PESTS AND DISEASES OF STINGLESS BEES

Trigona iridipennis Smith (Meliponinae: Apidae)

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by

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(2012-11-172)

THESIS

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2015

DECLARATION

I, hereby declare that this thesis entitled **"PESTS AND DISEASES OF STINGLESS BEES *Trigona iridipennis* Smith (Meliponinae: Apidae)"** 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 of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Date: 16/04/15 **(2012 -11- 172)**

CERTIFICATE

Certified that this thesis entitled **"PESTS AND DISEASES OF STINGLESS BEES *Trigona iridipennis* Smith (Meliponinae: Apidae)"** is a record of bonafide research work done independently by Ms. Jayalekshmi C.R. (2012-11-172) 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 her.

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We, the undersigned members of the advisory committee of Ms. Jayalekshmi. C. R. (2012-11-172), a candidate for the degree of **Master of Science in Agriculture** with major in Agricultural Entomology agree that this thesis entitled **"PESTS AND DISEASES OF STINGLESS BEES *Trigona iridipennis* Smith (Meliponinae: Apidae)"** may be submitted by Ms. Jayalekshmi C. R. in partial fulfilment of the requirement for the degree.

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LIST OF ABBREVIATIONS AND SYMBOLS USED

%	Per cent
Mm	Micro meter
Ml	Micro litre
@	At the rate of
°C	Degree Celsius
CD	Critical difference
Cm	Centimeter
EC	Electrical conductivity
et al.	And other co workers
Fig.	Figure
G	Gram
HMF	Hydroxy Methyl Furfural
h.	Hours
i.e.	that is
l.	Litre
Ml	Milli litre
M	Meter
Mm	Milli meter
Kg	Kilo gram
g	Gram
Mg	Milli gram
Sec	Seconds
IARI	Indian Agriculture Research Institute
sp. or spp.	Species (Singular and plural)
viz.	Namely
$mScm^{-1}$	milli Siemens per centimeter

Ft	Feet
p^H	Negative logaritham of hydro carbon ions
Ppm	Parts per million
Min	Minutes
Psi	Pounds per square inch
Meq	Milliequivalents
nos.	Numbers

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1. INTRODUCTION

1. INTRODUCTION

The honey bees belong to the family of eusocial insects which also include bumble bees and the tropical stingless bees. The true honey bees, bumble bees and stingless bees are a class of hymenopteran insects, constitute the family Apidae and comes under the sub- families Apinae, Bombinae and Meliponinae respectively. Meliponinae includes eight genera, having 15 sub-genera and more than 500 species (Wille, 1983). *Trigona* is the largest and most widely distributed genus, 130 species under ten sub-genera. The various genera include *Trigona, Plebia, Tetragona* and *Nanotrigona* (Camargo *et al*., 1988).

Honey bee is one of the few insects that are directly beneficial to mankind. Human's association with honey bees, dates back to several thousands of years. They are important and effective pollinators of many crops. Stingless bees visit flowers of plants including crops and forest trees as well as shrubs and herbs to collect nectar, pollen, wax, resins, oils and other plant substances. The frequency of their visit to the flowers and efficiency in effecting pollination result in high quality and quantity yields of fruits and seeds. However, as a result of their small size and large diversity, they are adapted for visiting small flowers/ florets not visited by *Apis* spp. and found to be one of the most effective and efficient pollinators especially of tropical flora and medicinal plants. Stingless bees can be employed in pollination of greenhouse plants/crops in both temperate and tropical regions (Muthuraman and Saravanan, 2004). Globally bees pollinate more than 400 crop species. The state is having 127 stingless bee foraging plants, identified by AICRP (2013). Many fruit crops and vegetables have been identified which need bee pollination to increase their qualitative and quantitative attributes.

Tetragonula (*Trigona*) *iridipennis* Smith is a common stingless bee which is small to medium sized with vestigial sting. They are sometimes called 'dammer' bees as they collect a kind of resin for constructing their nest along with wax produced from their body. *Tetragonula* (*Trigona*) refers to their triangular shaped abdomen and *iridipennis* refers to their iridescent wings. Stingless bees are the smallest of the honey producing bees. The stingless bees are living in permanent colonies, nesting in old walls, logs, crevices and such other concealed places. But the sting is greatly reduced without an effective tip. Hence, the defense behavior is by chasing and biting the intruders. They become entangled in the hairs and get into the nose, ears, and eyes of the intruders.

In Kerala stingless bees are reared as backyard beekeeping practice mainly for honey production. They are kept in wooden box, mud pots, bamboo hollows or coconut shells. The stingless bees are hardy and easy to handle. The nests are constructed using wax in a mixture with resins, mud, faeces, or other materials collected by the bees. The nest has one entrance tube made of cerumen. The entrance of the nest may be a simple hole, often extended from the nest as an external tube.

Honey is the natural sweet substance produced by honey bees from the nectar of blossoms or from the secretion of living parts of plants or excretion of plant sucking insects on the living parts of plants, which bees collect, transform combine with specific substances of their own, store and leave in honey pots to ripen and mature (Codex Alimentarius Commission, 1994). Stingless bees are poor honey gatherers. They store only limited quantity of honey. Stingless bee honey cannot be called honey, the term divine elixir has been suggested for Meliponinae honey. Honey is considered as food of the Gods in most folklores. Honey can be said as the miracle food, because of its nutritional and health benefits bestowed to human beings since centuries.

Stingless bee honey is popular for its antioxidant and antibiotic properties, hence its effectiveness in healing wound and fighting both internal and external infections. Stingless honey fetches higher price (Upto Rs. 1500 per Kg) in the market.

But, over time, lot of threats paved way for the decline of stingless bee population in our state. Though stingless bees are providing expensive honey, the elixir of life and an efficient pollinator the native population is diminishing. The decline may be due to the non judicious use of pesticides, electromagnetic radiations from mobile towers, destruction of habitats and nesting sites, destruction of bee friendly plants due to deforestation, mechanization, urbanisation and industrialization. Global warming and climate change together with the above factors paved way for the incidence of pests and diseases in stingless bee colonies. However, little studies have been conducted on the pests and diseases associated with stingless bees in our state. In this context, the present investigation was carried out with the following objectives:

- To document and identify the pests and diseases associated with stingless bees.
- To assess the extent of damage and yield loss
- To assess the nest architecture and defense mechanism

2. REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The review of literature pertaining to the documentation of the pests and diseases of stingless bees, assessing the extent of damage and yield loss due to pests and diseases, nest architecture and defense mechanism are presented in this chapter.

Tetragonula (*Trigona*) *iridipennis* was first described from Ceylon by Smith (1954). The most common species of stingless bee found in India was known by name *Melipona iridipennis* Smith. Later this species was transferred to the genus *Trigona* as *Melipona* was found to be restricted only to neotropics (Michener, 1974). It was distributed only in India and Sri Lanka (Sakagami, 1978).

Stingless bees are taxonomically different from honey bees. The honey bees (Apinae), bumble bees (Bombinae) and stingless bees (Meliponinae) come under the family Apidae. The species coming under Meliponinae are divided into two tribes *Trigonini* and *Meliponini*. All Asian and African stingless bee species belong to the tribe *Trigonini*. The different genera under this tribe include *Trigona, Plebia, Tetragona* and *Nanotrigona*. The genus *Melipona* consists of about 40 species, medium to large sized bees, which occurs only in neotropics (Camargo *et al*., 1988). Crane (1992) listed 14 species of *Melipona* and 21 species of *Trigona* that are being reared by traditional beekeepers. The group with the morphology considered to be antique, is *Trigona* the largest and most widely distributed genus in South East Asia (Velthius, 1997).

