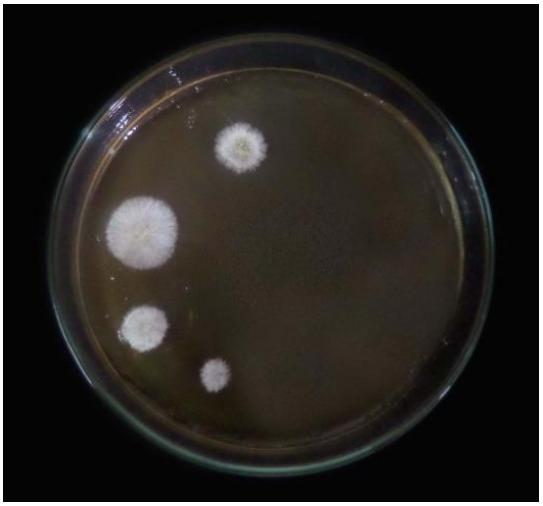

Plate 8 a. Fungal colony isolated from stingless bee honey (Pullampara)

Plate 8 b. Bacterial colonies isolated from stingless bee honey (Panacode)

Plate 8. Colonies of microbes isolated from stingless bee honey

Maximum number of bacterial colonies were observed in the honey sample from midland location, Panacode (4.50 x 10^2 cfu ml^{-1}) followed by upland location, Cheruvalloor (2.88 x 10^2 cfu ml^{-1}). Fungal colonies were isolated from the honey samples of three locations, Pullampara (1.33 cfu/ml), Palamukku (3.67 cfu ml^{-1}) and Peppara (1cfu ml^{-1}).

The bacterial and fungal isolate(s) obtained from the honey during microbial load assessment were subjected to identification. The bacterial isolates were *Bacillus vallismortis* and *Staphylococcus lentus* which were identified as gram positive cocci while the fungus identified in both the honey samples collected from Pullampara, Palamukku and Peppara was *Penicillium* sp. The microbial load of these isolates was within the admissible levels.

4.4.4. Cluster analysis of physico-chemical and antioxidant properties of Stingless Bee honey

Cluster analysis of physico-chemical and antioxidant properties of honey from thirty locations resulted in three major clusters (Fig 4.). Cluster 1 includes seven midland locations (Perukavu, Malayam, Ooyur, Pangode, Pathanapuam, Palamukku and Kilimanoor) and seven upland locations (Cheruvalloor, Ottasekharamangalam, Pathadi, Anappetti, Mulluvenghamoodu, Peppara and Peringammala). The second cluster had five midland location (Vattappara, Pullampara, Kadakkal, Panacode and Balaramapuram) and three upland locations (Tholicode, Bharathanoor and Nettayam) While third cluster comprises of three midland locations (Vamanapuram, Ooroottambalam and Poojappura) and five upland locations (Vellanad, Thekkumoodu, Kavanakonam, Arippa and Madathara).

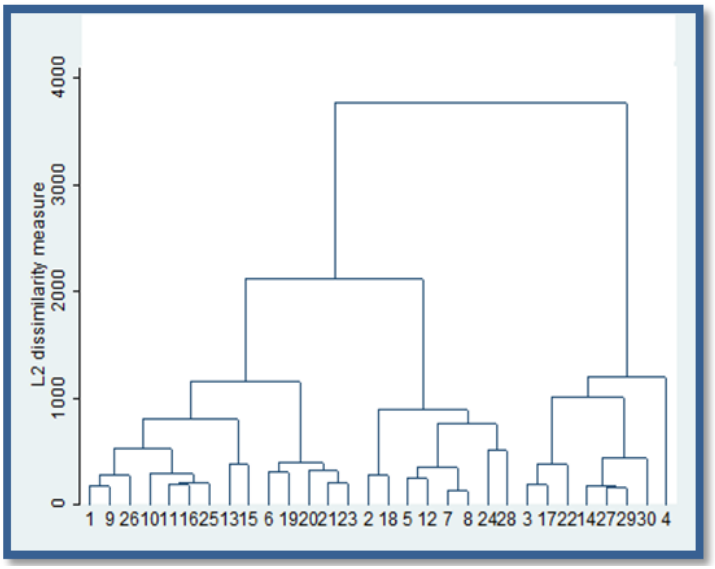

Fig. 4. Dendrogram showing cluster analysis of locations based on physico - chemical and antioxidant properties of stingless bee honey samples

Sl. No.	Midland locations	Sl. No.	Upland locations
1	Perukavu	16	Ottasekharamangalam
2	Vattappara	17	Vellanad
3	Vamanapuram	18	Tholicode
4	Poojappura	19	Anappetti
5	Pullampara	20	Mulluvenghamoodu
6	Kilimanoor	21	Peppara
7	Panacode	22	Thekkumoodu
8	Balaramapuram	23	Peringammala
9	Malayam	24	Bharathanoor
10	Ooyur	25	Pathadi
11	Pangode	26	Cheruvalloor
12	Kadakkal	27	Kavanakonam
13	Pathanapuram	28	Nettayam
14	Ooroottambalam	29	Arippa
15	Palamukku	30	Madathara

Discussion

5. DISCUSSION

Investigations were conducted at the AICRP on Honey Bees and Pollinators, Department of Agricultural Entomology, College of Agriculture, Vellayani to study the morphometric variations of stingless bees with respect to the upland and midland physiographic regions of southern Kerala and to evaluate the physical, chemical and antioxidant properties of their honey during the period 2013-2015. The results generated in the present study are discussed below:

5.1. NEST ARCHITECTURE AND DEFENCE MECHANISM

In the present study, the mean number of guard bees was more in midland (7.6) than upland locations (6.4). The number of guard bees in a hive varies based on stingless bee species, presence of natural enemies, size of the nest entrance and colony behaviour or level of aggressiveness of guard bees and forager bee traffic (Couvillion *et al*., 2008). Hence the variation in number of guard bees in different physiographic regions is not focused on a single factor but is related to the biotic and abiotic factors around the nest. Though studies on variations in guard bee population between physiographic regions were not much attempted in our state earlier, Jayalekshmi (2015) revealed that the mean number of guard bees in *T. iridipennis* colonies ranged from 6.00 to 9.00 numbers per colony.