T. iridipennis is a gentle species and it can be manipulated with ease. The species found in Karnataka (Biesmeijer, 1993) Kerala (Raakhee and Devanesan, 2000) and Tamil Nadu (Swaminathan, 2000) have been reported as *T. iridipennis*.

2.1. DOCUMENTATION OF THE PESTS AND DISEASES OF *T.iridipennis*

2.1.1. Pests

A dipteran fly *Hermetia illucens* L. which thrived on decaying organic matter was newly identified as a pest of stingless bee *T. iridipennis* in Kerala. Both the maggots and adults were observed from within the stingless bee hives (Nisha, 2002). AICRP (2004) also reported the dipteran fly *H. illucens* as a pest of stingless bees in Kerala.

Simoes *et al.* (1980) described *Mealonctia sinistia* Borgmeier as an endoparasitic phorid fly of *Nannatrigona (Scaptotrigona*) *postica* Latreille in Brazil. Sommeijer (1999) reported phorid fly (*Pseudohypocerrspo* sp.) as a major pest of stingless bee in America, which fed on pollen and brood. The maggots and adults of the phorid fly *Megaselia scalaris* Loew affected the nested colonies of all the five stingless bee species in Kakamega forest, Kenya (Kiatoko, 2012).

Studies conducted at the Kakamega forest, Kenya showed that the brood of *Meliponula bocandei* Cockerell, *Meliponula ferruginea* Cockerell (reddish brown), *Meliponula ferruginea* Cockerell (black), *Meliponula lendliana* Friese and *Hypotrigona gribodoi* Magretti stingless bee species were vulnerable to invasion by *A. tumida* grubs (Kiatoko, 2012). He also reported other coleopteran pests like *Rhizoplatys mucronatus* Beauvois and *Tenebroides mauritanicus* Linnaeus from the pollen pots of feral colonies of *M. bocandei* and *M. ferruginea* from Kakamega forests, Kenya.

It was asserted by Flechtmann *et al*. (1974) that a small whitish mite *Neotydeolus therapeutikos* Flechtmann and de Camargo was found in the nests of *Trigona postica* Latreille in Brazil. *Meliponopus palpifer* G.N. a phoretic deutonymph (hypophus) was noticed in *Melipona seminigra* Merrillae from Brazil (Fain and Fletchmann, 1985). *Neocypholaelaps phooni* Baker a new species of mite found in nests of stingless bees in Malaysia was reported by Baker and

Delfinado (1985). Baker and Baker (1988) observed the incidence of two mites belonging to the genus *Eumellipites* in the nests of *Tetragonula* (*Trigona*). A new species of mite *Neocypholaelaps malayensis* Pilsbry found in the nests of *Trigona itama* Cockerell and *T. iridipennis* in Malaysia was described by Delfinado *et al*. (1989). Hallim and Sommeijer (1994) reported robber bees *Lestrimelitto* sp. as a major pest of *Melipona trinitatis* Cockerell. Smiley *et al*. (1996) reported a new species of mite *Hypoaspis* (Mesostigmata: Laelapidae), as a pest of stingless bee *T. iridipennis* from Malaysia.

Nisha (2002) reported a mite *Amblyseius* infestation in bamboo hives of stingless bees in Kerala. New species of mite, *Carpoglyphus lactis* L. was reported by Vijayakumar *et al.* (2013) from the nests of *T. iridipennis* in Tamil Nadu.

2.1.2. Insect Predators

The females of the assassin bug *Apiomerus pillipes* Santschi (Reduviidae, Harpactorinae) were predators of the genus *Melipona* (Silva and Gl-Santana, 2004) in Brazil. Wattena and Jongjitvimol (2007) reported that *Pahabengkakia piliceps* Miller (Assassin bug) (Hemiptera: Reduviidae: Harpactorinae) as a predator of the stingless bee *Trigona collina* Smith in Thailand. The *Acanthaspis siva* Distant (Reduviidae: Hemiptera) was reported as a predator of stingless bees and honey bees in Kerala (Premila *et al.*, 2013).

Ants belonging to subfamilies of Dorylinae and Ecitoninae, which included the army ants, were fearsome predators of honey bees and stingless bees (Hamida, 1999). Raakhee (2000) observed *Solenopsis geminata* Wheeler (ants) entering the weak colonies of stingless bee hives to rob pollen stock from colonies in different locations of Kerala. Ants (*S. geminata*) were seen entering the hive especially in initial stages when the colonies were just divided (Devanesan *et al*., 2009). The ant *Myrmicaria* sp. as a predator of stingless bee species in Kenya was reported by Kiatoko (2012).

George (1934) reported a wasp, *Megachile* spp. which visited the nest and snatched wax from the entrance of *T. iridipennis*. A species of wasp *Megachile disjuncta* Fabricius was observed as a predator of *T. iridipennis* in Kerala (Nisha, 2002). A wasp, *Philanthus* sp. (Hymenoptera: Crabronidae) which was also referred to as bee wolf was observed as a major predator of *M. ferruginea* (black) in Kenya (Kiatoko, 2012).

The centipede *Scolopendra hardwicki* Newport (Chilopoda) was a specialized predator of stingless bee *T. iridipennis* in Tamil Nadu (Vijayakumar *et al.*, 2012).

2.1.3. Spider Predators

Spiders are major predators of stingless bees. Craig *et al.* (1996) reported that *Nephila clavipes* L. a large orb spinning spider feeds on *Trigona fluviventris* Friese by making large yellow webs. *T. fluviventris* is attracted to yellow colour and hence they are trapped in the webs of *N. clavipes* more frequently than in any other spider web. Wattena and Jongjitvimol (2007) reported that *Nephila maculate* F. a golden orb web spider and *Argiope* sp. a golden garden spider were predators of *Trigona collina* Smith nests in Thailand. Different species of spiders (*Thomisus* spp.) were found to predate on stingless bees in Kerala (Devanesan *et al*., 2009).

Large animals like civets (*Paradoxurus hermaphrodites* Pallas), bears *(Melursus ursinus* Shaw), honey badgers (*Mellivora capensis* Storr), tamandus (*Tamandua tetradactyla* Linnaeus), tayras (*Eira barbara* Linnaeus) and chimpanzees (*Pan troglodytes* Blumenbach) were also reported as important enemies of stingless bees (Roubik, 1995).

The African blue fly catcher bird, *Elminia longicauda teresita* Antinori was reported as a major hunter of forager stingless bees, *M. ferruginea* (black) and *M. ferruginea* (reddish brown) by Kiatoko (2012) in Kenya.

The stingless bee *M. lendliana* was one of the predators that frequently robbed propolis from *M. bocandei* hives which were put in place to seal gaps in their hives (Kiatoko, 2012). He also reported that the honey bee *A. mellifera* robbed the honey stored in pots in the nests of the five stingless bees at the Kakamega forest in Kenya.

2.1.4. Diseases

Anderson and Gibbs (1982) reported that *Trigona* pupae were infected by Kashmir bee virus. Five *Bacillus* species have been reported as being associated with stingless bee colonies of *Trigona hypogea* Silvestri (Iaponte, 1996). A type of yeast was found to infect the hives of three species of stingless bees *M. ferruginea* (reddish brown), *M. ferruginea* (black) and *M. lendliana* in Kenya (Kiatoko, 2012).

2.2. ASSESSMENT OF DAMAGE AND YIELD LOSS

Literature pertaining to assessment of damage on brood cells, pollen pots, honey pots and nest due to pests and diseases of stingless bees are meager. Similarly studies on the honey yield and quality from infested and healthy stingless bee colonies are limited. Hence the literature related to true honey bees (*Apis* spp.) and the available literature related to stingless bees is reviewed here.