Regarding the design of the hive entrance tubes, slit like (elliptical) nest entrance was maximum (73.33 %), when compared to the round (20.00 %) and multiple entrance (6.66 %) (Fig. 5). Similar findings on nest entrance designs (slit and round entrance) has already been reported with *T. iridipennis* by Pavithra *et al.* (2013) from Karnataka, who observed that the stingless bees preferred oval or elliptical type nest entrance followed by circular or round type nest entrance. While Jayalekshmi (2015) in her studies on *T. iridipennis* at southern Kerala reported that the stingless bees preferred round type nest entrance.

According to Couvillon *et al*. (2008), stingless bee colonies with smaller nest entrance was more defensive against natural enemies. They also added that the area of the nest entrance with elliptical or slit like nest entrance was less when compared to that of the round like or circular nest entrance with same width.

Camargo and Pedro (2004) working with the stingless bee, *P. lurida* recorded pronounced differences in the nest entrance designs with respect to the geographical locations. Though slight variations in nest entrance design were observed between physiographic regions, in the present study, it was not much prominent. The various agro climatic conditions and diverse flora present in southern Kerala plays a leading role in the selection pressure of the bees for their nest entrance construction and defense mechanism. Biesmeijer *et al*. (2007) also reported that the variations were due to the selection pressure in stingless bee colonies for foraging requirements, adequate defense and maintenance in terms of nest entrance architectural parameters including size, number, shape and conspicuousness.

The multiple nest entrance recorded during the study from each physiographic region also varied; the stingless bee colony from midland location, Poojappura was constructed with two round type nest entrances (1cm diameter) but they permanently closed the second nest entrance just below the first one and used it as a landing platform. While that of Tholicode, the upland location, a single tubular nest entrance flattened at the apex and two slit like entrance holes with 1cm width constructed at the tip was observed. Reports on the multiple entrances are much limited throughout the world, though Roubik (2006) has reported such type of entrances with the genus *Lepidotrigona, Plebeia, Scaptotrigona* and *Tetragona*.

Considering the hive entrance length of 30 colonies, 10 colonies were categorized under less than 1 cm, 14 colonies had 1-3 cm and six colonies had greater than 3 cm hive entrance length. In the case of hive entrance width, maximum colonies (21) were in the range of 1-2 cm, 8 colonies had < 1 cm whereas only one

colony had their hive entrance width greater than 2 cm. The results of the study confirm with the findings of Jayalekshmi (2015) in which the length and width of hive entrance tube of *T. iridipennis* observed was in between 1.56 to 5.48 cm and 1.10 to 2.08 cm respectively. It was found contradictory with the reports of Danaraddi (2007) where the length and width of *T. iridipennis* varied from 0.56 to 1.45 cm and 0.02 to 0.06 cm respectively. This may be due to the selection pressure of bees, *T. iridipennis* colonies (Biesmeijer *et al*., 2007) where the length and width of hive entrance was selected by worker bees during nest entrance construction with respect to the external environment.

In addition to this, studies on the relation between hive entrance width and guard bees revealed that the guard bees were more in the hive entrance with > 2 cm width (Fig 6). This forms a part of the defensive behaviour by bees where more number of guard bees is found aggregating at the hive entrance opening to cover the exposed area.

With regard to the height, the present study has not much impact since domesticated feral colonies were selected for the study. Maximum number of colonies (14) was placed at a height of > 2 metre category from the ground level. Though the above said height had a positive effect against predator attack, most of the feral colonies in the southern region prefer a height of < 2 m (Danaraddi *et al*., 2007) when compared to that of the Northern region where Khan and Srivastava (2013) reported that most of the colonies of *T. laeviceps* were observed in between 3 to 6 m. The results of the present study conducted using domesticated feral colonies is in line with the findings of Jayalekshmi (2015) who reported that the farmers maintained maximum number of *T. iridipennis* colonies at a height of 1.8 to 3m (36 %) in Thiruvananthapuram while greater than 3.6 m (46 %) in Kollam district.