2.2.1. Extent of Damage by Pests, Predators and Diseases

2.2.1.1. Insect Pests

The larvae of dipteran fly, *H. illucens* massively fed on the pollen, honey, brood and cerumen deposits of *T. iridipennis* (Nisha, 2002).

The impact of infestation of *A. tumida* was severe as it resulted in rotting of the nests, which dried up over time. Adult bees were also found to be affected by the infestation as the bees abandon the severely infested hives (Kiatoko, 2012).

The larvae of coleopterans such as *Cetonia apaca* L. and *Cetonia morio* L. belonging to the family Cetonidae were reported to harm stingless bees by entering the hives to feed on honey and dig galleries in to the wax (Leonard, 1983). The commercial honey production was severely affected in parts of North Africa when there were massive invasions of coleopteran beetle (Hamida, 1999).

Nisha (2002) reported a mite *Amblyseius* sp. feeding on the pollen leading to the destruction of the entire colony. *C. lactis* mites were found to infest the pollen store initially and subsequently spread over the brood cells (Vijayakumar *et al.*, 2013).

2.2.1.2. Insect Predators

The nymphs of assassin bug *P. piliceps* occupied the nest entrance and killed the forager bees. The nymphs and adults of *P. piliceps* captured, immobilized and fed on the prey. Bugs used their legs to capture and immobilize returning foragers. They used their rostrum (beak) to pierce the thorax and abdomen or mesoscutellum of their prey in order to access the haemolymph (Wattena and Jongjitvimol, 2007). Premila *et al.* (2013) reported that the nymphs of the reduviid predator, *A. siva* preyed upon the adults of stingless bees by camouflaging themselves by attaching bits of debris over the body. The bugs fed on the adult workers of *Apis* and stingless bees by sucking body fluid.

Hamida (1999) reported that ants can be a major predator of honey bees and stingless bees in many ways. Ants belonging to sub families of Dorylinae and Ecitoninae, which includes the army ants are fearsome predators of honey bees and can destroy a whole apiary within a few hours. Unlike other ants they do not prefer honey or pollen; instead they prey on adults, larvae and eggs of honey bees. The ant, *Myrmicaria* sp. was found to capture the stingless bee species in the newly nested hives where the gaps were not obtruded completely (Kiatoko, 2012).

The wasp, *Philanthus* sp. (Bee wolf) hunt forager bees flying out and returning into their hives by hovering diagonally closer (up to 7 cm) to the nest entrance of the targeted colony (Kiatoko, 2012).

The centipede, *S. hardwicki* damaged the wooden hives of stingless bee colonies and regular entry of this centipede reduced the strength of worker bees (Vijayakumar *et al*., 2012).

2.2.1.3. Spider Predators

Spiders construct their webs towards sources of abundant prey. *Argiope* spiders generally build their webs in stingless bee habitat especially near the ground and hollow logs where the stingless bees build their nests. This increases the chance of encounter with the spider web whilst foraging and returning to the hive (Rao *et al*., 2008). Devanesan *et al.* (2009) observed that *Thomisus* spiders were attacking the stingless bees at the hive entrance as well as from their foraging sites.

2.2.1.4. Diseases

The pillars and involucrums which were the main components in a stingless bee nest were vulnerable to infestation by a type of yeast in three species of stingless bees *M. ferruginea* (reddish brown), *M. ferruginea* (black) and *M. lendliana*. The yeast was reported to infect 20.70 per cent of the nested colonies of *M. ferruginea* (black), 20.20 per cent of the nested colonies of *M. ferruginea* (reddish brown) and 33.30 per cent of the established colonies of *M. lendliana*. Up to 16.70 per cent of the infected nests of *M. ferruginea* (black) and 17.60 per cent of the infected nests of *M. ferruginea* (reddish brown) and 33.30 per cent of *M. lendliana* absconded due to this infection (Kiatoko, 2012).

2.2.2. Assessment of Honey Yield and Quality Parameters

Stingless bee honeys were produced in the tropics and had a good reputation in traditional medicine (Dold *et al.*, 1937). Phadke (1968) reported that *Trigona* honeys do not granulate even after three years in spite of highly dissolved solids. Honey of *Melipona* are predominantly made of nectar from flowers whereas it is not so in the case of *Trigona*. Honey production increased with increase in number of forager bees (Marceau *et al*., 1990). Honeys of *Trigona* were highly resistant to unwanted fermentation (Crane, 1992). They have broad dietary habits and they use materials other than nectar such as fruit juice, honey dew, fungus and liquids from dead animals to make honey (Bruijin, 1993). According to Carvalho *et al*. (2006) honey composition may vary depending on the flora, location, harvest season, management and the type of bee.

Trigona bees produced meager quantities of dense, darkish, sour honey up to a maximum of 100 ml/ colony (Ramanujam *et al*., 1993). Raakhee (2000) observed the yield of stingless bee honey ranged from 9 ml to 65 ml while Nisha (2002) reported it as 120-350 ml/hive. Sureshkumar *et al.* (2012) reported that a single colony of *T. iridipennis* reared by Kani tribes produced 600 -700 g of honey per year.

The stingless bees visited small flowers that may be poisonous and produced honey which was heat producing and caused acidity when consumed. This is the reason for the medicinal property of *Trigona* honey (Kamal and Pulak, 1994). Ancient Indians used *Trigona* honey for wound, ulcers, kidney disorders and intestinal infection (Bruijin, 1996). Boon (2002) reported that honey of stingless bees had been attributed with medicinal property due to resin chemicals leached from storage pots within the hive, making the honey strongly acidic and sour.

White *et al*. (1962) reported that colours of honey form a continuous range from very pale yellow through ambers to a darkish red amber to nearly black. Schutte and Remy (1982) pointed out that there was a positive correlation between the depth of colour and ash content in honey of *T. iridipennis.* Eupatorium honey was light in colour when fresh and changed to amber colour as it matured with a pleasant aroma and flavour (Thapa and Wongsiri, 1997). The studies conducted at AICRP, Vellayani centre indicated that the *Trigona* honey from different tracts of Kerala varied widely in colour ranging from light yellow to dark amber colour with all intermediate shades (Nisha, 2002 and AICRP, 2004).

Higher value of acidity in stingless bee honey was reported by Vit (1992). Premila *et al.* (2007) reported that the acidity of stingless bee honey ranged from 0.178-0.298 meq Kg^{-1} in the samples collected from different locations of Kerala. The acidity of Pakistan honey samples was 29.20 meq Kg^{-1} (Shafiq *et al.*, 2014).

Vit (1992) asserted that stingless bee honey had higher value of moisture content. The mean moisture content of *T. iridipennis* honey was reported as 24.17 per cent (Swaminathan, 2000); 18.96 to 20.48 per cent (Nisha, 2002) and 18.90 to 20.40 per cent (Premila *et al.,* 2007). The moisture content of Nigerian honey samples was 14.09 per cent (Omafuvbe and Akanbi, 2009) and that of Zambia honey was 14.90 to 16.40 per cent (Nyau *et al*., 2013).

Electrical Conductivity of honey from Nigeria ranged from 0.05 to 0.41 (Buba *et al*., 2013) while that of Indian honey varied from 152.33 to 371.66. (Kumar *et al*., 2013).

Buba *et al*. (2013) reported that the hydroxy methyl furfural (HMF) of honey varied from 5.99 to 17.22 mg kg^{-1} from North East Nigeria. The HMF of *Apis* sp. was reported to be 1.75 to 27.87 mg kg^{-1} (Kumar, 2013) whereas that of Pakistan honey was 13.87 mg kg^{-1} (Shafiq *et al.*, 2014).

It was reported by Raakhee (2000) that the glucose and fructose content of *Trigona* honey were 37.10 per cent and 41.60 per cent respectively. Nisha (2002) studied the reducing sugar, glucose, fructose and sucrose content of stingless bee honey which varied from 77.12 to 69.85 per cent, 35.92 to 33.53 per cent, 38.16 to 39.74 per cent and 1.23 to 1.81 per cent respectively. Studies conducted by Kumar *et al*. (2013) showed that the per cent of glucose and sucrose in stingless bee honey was 62.24 to 70.24 per cent and 1.76 to 2.56 per cent from different locations of India. In honey, the total sugar of 61.67 to 72.42 per cent and that of non reducing sugar contents varied from 1.95 to 3.93 per cent from Pakistan (Fahim *et al.,* 2014) while the glucose content of Pakistan honey samples was 32.01 g Kg^{-1} (Shafiq *et al.*, 2014).

Nisha (2002) studied the pH of *Trigona* honey which ranged from 3.76- 4.40 in Kerala. The stingless bee honey was more acidic and the pH value ranged from 3.70- 4.40 (Premila *et al*., 2007). Kumar *et al* (2013) reported that the pH of honey ranged from 3.30- 4.13 in India. The Nigerian honey contained pH of 3.86 (Omafuvbe and Akanbi, 2009) while that of Zambia honey it ranged from 4.26 to 4.44 (Nyau *et al*., 2013).

It was reported by Huidobro *et al.* (1995) that the diastase and invertase activity indicates freshness of honey. Diastase activity (g of starch hydrolysed per 100 g honey per hive) was much high in *Trigona* (6.60 to 35.60) and invertase activity (µ moles p-nitrophenyl glucopyranoside per kg honey per minute) varied from 15.90 to 214.30 (Patricia and Patrizio, 1996). The diastase and invertase value of stingless bee honey ranged from 0.90 to 23.00 and 19.80 to 90.10 respectively (Souza *et al*., 2006). According to Carvalho *et al.* (2006) the diastase value of *M. scutellaris* and *M. quadrifasciata* honey ranged from 1.73 to 3.01 and 1.34 to 2.14 respectively. Oddo *et al*. (1990) reported that the diastase activity and invertase activity of *T. carbonaria* was 0.40 ± 0.50 and 5.70 ± 1.50. The diastase activity of honey from north east Nigeria ranged from 8.00 to 13.00 (Buba *et al*., 2013)

2. 3. NEST ARCHITECTURE AND DEFENSE MECHANISM

The nesting nature of stingless bees was more complex and diverse than that of *Apis* spp. with great variation in forms, size and place of construction (Schwarz, 1948). Stingless bees exhibit extraordinary variation in nest architecture within and among species. *Trigona denoiti* Smith build combs inside the nests of termites (Smith, 1954). The bees that build exposed nests such as *Trigona spinipes* Fabricius used leaves and other vegetative parts mixed with resin to construct nests. Most stingless bees build their nests in empty trunks or in hollow branches or even in soft and hard soil (Pooley and Michener, 1969). *Trigona oryani* Darchen builds nest within nest of the ant *Crematogaster* sp. inside cavities produced by depredation of the diurnal pangolin (Darchen, 1971).

Schwaziana quadripunctata Lepeletier, *Paratrigona sp*, *Melipona quinquifasciata* Lepeletier and *Geotrigona* sp. constructed their nests under root cavities (Sakagami, 1982). Nests of most species were built within protective cavities such as hollow trees or in the ground (Sommeijer, 1999).

The most commonly described traditional hive for beekeeping with stingless bees, especially *Melipona beecheii* Bennett in Yacatau was a horizontal hollowed log, closed at each end with a disc of wood (Crane, 1985). Central Bee Research and Training Institute, Pune recommended wooden boxes of 10 × 12.50 × 10 cm size with a glass top covered by a wooden lid for rearing *T. iridipennis* (Percy, 1989). The nests of stingless bees were more elaborate and complex than those of *A. mellifera*. Mishra (1995) also asserted that *Trigona* was domesticated in hives. Large boxes of 100 × 40 × 40 cm was used for housing *Melipona trinitatis* Cockerell in Trinidad and in Costa Rica, the small bee *Tetragonisca angustula* Latreille were kept in boxes with average capacity of three litres (Brujin, 1996). *T. carbonaria* were reared in hives consisting of two boxes, an inner box and an outer box. The inner box was designed with three stores to contain a brood space, food storage space and feeding space. For propagating the colony the brood space was divided. The outer box equipped with heater system keeps the hive at a fixed temperature (Dollin, 2001). He recommended the use of bamboo and wooden box duplication method, as an alternative for propagating *T. carbonaria*.

Coconut shells, small earthen pots and bamboo bits were being used in Kerala for rearing stingless bees from olden times and even now people are using the same. Wooden hives of size 3 × 14 × 15 cm were found superior to bamboo hive and earthen pots for rearing *T. iridipennis* (Raakhee, 2000). The studies conducted in Australia showed that, majority of the stingless bee colonies were kept in Original Australian *Trigona* Hive (OATH) (Heard and Dollin, 2000). Nests of *H. gribodoi* were observed in the trees and wall cavities; while those of *M. lendliana* species were observed in the underground hollows and tree cavities. *M. ferruginea* (reddish brown) and *H. gribodoi* preferred hollows in walls of houses for nesting compared to the hollows in the other nesting site (Kiatoko,

2012) in Kenya. Sureshkumar *et al*. (2012) reported that Kani tribes of Tamil Nadu used bamboo bits to rear stingless bees.

Lindauer (1957) recorded that *T. iridipennis* placed scattered droplets of sticky matter in the hive and fresh resinous matter at the mouth of entrance tube. He also observed that the stingless bees deposit irregular rows of pointed lumps of resin in front of the colony entrance which helped to prevent the invasion of ants. The stingless bee nest had an entrance which was build mostly of wax and mud, which is sometimes sticky. Nests were notable points of bee activity; nesting biology was a highly visible aspect of stingless bee behaviour (Michener, 1974). Starr and Sakagami (1987) reported that the brood cells were arranged in clusters, not in organized combs and were not surrounded by involucrum. The form of the entrance varied from one species to another and it was useful in the orientation of the bees and defense of the nests (Sakagami and Inove, 1989). The flight entrance was lined with cerumen or propolis and with entrance tube 6 to 25 mm long was reported in *Trigona gribodi* Margretti (Crane, 1992). The stingless bees void their excreta in the hive on the top of the heap, in contrast to *Apis* colonies in which workers always defecate outside the nest (Brujin, 1993).

The main building material was cerumen, a mixture of beeswax and plant resins. Extensive use was also made of batumen, a mixture of mud, plant resins and animal faeces (Sommeijer, 1999). He also reported that nests were immobile fixtures and potentially long-lived, much like trees in forests where *Meliponines* live. The narrow nest entrance of *Melipona* and other genera allowed the nest to be defended by one or only a few guards positioned in the mouth of the entrance tube which was often rather elaborate. Dispersion (spatial arrangement) of colony resources and stress sources had much significance to nest architecture of *Meliponines* to these critical factors which defined their nesting biology (Camargo and Pedro, 2002; Biesmeijer *et al*., 2005). In many hives piles of yellowish brown coloured matter could be seen at a distance from the brood, cocoons as well as faecal material. Resin in the form of creamy droplets could also be observed in the hive.

Small lumps of resin were found to be attached near the hive entrance and other parts of the hive (AICRP, 2004). Devanesan *et al.* (2009) observed that the openings of the nests of *T. iridipennis* were through resin tubes of varying shapes, length and sizes, normally built of wax and mud, sometimes sticky in nature. The stingless bees mostly used nest enclosure materials such as resins, mud and wax (Pavithra *et al*., 2013).