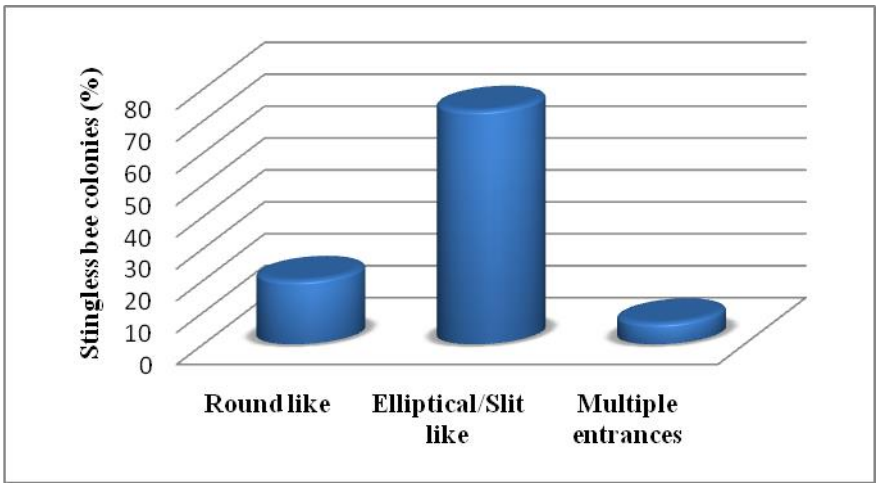

Fig. 5. Diversity of nest entrance design in stingless bee colonies

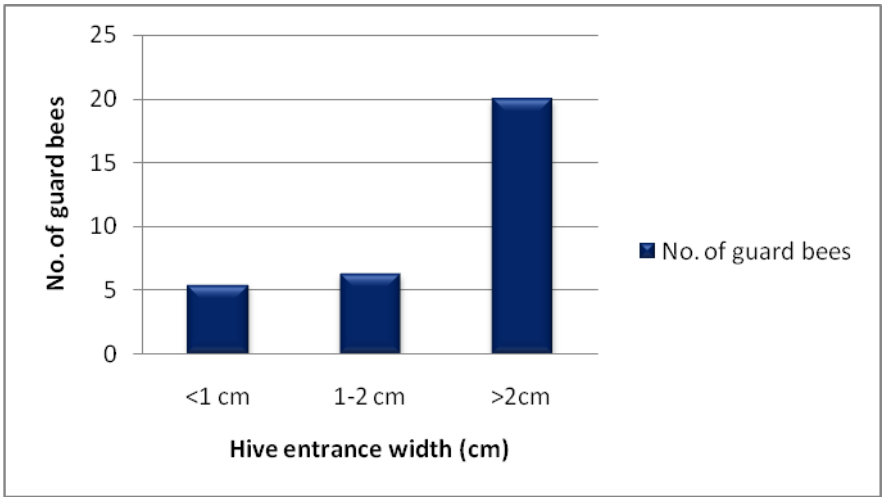

Fig 6. Influence of the number of guard bees to the hive entrance width in stingless bee colonies

An attempt was also made to analyse the hive entrance construction materials of stingless bee colonies. It was found that *T. iridipennis* used bee wax, plant resins, dry leaves and vegetative parts, wood particles, bee cadavers, sand and soil particles irrespective of the midland and upland regions. The findings of present study were in line with a handful of earlier reports from Kerala where Devanesan *et al*. (2009) also revealed that the nest entrance was constructed with wax or cerumen, other materials such as geopropolis, faeces and clay. Another study from Kerala on the nest entrance of *T. iridipennis* by Jayalekshmi (2015) revealed that stingless bees constructed different types of hive entrances using natural materials such as resins, waxes, tiny chips of wood, saw dust, sand, bee cadavers, leaves and vegetative parts, cerumen and dried bark of trees.

The present findings were also in agreement with the findings of Pavithra *et al*. (2013) where the nest entrance of *T. iridipennis* in Karnataka, was made up of bee wax, resin, wooden pieces, sand, mud, stone, cow dung, animal feces, pollen, tar, blue paint and grease. The study conducted by Biesmeijer *et al.*, (2007) in the nest of a different genus of stingless bee, *Scaura latitarsis* (Friese) at Brazil revealed that the entrance was constructed only with wax and resin mixture while that of *T. fiebrigi* and *T. clavipes* with pure wax and propolis respectively (Lima *et al.,* 2013). From this we can infer that the stingless bee workers are using the commonly available raw materials from their hive surroundings.

5.2. FORAGING PLANTS VISITED BY STINGLESS BEES

In order to assess the food (nectar and pollen) sources of stingless bees, studies on the foraging plants (categories) visited by bees around their hive in fifteen locations each of the midland and upland physiographic regions were studied. It was revealed that out of the fifty foraging plants, based on food source, maximum number of plants was under the category of both nectar and pollen providers (44 %) followed by nectar (28 %) and pollen providers (28 %) (Fig.7). Similar foraging plants were

also reported from Kerala and India (Premila *et al*., 2007; Raju *et a*l., 2013). But the nectar or pollen production by the foraging plant species is influenced by temperature (Yuan *et al*., 2009; Scaven and Rafferty, 2013), where they varies in physiographic regions with different climatic conditions. In addition to the nectar production, its composition and concentration were also influenced by temperature (Pacini *et al*., 2013).

Ornamental plants contributed maximum food sources (26 %) followed by the fruit crops (18 %) whereas medicinal plants and vegetables possessed an equal contribution of 14 per cent (Fig. 8). Since the hives selected for the study were homestead oriented, ornamentals and fruit crops were the major flora visited by stingless bees irrespective of the locations.

5.3. MORPHOMETRIC VARIATIONS OF STINGLESS BEES

The honey bees, in general are considered as an efficient pollinator compared to other insects based on their morphological adaptations especially in their mouth parts, wings and hind legs. The proboscis aid in the nectar collection and trophallaxis, wings are important for flight during foraging and thermal regulation of comb whereas the hind-leg bears the pollen basket and pollen brushes, an adaptation for their efficiency in pollen collection and transportation. With regard to the morphometric variations in the present study, the following parameters *viz*., proboscis length (1.049 mm), length (2.854 mm) and width (1.056) of stingless bee forewing, femur length (0.782 mm), metatarsus length (0.404 mm) and third sternite width (1.148 mm) obtained from midland were found to be statistically superior to the upland samples. Thus the present findings is in agreement with the above and this is also supported by Byrne *et al*. (1988) who reported a noticeable positive correlation between worker bee size, particularly in the wing area and flight distances. While Kuberappa (2005) reported significant variations in stingless bees (*T. iridipennis*) only in their total body length and head length whereas there were no modifications

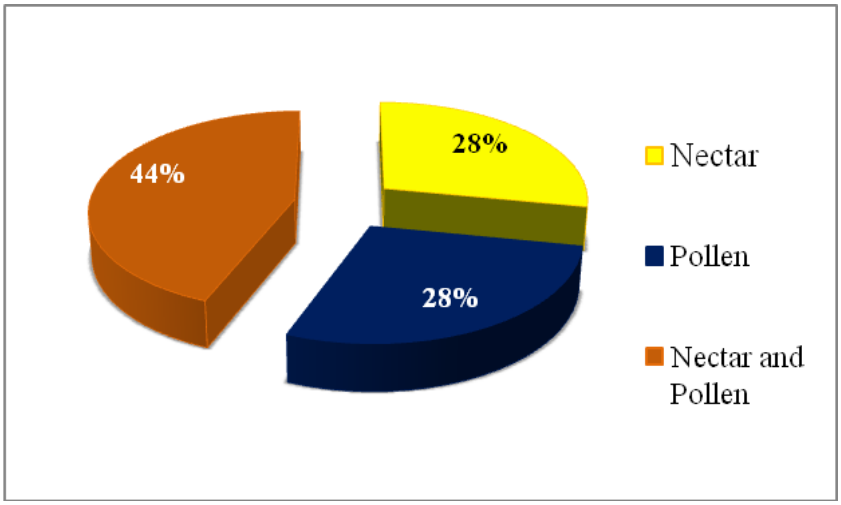

Fig. 7. Percentage contribution by foraging plants to stingless bees based on their food sources

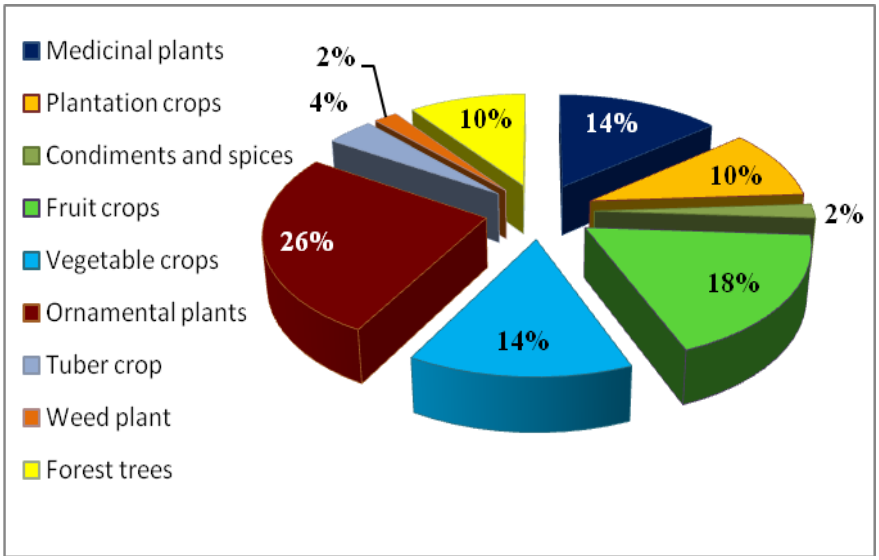

Fig. 8. Percentage contribution by categories of foraging flora to stingless bees

in parameters which improve their pollinator efficiency. Thus the morphometric variations in stingless bee may be due to environmental factors (Ruttner, 1988) or availability of food resource during their maturity phase (Pignata and Diniz-Filho, 1996).

The study on the morphometric parameters of stingless bee samples recorded the measurements like head length (0.957 - 1.086 mm), head width (1.163 - 1.342 mm), distance between ocelli (0.278 - 0.316 mm), dorsal ocello occular distance (0.172 - 0.272 mm), antennae length (1.427 - 1.633 mm), proboscis length (0.883 - 1.502 mm), forewing length (2.648 mm - 3.284 mm), forewing width (1- 1.183 mm), number of hamuli (5 Nos.), length of tibia (1.037 - 1.260 mm), femur length (0.721 - 0.918 mm), metatarsus length (0.355 - 0.480 mm), metatarsus width (0.175 - 0.257 mm), third tergite length (0.468 - 0.527 mm), third sternite width (1.045 - 1.294 mm) and lateral width of fourth tergite tomentum (0.059 - 0.088 mm). Similar results were also documented by Raakhee (2000) where she reported that the proboscis length, length of forewing, width of forewing, length of femur of *T. iridipennis* were 1.3, 3.55, 1.30 and 1.25 mm respectively. The number of hamuli observed in the earlier reports (Raakhee, 2000; Rasmussen, 2013) was also in conformity with the present findings.

The findings also corroborate with the study conducted by Rasmussen (2013) in India where he reported that length of metatarsus, width of metatarsus in the workers of *T. iridipennis* was 0.50 and 0.29 mm respectively while the parameters like length and width of head (1.30 and 1.60 mm), length of antennae (1.81 mm), length of tibia (1.55 mm), length and width of forewing (3.80 and 1.32 mm) measured from the type specimens of *T. iridipennis* was slightly higher than the present findings. These variations were in conformity with the earlier findings, where the worker body size was considered as an adaptation to foraging activity and floral resource utilization (Roubik and Ackerman, 1987; Baumgartner and Roubik, 1989).

The investigation on morphometric parameters among the locations also showed significant variations. The stingless bees from the midland location, Malayam were significantly different from rest of the samples in their proboscis length (1.502 mm), femur length (0.918 mm), length (0.480 mm) and width (0.257 mm) of metatarsus, length (3.284 mm) and width (1.183 mm) of forewing. Similar results were also observed in the *M. mandacaia* population by Kuhn-neto (2009) who reported that two types of forager bees with different body size were seen in the same native habitat under natural conditions. He also added that larger foragers can visit the flora of greater distances than smaller foragers. In the case of *M. quadrifasciata*, average worker size varies with colony conditions like food resource storage and the difference in food intake can contribute the body size of the bees (Ramalho *et al*., 1998).