The nests of individual species were recognizable from nest entrances and their site of construction. Inside the nests, the shape and arrangement of brood cells and food storage containers varied. Honey and pollen were stored in separate pots. Stored nectar or ripened honey were placed in nest cavity extremes (for storage during heavy flowering periods), while pollen and some honey surround the brood area. However, robber bee genera *Lestrimelitta* sp. and *Cleptotrigona* sp. collected and stored honey and brood in mixed provisions (Sakagami, 1982).

Roubik (2006) studied the stingless bee architectural features and behavior so as to decrease their vulnerability to pests. Nests were constructed in bamboo stem cavities of 0.70 to 3.01 cc volume. The entrance tube was simple and of medium length. The tube aperture is longer and narrower in *Trigona sapiens* Cockerell than *Trigona fuscobalteata* Schwarz. Pavithra *et al*. (2013) observed the oval shaped and medium sized nest entrances in Karanataka while Vijayakumar (2014) observed funnel shaped entrance tube of 5.8± 2.00 cm in Nagaland.

Regarding the defense mechanism the sting of stingless bees were vestigial and non functional, instead they exhibited various other means of defense. Some species adopted aggressive ways of defense like biting (*Trigona cupia* Smith and *Tetragonula gusipennis* Smith), emitting a caustic liquid from mouth (all species of *Oxytrigona*) and releasing unpleasant odour (*Trigona capitata* Zexmeniae and *Melipona marginata* Lepeletier) (Dollin, 1996). However the most common strategy of defense was to make their nests and the entrance invisible to intruders (Bruijin, 1996).

Defending bees normally came from the entrance area and nests were equipped with suitable architectural features to allow their aggregated presence. Defensive strategies also included release of alarm chemicals of mandibular gland in the air within and outside the nest (Wittman *et al*., 1990). *T. iridipennis* defended its nests by sealing up all necessary nest openings and sometimes they also crawled over the persons and gave tiny nips with their mandibles (Dollin, 1997). The manner of protecting the open entrance of the nests at night hours varied within the stingless bee species. The presence of a protective barrier at the open entrance of the nest to guard against intrusion during the night was found in three bee species namely, *M. bocandei*, *M. lendliana* and *M. ferruginea* (reddish brown). In *H. gribodoi* and *M. ferruginea* (black) species, the open entrances of nests were never closed during the night hours (Kiatoko, 2012).

Tetragonula laeviceps Friese mostly constructed the colonies on terrestrial shelters at heights of 10 to 20 ft (52.30 colonies), 77 per cent were recorded in main trunk below 10 ft height from the ground level. Nine per cent colonies were located at 20 to 30 ft height from the ground level in Pantnagar (Khan and Srivastava, 2013). The study conducted by Pavithra *et al*. (2013) revealed that stingless bee mostly preferred middle elevation range of 11 to 15 feet for nest building in Karnataka.

3. MATERIALS AND METHODS

3. MATERIALS AND METHODS

A survey was conducted in five Southern districts of Kerala *viz*. Thiruvananthapuram, Kollam, Pathanamthitta, Kottayam and Idukki for a period of one year from July 2013 to June 2014. The pests and diseases of stingless bee *Tetragonula* (*Trigona*) *iridipennis* Smith was documented in two periods *viz*., colony division (July - December 2013), honey harvesting (January – July 2014). The survey was conducted in the apiary of ten stingless beekeepers from each district, possessing a minimum of 50 bee colonies. The year long observations and documentation were carried out in ten stingless bee hives that were marked at random from the selected apiaries. Care was taken to maintain those hives at the same position during the entire period of the study.

Preliminary information was gathered through observations and from interviewing the farmers. The questionnaire contained mainly queries regarding the beekeepers such as numbers of stingless bee colonies in possession, incidence of pests and diseases, location of hives, type of hives, size and shape of hives, height at which the hive is placed above ground level and years of experience in stingless beekeeping. The details of the questionnaire are attached as Appendix 1.

3.1. DOCUMENTATION OF PESTS AND DISEASES OF *T. iridipennis*

Survey was conducted in the apiaries of ten stingless bee keepers of each district. The infected/weak colonies were identified at random and marked for documenting the pests and diseases of *T. iridipennis* from the ten hives of selected ten stingless beekeepers for the year long observation and documentation.

3.1.1. Pests and Predators of Stingless Bees

The pests/ predators associated with stingless bees (seen inside the hive, hive entrance and during foraging), their nature of damage and symptoms produced on infested colonies were recorded. The infested colonies were thoroughly examined for pests and its different life stages and then brought to the laboratory, observed daily to get pests if any. The pests thus collected were documented and preserved in 70 per cent alcohol. The specimens were identified by Division of Entomology, IARI, New Delhi.

The spider predators observed from the stingless bee hives and during foraging time were documented and preserved in 70 per cent alcohol. The spiders were identified by Dr. A.V. Sudhikumar, Assistant Professor and Research supervisor, Christ College, Irinjalakuda, Thrissur. The insect predators were identified by Dr. V. Shashidhar Viraktamath, Professor, UAS, Dharwad.

3.1.2. Diseases of Stingless Bees

The disease infected stingless bees, brood, honey and pollen pots were observed and collected from the apiaries and brought to the laboratory for isolation, purification, identification and testing their pathogenicity.

The dead cadavers of bees, infected brood and nest materials were used for isolation of pathogens. The cadavers of bees and nest materials were surface sterilized in 0.1 per cent sodium hypochlorite for 10 minutes. The specimens were then rinsed twice in sterile distilled water for 2 minutes and placed on a sterile blotting paper to remove excess moisture. The specimens were then placed in Petri plate containing Potato Dextrose Agar (PDA). The plates were then incubated in dark at 30±2°C in a BOD incubator. The hyphal growth emerging from the isolation material was transferred to Petri plate containing PDA after 2-3 days. Hyphal tip culture was followed to get pure cultures of the pathogens.

The single spore isolation technique by Booth (1977) was followed. A drop of sterile water was placed on a sterile slide under dissecting microscope. An accumulation of spores obtained on the wet tip of a needle from the aerial mycelium was introduced into a water drop on the slide. Then the spores were observed to flow from the tip of the needle into the drop of water. The spore suspension on the slide was then picked up by a sterile loop and streaked across a clear agar plate, the position of the streaks were marked by a glass marker at the bottom of the Petri plate. The plate was then incubated for 12-16 h at 25 ± 2^0C in a BOD incubator. By following the lines under 100 X objective of a compound microscope, germination of the conidia was observed and those which were suitably positioned were removed by using a one mm cork borer on to a Petri plate containing Potato sucrose agar (PSA) medium.

3.1.2.1. Identification and Microscopic Characters

The identification of the isolates was carried out by culturing on PDA to study the cultural and morphological characters of the different strains. The spore shape and size were observed under Motic BA 210 compound microscope under 400 X objective magnification. The photographs of spores, phialides and chlamydospores were taken using Motic Image Plus (Version 2.0 ML).

3.1.2.2. Pathogenicity Test

The pathogenicity of isolated strains was carried out to prove Koch's postulates on adult stingless bees, brood and nest materials using standard procedures (Flores *et al*., 2004) with slight modifications. Spore suspension of the test pathogen (1.25 × 10^7 spores/ml) was prepared in sterile distilled water and sprayed (10 ml) on the brood cells, pollen pots and honey pots. Three replications were maintained for each strain and an absolute control using sterile distilled water was also kept. Regular observations were taken every day for infected or dead bees and recorded the mortality.

The fungal pathogens were identified by National Fungal Culture Collection of India (NFCCI), Agharkar Research Institute, Pune.

3.1.3. Population of Total Predators in Five Districts

The population of total predators (insect predators and spider predators) on hives and near surroundings were assessed from randomly marked colonies as described in 3.1 at two periods *viz.*, colony division (July - December, 2013) and honey harvesting (January - July, 2014).