The results obtained from the cluster analysis based on morphometric parameters of stingless bees resulted in three major clusters, of which two major clusters contain both midland and upland stingless bee samples while the stingless bee sample from third cluster contain only one midland stingless bee honey samples (Poojappura). It was also recorded that the second sub cluster of cluster 1 contain only one stingless bee sample which was collected from midland location Malayam. Thus morphometric variations are mainly concentrated in the midland physiograhic region; this may be due to the influence of homestead conditions particularly the flora. Variation and similarities of stingless bee body parameters within and between locations may be due to the influence of surrounding environmental factors and foraging plants (Ramalho *et al*., 1998).

5.4. QUALITY PARAMETERS OF STINGLESS BEE HONEY

5.4.1. Physico-chemical properties of stingless bee honey

In the present study, significant variations in physical properties *viz*., pH, moisture content, EC, total dissolved solids, colour intensity, reducing sugar and

chemical properties *viz*., flavanoids, total phenols, diastase and invertase activity of stingless bee honey samples was observed in between the midland and upland location. Though the flora visited by the bees, particularly around the hives, mainly determines the physico-chemical properties of honey; the stingless bee activity right from the collection and processing of nectar up to the storage in honey pots and the climatic conditions within and around the hive also had a great impact. The parameters *viz.*, pH (3.45), EC (20.16 µS cm^{-1}), total dissolved solids (29.85 ppm), colour intensity (880.57 mAU), reducing sugar (69.96 %), flavanoids (220.09 catechin kg^{-1}), total phenols (879.84 mg catechol kg^{-1}), diastase (31.20 DN) and invertase activity (136.15 IN) of the honey samples collected from upland was found to be superior over midland locations whereas the moisture content (20.45 %) recorded from midland was significantly higher than the upland.

The pH of stingless bee honey from midland and upland were 3.33 and 3.45 while it ranged between 2.87 to 4.04 among the thirty locations. This is in accordance with the earlier reports as 3.98 (Raakhee, 2000), 4.14 Nisha (2002) and 3.76 (Jayalekshmi, 2015). Earlier reports on the reason behind this acidic pH are limited, whereas certain reports (Boon, 2002) cite that the leaching of the polyphenols from the honey pots (made up of cerumen which is a mixture of bee wax, plant resin and gums) to the honey stored in it provides the acidic pH to honey. This is not much applicable to Indian bee honey where the honey is stored in cells made up of pure bee wax (produced from the wax glands situated in abdominal sternites). In effect, the foraging plants visited by the stingless bees had a certain role in determining the pH of honey.

The moisture content in the investigated honey samples ranged from 17.37 to 22.43 which were within the recommended range of ≤ 30 per cent (Vit *et al.*, 2006). Variation in moisture content in honey may be due to its composition, floral origin and hygroscopic nature (Dizaji *et al*., 2014) which was supported by

Zamora *et al.,* (2006) who reported that water content in honey depends upon several factors like level of relative humidity during honey production, maturity of honey, temperature in hive and extraction technique used. From the present study the moisture content in midland (20.45 %) and upland (19.58 %) honey samples were also documented which were almost in line with that recorded by Raakhee (2000) and Jayalekshmi (2015) as 20.70 and 19.02 per cent respectively.

Electrical conductivity of stingless bee honey samples collected from Kerala ranged from 28.46 to 41.76 µS cm^{-1} (Jayalekshmi, 2015) while in the present investigation it was in between 8.76 to 30.92 µS cm^{-1}. Electrical conductivity depends on botanical origin of honey along with other factors like mineral content, organic acids, some complex sugars and proteins (Terrab *et al.*, 2003). Thus, the variations in any of these factors could contribute a wide fluctuation in the value of EC. Total dissolved solids (TDS) is a measure of the combined content of all inorganic and organic substances in honey in the molecular, ionized or micro granular (colloidal solution) suspended forms. Thus TDS always exhibits a positive relation with EC where both are determined by the same factors. The total dissolved solids in honey samples in the present experiment ranged between 16.22 and 68.02 ppm.

Inconsistency has been observed in the distribution of colour intensity among the thirty honey samples. The colour intensity ranged from 131 to 1877.67 mAU. The is due to the variation in the flora visited by bees which is supported by Terrab *et al.* (2003) and Khalil *et al.* (2012) where they reported that honey colour is one of the most variable attributes which depends upon their botanical origin, along with other factors like ash content, temperature and time of storage as well as the presence of antioxidant pigments like flavanoids and carotenoids.

The level of sucrose or non reducing sugar differs according to the maturity degree and origin of the nectar compound of honey (Dizaji *et al.,* 2014). The total reducing and non - reducing sugars in the honey samples from 30 locations ranged

between 56.80 - 78.64 per cent and 1.50 - 4.23 per cent respectively which satisfied the quality of stingless bee honey (Vit *et al.,* 2006). This is in agreement with reports of Nisha (2002) where she reported that the *T. iridipennis* honey had 73.57 and 1.48 per cent total reducing sugar and sucrose content respectively. Similar results were also reported from Brazilian stingless bee honey of different entomological origin which was recorded as 58.00 to 75.70 per cent (total reducing sugars) and 1.10 to 4.80 per cent (non-reducing sugars) (Souza *et al*., 2006).