3.2. ASSESSMENT OF THE EXTENT OF DAMAGE AND YIELD LOSS DUE TO PESTS AND DISEASES

3.2.1. Assessment of the Extent of Damage Due to Pests

The extent of damage due to pests of stingless bees, *T. iridipennis* on brood, pollen, honey pots and nest were assessed from the randomly marked colonies as described in 3.1. Nest damage was recorded based on the per cent damage on brood cells, pollen pots and honey from the infested colonies. Damage of brood cells, pollen pots and honey pots was estimated by observing 5 cm^2 area of each using a scale and represented as the mean area damaged.

3.2.2. Assessment of Yield Loss of Honey

Based on the infestation observed, honey yield was assessed from three healthy and three infested colonies in selected apiaries of four districts *viz.*, Thiruvananthapuram, Pathanamthitta, Kottayam and Idukki. The worker bees were transferred from the hives to empty bottles. Then the hives were opened and the honey pots were scooped out and extracted the honey, filtered and weighed. The percentage reduction in honey yield due to pests and diseases was worked out.

3.2.3. Assessment of Quality Parameters of Honey

The physio-chemical characters such as colour, acidity, moisture content, electrical conductivity (EC), hydroxyl methyl furfural (HMF), sugars, pH, diastase and invertase activity of honey collected from the healthy and infested colonies were analyzed by following the methodology and procedures suggested by the Association of the Official Agricultural Chemists of U.S.A (A.O.A.C).

3.2.3.1. Colour

The variation in colour among different honey samples was recorded through visual observation based on USDA (1985) P fund colour scale.

3.2.3.2. Acidity

Acidity of the honey samples was determined by following the procedure described in USDA (1985). The procedure is as follows.

Two grams of honey sample was dissolved in 50 ml distilled water and mixed thoroughly. The sample was boiled for 10-15 min. and the volume was made up to 100 ml. From this, 25 ml solution was taken in a conical flask, four to six drops of phenolphthalein indicator was added and titrated against standard Na OH (0.5 N). A blank was run simultaneously using water instead of honey. Total acidity was then calculated using the formula:

$$\text{Acidity (as formic acid) in 100 g honey (\%)} = \frac{V \times N \times 0.023 \times 100}{25 \times 100}$$

Where, V= Volume of sodium hydroxide used

N= Normality of sodium hydroxide

3.2.3.3. Moisture Content

Moisture content in honey samples were determined as per the procedure described by A.O.A.C. (1990). Twenty five gram of honey was weighed and taken in a clean and dry Petri plate. The Petri plate was then kept in a hot air oven at 100^0C for overnight. The sample was then cooled in a dessicator. The above mentioned procedure was continued until the honey attained a constant weight. Moisture content was expressed as percentage.

Moisture content was determined as follows

Initial weight of honey = A

Final weight of honey = B

Per cent dry weight = B/ A × 100 = C

Moisture content (D) = 100 – C

3.2.3.4. Electrical Conductivity (EC)

Electrical Conductivity was determined using an electrical conductivity (EC) meter, following the procedure described by A.O.A.C. (1990). Twenty gram of honey was dissolved in 100 ml water thoroughly mixed to form a solution. The probe of electrical conductivity cell was immersed in the sample at 20^0C to get the reading, which was expressed in milli Siemens per centimeter ($mScm^{-1}$).

3.2.3.5. Hydroxy Methyl Furfural (HMF)

HMF was determined by following the procedure described by A.O.A.C (1990). Spectrophotometric method was followed for determination of HMF content in the honey sample. Five gram of honey was accurately weighed in a small beaker and transferred to a 50 ml volumetric flask and made up the volume. 0.50 ml of Carrez solution- I was added to the volumetric flask followed by thorough mixing. 0.5 ml of Carrez solution -II, was added to the solution and the volume was made up with water. A few drops of alcohol (95 %) were added to suppress the foam. The first 10 ml filtrate was filtered through a filter paper and it was discarded.

Five ml of the filtrate was pipetted into each of two 18 x150 mm test tubes. Five ml water was added to the tube with test solution and five ml Na HSO_3 solution was added to the other test tube. The samples were mixed well (vortex meter used) and the absorbance value (A) of test solution was determined against reference at 284 and 336 nm in one cm cells. The test solution was diluted with water and the reference solution with 0.1 % NaHSO3 when the (A) value of the solution was > 0.6 and the dilution was corrected.

$$\text{Mg hydroxyl methyl furfural in 100 g honey} = \frac{(A_{284} - A_{336}) \times 14.97 \times 5}{\text{Weight of test sample}}$$

Constant factor = 14.97

3.2.3.6. Sugars

Estimation of reducing and non-reducing sugars was determined as per the procedure described by A.O.A.C. (1990).

3.2.3.6.1. Estimation of Reducing Sugars- Glucose (%)

Five ml of Fehling solution A and B was taken in a clean conical flask and diluted with 10 ml of water. Few porcelain pieces or glass beads were added in to the conical flask and it was heated on wire gauze. When the solution started boiling, the sample honey solution was added to it from a burette. When the color of the Fehling solution nearly faded, two drops of methylene blue indicator was added. The titration was continued while adding honey sample solution in drops. The end point was indicated by the disappearance of blue color of the indicator and appearance of bright red color of cuprous oxide. The titration was carried out with another sample till concordant values were obtained.

$$\text{Percentage of glucose in the solution} = \frac{100}{V} \times 0.05$$

3.2.3.6.2. Estimation of Non Reducing Sugars - Sucrose (%)

Two millilitre of sample solution was taken in a 250 ml beaker and diluted with 100 ml water. Five ml concenterated hydrochloric acid was added in to the solution with constant stirring and contents were kept in thermostat maintained at 60-70^{0}C for about 10 min. The beaker was then removed from the thermostat and a piece of blue litmus was dipped into it. Sodium hydroxide solution was added until the acid was nearly neutralized. The neutralization was completed by the addition of sodium carbonate. The solution was filtrated in to a 250 ml measuring flask and made up using distilled water. Ten ml of Fehling solution was titrated against this solution from the burette, until brick red color appeared.

$$\text{Weight of sucrose in 100 cc of the sample solution} = \frac{250 \times 0.05 \times 5 \times 0.95}{V} \text{ g}$$

3.2.3.7. pH

pH was measured using a pH meter by taking a solution containing 10 g of honey sample in 25 ml of distilled water (AOAC, 1990).

3.2.3.8. Diastase Activity

Diastase activity was determined as per the procedure described by Codex Alimentarius Commission, CAC (2001).

Diastase activity was measured using a buffered solution of soluble starch and honey, which was incubated in glass tubes in a bath with controlled temperature. The time required to reach a specified reaction end point (absorbance < 0.235 nm) was determined spectrophotometrically (Metrolab 1700 UV/VIS Spectrophotometer). The diastase activity, as Goethe's degrees, is expressed as ml of one per cent starch hydrolyzed by the enzyme in one gram in one hour, called the diastase number (DN).

3.2.3.9. Invertase Activity

The invertase activity was determined as per the procedure described by CAC (2001).

3.3. NEST ARCHITECTURE AND DEFENSE MECHANISM

The following architectural features were recorded from the selected apiaries as given in 3.1 using standard protocols (Nisha, 2002; Danaraddi, 2007 and Lima *et al.*, 2013).

3.3.1. Type of Hives

The total number of hives was counted and was categorized based on the materials used for domestication of stingless bees as wooden boxes, mud pots, bamboo hives and PVC pipes. The number of each type of hives and pest/disease incidence in the hives were recorded and expressed as percentage.

3.3.2. Design of Hive Entrance

Designs of hive entrance observed were categorized based on AICRP (2013) as given below:

a. Long tubular : Tubular structure projecting out of hive entrance which is more than five cm in length.

b. Short tubular : Tubular structure projecting out of hive entrance which is less than five cm in length.

c. Round : Complete round shaped entrance slightly projecting outward.

d. Cryptic : No tubular structure projecting out and not round.

The number of each entrance tubes in the four types of hives was counted from five districts and is expressed in numbers.

3.3.3. Length and Width of Hive Entrance

The diameter of the entrance to each nest was measured using a centimeter scale, vertically (length) and horizontally (width), and expressed in centimeter.

3.3.4. Number of Guard Bees

The number of bees that were present at the nest entrance was recorded as the number of guard bees.

3.3.5. Materials Used for Constructing Hive Entrance

The different materials used for the construction of nest entrance were recorded.

3.3.6. Hive Entrance Closure

Presence or absence of involucrum at the hive entrance was observed during the period of survey. The details regarding closure of hive entrance was based on the information from farmers.

3.3.7. Height from the Ground Level

The height of the hive from the ground level was measured using a meter scale and categorized as 3-5ft, 6-8ft, 9-11ft and >12 ft.

The number of hives in each height category from each district was recorded and expressed in percentage. The pests/ predators and diseases incidence in hives at each height was also recorded and is expressed in per cent.

3.4. STATISTICAL ANALYSIS

The data obtained from the studies conducted in laboratory and survey work were subjected to student's t test and completely randomized design (CRD) after appropriate transformations wherever needed.

4. RESULTS

4. RESULTS

The results on the incidence of pests and diseases of stingless bees Tetragonula (*Trigona*) *iridipennis* Smith (Meliponinae: Apidae) conducted during the period 2012-14 under the aegis of the All India Co-ordinated Research Project (AICRP) on Honey bees and Pollinators, Department of Agricultural Entomology, College of Agriculture, Vellayani are presented here under.

4.1. DOCUMENTATION OF THE PESTS AND DISEASES OF *T. iridipennis*

In the survey conducted in five Southern districts of Kerala *viz*., Thiruvananthapuram, Kollam, Pathanamthitta, Kottayam and Idukki, the following pests, predators and diseases of stingless bees were documented. The incidence of pests and predators in five Southern districts of Kerala are presented in Table 1.

The pests recorded were dipteran fly *Hermitia illucens.* L from Pathanamthitta district and nitudilid beetle *Aethina* sp. from Kottayam and Idukki districts. The two predators recorded were reduviid bug *Acanthaspis siva* Distant and assassin bug *Sycanus* sp. from Thiruvananthapuram and *Sycanus* sp. alone from Kollam district. Eight different predatory spiders were observed. They included cream crab spider *Thomisus projectus* Tikader which was recorded from all the five districts but the white crab spider *Thomisus lobosus* Tikader was recorded from Thiruvananthapuram and Pathanamthitta. Only grey sphere spider *Neoscona nautica* Koch was documented from Pathanamthitta and Idukki whereas common wall jumper *Menemerus bivittatus* Dufour was observed from all the districts except Kollam. The common zebra jumper *Plexippus paykulli* Audouin was observed from Kottayam and Idukki. Two tailed spider *Hersilia savignyi* Lucas was recorded form Kollam and Idukki. The common house spider *Heteropoda venatoria* Linnaeus was recorded from Thiruvanantha puram, Pathanamthitta and Idukki. Box spider *Crossopriza lyoni* Blackwall was documented from all the five districts.

Table 1. Incidence of pests and predators of stingless bees in five Southern districts of Kerala

Pests/ Predators					Districts				
	Common name	Scientific name	Family	Order	Thiruvananthapuram	Kollam	Pathanamthitta	Kottayam	Idukki
Pests	Black soldier fly	*Hermitia illucens* L.	Stratiomyidae	Diptera	-	-	+	-	-
	Nitidulid beetle	*Aethina* sp.	Nitidulidae	Coleoptera	-	-	-	+	+
Predators	Assassin Bug	*Sycanus* sp.	Reduviidae	Hemiptera	+	-	-	-	-
	Reduviid bug	*Acanthaspis siva* Distant			+	+	-	-	-
Spider predators	Cream crab spider	*Thomisus projectus* Tikader	Thomisidae	Araneae	+	+	+	+	+
	White crab spider	*Thomisus lobosus* Tikader	Thomisidae		+	-	+	-	-
	Grey sphere spider	*Neoscona nautica* Koch	Araneidae		-	-	+	-	+
	Common wall jumper	*Menemerus bivittatus* Dufour	Salticidae		+	-	+	+	+
	Common zebra jumper	*Plexippus paykulli* Audouin	Salticidae		-	-	-	+	+
	Two tailed spider	*Hersilia savignyi* Lucas	Hersilidae		-	+	-	-	+
	Common house spider	*Heteropoda venatoria* Linnaeus	Sparassidae		+	-	+	-	+
	Box spider	*Crossopriza lyoni* Blackwall	Pholcidae		+	+	+	+	+

\+ Incidence of pests and predators

\- No incidence of pests and predators

4.1.1. Pests

4.1.1.1. Black Soldier Fly Hermitia illucens

The adult fly was smoky black winged, measuring 15-20 mm long. Wings were held over the back when at rest. The fly was easily recognized by their two translucent spots on first abdominal segment (Plate 1). The fly thrived on decaying organic matter and occasionally found entry into the stingless bee hives through the entrance tube, especially on weak colonies. The adult fly did not feed on the brood, pollen and honey pots but only the larval stage of the pest damaged the colonies. The maggots were dull, whitish color, plump, firm and leathery with tiny yellowish black head (Plate 1). The flattened body was composed of a strongly sclerotized head capsule, three thoracic and eight abdominal segments. The pupae were enclosed in a puparium (Plate 1).

4.1.1.2. Nitidulid Beetle Aethina sp.

The beetle was a destructive pest of stingless bee colonies, causing damage to brood cells, pollen and honey pots. The adult beetle was dark brown and about 5 mm in length. Adults were broad, flattened and oval in shape. Antennae clubbed and elytra are shortened (Plate 2). The adults were found on the rear portion of the brood, pollen and honey pots of a hive. The grubs and adults of beetle were observed on the decaying hive materials. They completed their life cycle inside the damaged hives. Female beetles laid irregular masses of whitish eggs in cracks or crevices in a hive (Plate 2). The eggs hatched into white-colored grubs in 2–3 days that will grow to 10–11 mm length in 10–16 days (Plate 2). Grubs had three pairs of legs and dorsal spikes. The pupae were whitish in color with a length of 5 mm (Plate 2).

Adult of *H. illucens*

Maggot of *H.illucens*

Maggots of *H.illucens*

from a damaged hive

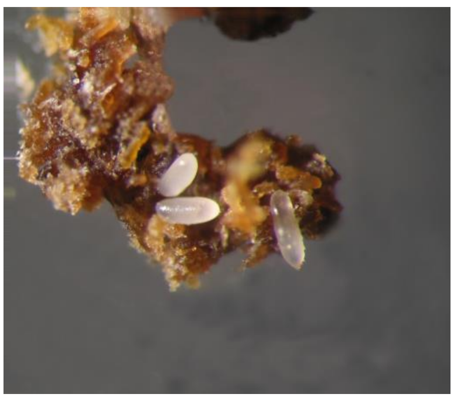
Pupae of *H.illucens*

from a damaged hive

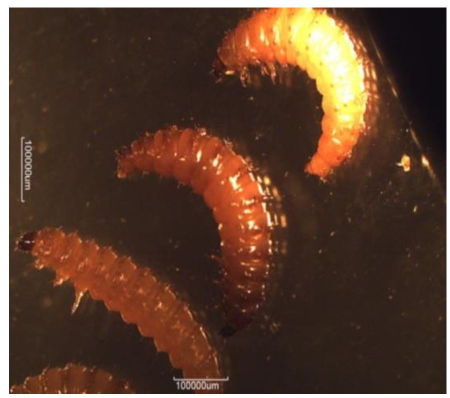
Plate 1.Different life stages of *H.illucens*

Dorsal view of adult of *Aethina* sp.