The protein content in stingless bee honey samples of the present investigation ranged between 0.71 to 0.92 per cent which is in tune with the earlier reports by Phadke (1968) where protein content was 0.78 per cent. While Raakhee (2000) reported it as 1.49 per cent, which was higher than the one recorded in the present study and this may be due to the variation in bee cephalic gland secretions, pollen and other extraneous matter in honey (Simuth, 2001; Phadke, 1968). Thus the slight fluctuation of protein content in honey samples may be due to the variation of the bee incorporated materials in honey *viz.*, enzymes, hormones, antibodies and serum in the honey (Balasubramanyam, 2013).

In the present study proline content which is an indication of honey ripeness ranged between 995.16 and 1513.42 mg kg^{-1}. Studies from Thailand revealed that the proline content of *Trigona laeviceps* honey samples were higher, 1,723 mg kg^{-1}. (Chanchao, 2009) while it ranged from 4.20 ± 0.03 to 5.64 ± 0.04 mg kg^{-1}. in honey samples of *Trigona* sp. (Eswaran *et al.*, 2015) which was lower than the recorded value. The proline content is added to stingless bee honey mainly from the nectar and secretion from bees and hence variations in its content are mainly contributed by the nectar source. The ascorbic acid content in honey samples ranged between 1.60 to 5.20 mg kg^{-1} which was in accordance with that of AICRP (2011) where it ranged between 1.04 to 2.80 mg kg^{-1}. On the other hand, ascorbic acid content in stingless bee honey samples from *T. carbonaria* was 17.70 mg kg^{-1}. (Vit *et al.,* 2006).

Studies on flavanoid content in stingless bee honey samples are scanty. It ranged from 92.84 - 374.22 mg catechin kg^{-1} in the present study which is in agreement with the findings of Ahmed *et al.* (2014) where the flavanoid content ranged between 85.7-217.7 mg catechin kg^{-1} in the raw honey samples of *A. mellifera*. Polyphenol content in the investigated honey samples ranged from 460.16 to 1318.20 mg catechin kg^{-1}. The polyphenol content in the honey samples of *Melipona subnitida* Ducke was 60 mg catechin kg^{-1} (Bastos *et al.*, 2009) which was lower than that of the present study. Flavanoids and simple phenolic derivatives such as phenolic acids are among the most common classes representing the maturity of plant polyphenols (Bravo, 1998) and they are found to vary according to the floral and geographical origin.

Diastase activity of stingless bee honey samples in the study ranged between 18 - 46 Diastase number (DN) which is in agreement with the findings of Patricia and Patrizio (1996) where they reported diastase activity in *Trigona* sp. honey samples at a range of 6.60 to 35.60 DN. Contrarily, diastase activity in the honey samples of *T. carbonaria* and *T. iridipennis* was 40 ± 0.50 and 1.35-2.40 DN respectively (Oddo *et al.,* 1999; Jayalekshmi, 2015). Invertase activity of stingless bee honey samples ranged in between 98.48 - 182.77 Invertase number (IN). This result is in agreement with the findings of Jayalekshmi (2015) were the invertase activity of stingless bee honey (*T. iridipennis*) was recorded as 65.53 – 98.00 IN. The findings corroborate with the observation of Patricia and Patrizio (1996) made in the stingless bee honey samples of *Trigona* sp. where the invertase activity of honey samples varied from15.90 to 214.30.

Though the variability in enzyme activity found in the different honey types is probably due to a series of factors, such as: nectar collection period, physiological stage of the colony, abundance of nectar flow and its sugar content (a high flow of concentrated nectar lead to lower enzyme content); age of the bees (when the honey bee becomes a forager its glands produce more digestive enzymes); pollen

consumption, etc (Fluri *et al.,* 1982; Brouwers, 1982 &1983) also determines the diastase activity. While that of the invertase enzyme, it is mainly determined by the bees since it is produced from the hypopharyngeal glands.

5.4.2. Antioxidant content in stingless bee honey

In the case of antioxidant content, reports with regard to the stingless bee honey are scanty. The samples collected from upland locations (374.84 μM Fe (II) 100 g^{-1}) was superior over midland location in antioxidant content during the study. The antioxidant content in stingless bee honey sample ranged from 104.86 to 647.52 μM Fe (II) 100 g^{-1}. The major factors which can contribute to the antioxidant content in honey is botanical source, phenolics, peptides, organic acids, enzymes, maillard reaction products, and possibly other minor components (Gheldof *et al*., 2002; Berreta *et al*., 2005).

Cluster analysis of honey samples resulted three major clusters based on their physico-chemical and antioxidant properties. All honey samples were grouped in to different clusters irrespective of midland and upland physiographic regions. It indicated that the major factor which influenced honey qualities was the foraging plants around the hives rather than the physiographic region.

5.4.3. Microbial load in stingless bee honey

The study on microbial load in the stingless bee honey samples recorded bacteria and fungi while colonies of actinomycetes were not observed in any of the honey samples. Frazier (1994) suggested that the principal sources of microorganisms in honey are flower nectar and the nest inmates including the foraging bees. Similar reports were also documented by Snowdon and Cliver (1996) who documented the presence of bacteria and fungus in honey samples. They suggested that primary sources of microbial contamination are likely to be pollen, the digestive tracts of honey bees, dust and nectar.

The results from the present study revealed that the maximum numbers of bacterial and fungal colonies in the honey sample were 4.50 x 10^2 and 3.67 cfu ml^{-1} respectively, where the count was less than 10^3. According to Snowdon and Cliver (1996), total plate counts of microbial contaminants from honey samples can vary from zero to tens of thousands per gram for no apparent reason. They also reported that honey with fairly high standard microbial plate counts (10000/g) could be acceptable if other microbial criteria (eg. indicating the presence of yeast less than 1000 spores per gram or free from fecal contamination - *Escherichia coli* or *Streptococcus* D (sic)), and anaerobic sulfite reducers (as an indicator of *Clostridium perfringens*) were satisfied. This fact is in agreement with the present findings and all the honey samples were positioned under the admissible level. According to Doyle (1988), microorganisms are generally not a hazard at very low levels and ingestion of food with more than 10^5 cells of microbial load g^{-1} of food is required to produce illness.

The present study on the microbial load in honey samples revealed the presence of two gram positive rod shaped bacterial isolates (*Bacillus vallismortis* and *Staphylococcus lentus*) and one fungal isolate (*Penicillium* sp). The presence of actinomycetes in the honey samples are not recorded hitherto.According to Snowdon and cliver (1996) Bacillus spores are the common bacterial contaminant in honey whereas staphylococcus was rarely occurred in honey samples. They also added that common molds in honey samples were *Penicillium and Aspergillus* sp. Lurlina *et al.* (2009) suggested that *Bacillus* is a bee associated microorganism and the chance of their occurrence in honey was variable. Thus the results indicated that stingless bee honey is safe for consumption purposes.

Summary

6. SUMMARY

The present investigation on "Morphometric variations of stingless bees in southern Kerala and assessment of honey quality" was implemented as three separate experiments, which includes - nest architecture, defense mechanism and foraging plants of stingless bees at midland and upland physiographic regions, their morphometric variations and assessment of honey quality at the Department of Agricultural Entomology, College of Agriculture, Vellayani. The study was conducted with an objective to assess the morphometric variations among stingless bee populations, their documentation and to evaluate the physical, chemical and antioxidant properties of their honey. Fifteen stingless bee colonies were selected from each of the midland and upland physiographic regions of southern Kerala. Ten samples of stingless bee workers and one honey sample each were collected from the selected stingless bee colonies.

Among the thirty locations, maximum number of guard bees was observed at the hive entrance of midland location, Pullampara (20). It was also observed that the number of guard bees were more in midland locations (7.6) than upland locations (6.4). Studies on the design of hive entrance revealed the presence of three types of designs – round, slit (elliptical) and multiple entrances. Of this slit like (22) nest entrance was found to be predominant in both the midland and upland regions, when compared to the round (6) and multiple entrances (2). In the case of hive entrance length, maximum of stingless bee colonies (8 Nos.) in midland location was recorded at a range of 1-3 cm length while in upland location; seven colonies lied under the category of less than < 1 cm. Considering the hive entrance length of stingless bee colonies from thirty locations, maximum number of colonies (14) was observed under the category 1-3 cm length. Regarding the width of hive entrance in midland and upland physiographic regions, maximum number of colonies (21) was observed under the category 1 - 2 cm followed by < 1 cm (8) while only one colony (Pullampara) had hive entrance width > 2 cm. The result on height of hive from the

ground level revealed that maximum number of colonies (14) was placed at a height of > 2 metre category from the ground level.

Studies on the hive entrance construction materials revealed the presence of bee wax, plant resins, dry leaves and vegetative parts, wood particles, bee cadavers, sand and soil particles. Stingless bees at midland location, Malayam was constructed only with bee wax, plant resin and sand and soil particles.

The stingless bees in midland and upland physiographic regions visited fifty foraging plants which comprises medicinal plants (7), plantation crops (5), condiments and spices (1), fruit crops (9), vegetable crops (7), ornamental plants (13), tuber crops (2), weeds (1) and forest trees (5). Of these fourteen were nectar providers; fourteen were pollen providers while 22 plants were both nectar and pollen providers.

Significant variations were observed in the morphometric parameters *viz.*, proboscis length (1.409 mm), length and width of forewing (2.854 and1.056 mm), femur length (0.782 mm), metatarsus length (0.404 mm) and third sternite width (1.148 mm), where the stingless bee samples of the midland were superior to that of the upland locations. Among the thirty locations, morphometric parameters of stingless bee samples collected from midland location, Malayam was significantly different from remaining locations in terms of proboscis length (1.502 mm), femur length (0.918 mm), length (0.480 mm) and width (0.257 mm) of metatarsus, length (3.284 mm) and width (1.183 mm) of forewing.

Studies on the morphometric parameters of stingless bees recorded the parameters like head length (0.957 - 1.086 mm), head width (1.163 - 1.342 mm), distance between ocelli (0.278 - 0.316 mm), dorsal ocello ocular distance (0.172 - 0.272 mm), antennae length (1.427 - 1.633 mm), proboscis length (0.883 - 1.502 mm), forewing length (2.648 mm - 3.284 mm), forewing width (1- 1.183 mm), number of hamuli (5 No.s), length of tibia (1.037 - 1.260 mm), femur length (0.721 –

0.918 mm), metatarsus length (0.355 - 0.480 mm), metatarsus width (0.175 - 0.257 mm), third tergite length (0.468 - 0.527 mm), third sternite width (1.045 - 1.294 mm) and lateral width of fourth tergite tomentum (0.059 - 0.088 mm).

Significant variation in physico-chemical properties *viz*., pH, moisture content, EC, total dissolved solids, colour intensity, reducing sugar, flavanoids, total phenols, diastase and invertase activity of stingless bee honey samples was observed in between the midland and upland locations. The parameters *viz.*, pH (3.45), EC (20.16 µS/cm), total dissolved solids (29.85 ppm), colour intensity (880.57 mAU), reducing sugar (69.96 %), flavanoids (220.09 catechin kg^{-1}), total phenols (879.84 mg catechol kg^{-1}), diastase (31.20 Diastase Number) and invertase activity (136.15 Invertase Number) of the honey samples collected from upland was found to be superior over midland locations whereas the moisture content (20.45 %) recorded from midland was significantly higher than the upland. In the case of antioxidant content, honey samples collected from upland locations (374.84 µM Fe (II) 100 g^{-1}) were superior over midland location. Studies on microbial load in stingless bee honey samples revealed that bacterial colonies were recorded in all honey samples except the honey samples from Peringammala while fungal colonies were recorded only from three honey samples (Pullampara, Peppara and Palamukku). They were found within the admissible levels while actinomycetes were absent in all the honey samples.

Thus the present study clearly revealed that the stingless bees preferred slit like entrance and the hive entrance was found to be constructed with similar materials irrespective of the locations. There was a positive relation between width of the hive entrance and number of guard bees. Out of the 50 plants visited by stingless bees, 14 were pollen providers, 14 were nectar providers while 22 were both nectar and pollen providers. The stingless bee samples from midland was significantly superior over upland locations in terms of proboscis length, length and width of forewing, femur length, metatarsus length and third sternite width, which have a direct relation with

pollinator efficiency. While, superior qualities on physicochemical properties of stingless bee honey samples was observed in upland honey samples. Stingless bee honey quality parameters like moisture content, reducing and non reducing sugars and diastase activity were superior to the suggested quality standards and population of bacteria and fungi recorded from the stingless bee honey samples were within the acceptable level while no actinomycetes were recorded from any of the honey sample

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Abstract

MORPHOMETRIC VARIATIONS OF STINGLESS BEES IN SOUTHERN KERALA AND ASSESSMENT OF HONEY QUALITY

by

DIVYA K. K.

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Abstract of the thesis

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ABSTRACT

The investigation entitled "Morphometric variations of stingless bees in southern Kerala and assessment of honey quality " was conducted at College of Agriculture, Vellayani during 2013-15 with the objective to study the morphometric variations among stingless bee populations, their documentation and to evaluate the physical, chemical and antioxidant properties of their honey. A purposive sampling was conducted in the bee keeping pockets of upland and midland physiographic regions of southern Kerala from where ten stingless bee workers and one honey sample were collected from fifteen locations of each physiographic region. The nest architecture and foraging plants were also recorded at the time of sample collection.

Studies on the design of hive entrance revealed that out of thirty locations, slit like (elliptical) nest entrance was maximum (22), when compared to the round (6) and multiple entrance (2). The stingless bee hive at midland location, Pullampara had the maximum hive entrance width (>2 cm) and number of guard bees (20). Considering the length of hive entrance, maximum number of colonies were observed under the category 1-3 cm length and maximum number of colonies (14) were placed at a height of > 2 metre category from the ground level.

Bee wax, plant resins, dry leaves and vegetative parts, wood particles, bee cadavers, sand and soil particles were used by stingless bees for construction of their nest entrance. Fifty foraging plants were visited by stingless bees, of which 14 plants were categorized as pollen providers, 14 as nectar providers and 22 as both nectar and pollen providers.

Studies on morphometric parameters within the locations revealed that proboscis length (1.502 mm), femur length (0.918 mm), length (0.480 mm) and width (0.257 mm) of metatarsus, length (3.284 mm) and width (1.183 mm) of forewing in stingless bees recorded from the midland location, Malayam were significantly different from rest of the samples. The morphometric parameters *viz*.

length of proboscis (1.049 mm), length (2.854 mm) and width (1.056) of stingless bee forewing, femur length (0.782 mm), metatarsus length (0.404 mm) and third sternite width (1.148 mm) obtained from midland were found to be statistically superior to the upland samples.

Studies on physico-chemical and antioxidant properties of stingless bee honey showed that the parameters *viz*., pH (3.45), EC (20.16 µS/cm), total dissolved solids (29.85 ppm), colour intensity (880.57 mAU), reducing sugar (69.96 per cent), flavanoids (220.09 mg catechin /kg), total poly phenols (879.84 mg catechol/ kg), diastase (31.20 Diastase Number (DN)), invertase activity (136.15 Invertase Number (IN)) and antioxidant content (374.84 µM Fe II/100 g) of the honey samples collected from upland was statistically superior to midland whereas the moisture content (20.45 %) recorded from midland was significantly higher than the upland. The population of bacteria and fungi recorded from the stingless bee honey samples were found within the admissible levels while actinomycetes were absent in all honey samples.

From the study it was inferred that the stingless bees preferred slit like entrance and the hive entrance was found to be constructed with similar materials irrespective of the locations. Significant variations in morphometric parameters which determine the pollinator efficiency were observed between midland and upland locations. The stingless bee honey quality parameters were superior to the suggested quality standards in terms of moisture content, reducing and non reducing sugars and diastase activity.

Appendices

APPENDIX-I

Data-Sheet

Sl. No.	Parameters	Location (Midland/Upland)														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1.	Date of collection															
2.	District name															
3	Location name															
3a.	Altitude															
3b.	Latitude															
4.	Design of the hive entrance															
5.	Length and width of entrance tube															
6.	Materials used for constructing the hive/ hive entrance															
7.	Number of guard bees															
8.	Height from the ground level															
9.	Plants visited by stingless bees near the feral colonies															

APPENDIX-II

Composition of different media

a) 10 per cent fructose amended Sabouraud dextrose agar (SDA)

Dextrose	- 40 g
Peptone	- 40 g
Agar	- 20 g
Distilled water	- 1000 ml

b) Nutrient Agar (NA)

Peptone	- 5g
Nacl	- 5g
Beef extract	- 3g
Agar	- 20
Distilled water	- 1000 ml