Ventral view of adult of *Aethina* sp.



Eggs of *Aethina* sp.



Grub of *Aethina* sp.

Close up view of pupa of *Aethina* sp.

Plate 2. Different life stages of *Aethina* sp.

4.1.2. Insect Predators

4.1.2.1. Reduviid Bug Acanthaspis siva

Adult is black or brown coloured with elongated head; distinct narrowed neck and legs were prominent and segmented. The nymphs camouflaged themselves by attaching bits of bee cadaver onto their body and remained close to the entrance tube of the colonies (Plate 3). Long stylets were present.

4.1.2.2. Assassin Bug Sycanus **sp.**

Adult insects were orange or black in body colour (Plate 4). The abdomen was curved upward with a large black spot and was fully covered by wings. Bugs have stylets that were at least twice as thick as their antennae. However, the stylets were shorter, stouter and curved. The forelegs were raptorial. The adults and nymphs of *Sycanus* were observed around the hives, crevices and on tree trunks in the vicinity of stingless bee hives. The eggs were brown in colour and was laid in clusters and cemented to each other (Plate 4). Nymphs were wingless and reddish orange in colour (Plate 4).

4.1.3. Spider Predators

Eight different types of predatory spiders were documented from the hive entrance of stingless bee colonies and from the flowers foraged for nectar/ pollen.

4.1.3.1. Cream Crab Spider Thomisus projectus

Cephalothorax reddish brown, as wide as long, lateral projection present at the occular region. Lateral sides slightly darker than median portion. Abdomen was typical pentagonal shape, yellowish brown (Plate 5).

Adult of *A. siva*

Nymph of *A. siva*

Plate 3. Different stages of *A.siva*

Adult of *Sycanus* sp.

Eggs of *Sycanus* sp.

Nymph of *Sycanus* sp.

Different stages of *Sycanus* sp.

4.1.3.2. White Crab Spider Thomisus lobosus

Cephalothorax was longer than wide, brownish with a longitudinal dark brown patch laterally on either side. Mid-dorsal region bears a 'V' shaped white conspicuous marking. Abdomen white, wider than long, anterior end protruding over posterior end of cephalothorax and broadest behind the middle. Dorsum decorated with black spots, lateral protrusion bears black patches laterally. Ventrum is pale white (Plate 6).

4.1.3.3. Grey Sphere Spider Neoscona nautica

Cephalothorax longer than wide, blackish, cephalic region darker; thoracic region near posterior end brownish and excavated. Dorsum of abdomen was velvety black, with a mid dorsal slightly paler region with two lateral projections. A pair of whitish or greyish spots was present on the anterior half (Plate 7).

4.1.3.4. Common Wall Jumper Menemerus bivittatus

Cephalothorax 'U' shaped, both cephalothorax and abdomen was brown coloured, with dull brown bands along the margins (Plate 8).

4.1.3.5. Common Zebra Jumper Plexippus paykulli

Both the cephalothorax and abdomen were dull sand yellow colour; with an inconspicuous mid-dorsal band in the cephalic region while the abdomen had a conspicuous white spot about the middle. Abdomen longer than wide, nearly oval shaped with a narrowing posterior end (Plate 9).

4. 1. 3.6. Two Tailed Spider Hersilia savignyi

Cephalothorax and abdomen were flat and almost circular, slightly broader than long. Abdomen greyish with a darkish lanceolate cardiac area and dark transverse margins posteriorly. Posterior lateral spinnerets are very long, much longer than the abdomen (Plate 10).

Plate 5. *T. projectus*

Plate 6. *Thomisus loboscus*

Plate 7. *Neoscona nautica*

Plate 8. *Menemerus bivittatus*

4. 1.3. 7. Common House Spider Heteropoda venatoria

Cephalothorax dorsoventrally flattened and yellowish brown in colour. Abdomen elongated, oval and same in colour as that of cephalothorax. Females carry the white disc-shaped egg sac under the body with the help of palps (Plate 11).

4. 1. 3. 8. Box Spider Crossopriza lyoni

Cephalothorax wider than long, greyish white in colour, a dark band along mid longitudinal line and top part of the cephalothorax with deep excavation. Abdomen greyish off white with black and white patches in the sides and dorsum, triangular in shape when viewed from sides, with the spinnerets located in the lower projection of the triangle. A small conical extension was projected at the upper posterior end of the abdomen (Plate 12).

4. 1. 4. Diseases of Stingless Bees

The fungus could be isolated only from three out of 100 colonies in two districts, Thiruvananthapuram and Pathanamthitta. The fungus was isolated from abandoned hives, pollen pots, honey pots, propolis and also from the dead bees lying inside the hive floor. Two fungal isolates of *Paecilomyces variotii* Bainier was isolated from Thiruvananthapuram district while a single isolate of *Aspergillus niger* Tiegh was isolated from Pathanamthitta district. Incidence and cultural characters of isolates from stingless bee colonies are presented in Table 2.

4. 1. 4. 1. Cultural and Microscopic Characters

4.1.4.1.1. Paecilomyces variotii

The fungal growth was found in abandoned hives, pollen pots, honey pots, propolis and on the walls of the hive (Plate 13). No dead bees were found in the hives from which *P. variotii* was isolated.

Plate 9. *Plexippus paykulli*

Plate 10.*Hersilia savignyi*

Plate 11. *Heteropoda venatoria*

Plate 12. *Crossopriza lyoni*

Studies on the cultural characters on PDA showed that the fungus produced pale cream coloured colonies and they reached a growth diameter of 9.0 cm in 14 days. The colonies appeared floccose and powdery due to dense production of conidiophores (Plate 14). The colonies get darkened as it become older. Microscopic characters of the fungal growth revealed that the conidiophores appeared in dense, vertically arranged whorls or branches. Each conidiophore bears single to many phialides, 30-100 µm in length. Phialides appeared solitary or in whorls, variable in length (10-25 x 2.0-5.0 µm). It appears cylindrical at the base and tapers abruptly into a long cylindrical bottle shaped necks (Plate 15). Some phialides appear slightly bend towards the end. Conidia are hyaline to light yellow, smooth-walled, shape varies from subglobose to ellipsoidal, (2.0-5.0 x 2.0-4.0 µm in length). Chlamydospores appear in old cultures, singly or in short chains globose, thick-walled, 4.0-8.0 µm in diameter.

4.1.4.1.2. Aspergillus niger

The fungus was isolated from dead bees lying inside the hive floor and was not found to infect or contaminate the hive or its components like brood cells, honey pot or pollen pot (Plate 16).

The fungus produced white mycelium on PDA, covered 9.0 cm in 12 days (Plate 17). Conidiophores were hyaline and 500- 1000 µm in length. Thus the microscopic characters showed that the fungus possessed large globose conidial head (vesicle) and was dark brown in colour (up to 75 µm in length) (Plate 18 and 19). Metulae was club shaped 5-10 µm long. Conidia were globose to subglobose 3-4 µm with rough surface.

4.1.4. 2. Pathogenicity Studies

The fungus isolated, after morphological characterization, was subjected to pathogenicity studies.

Table 2. Cultural and microscopic characters of fungal isolates from stingless bee colonies

Sl. No	Isolates	Districts	Number of infected colonies *	Source	Cultural characters	Microscopic characters	NFCCI accession code
1.	*Paecilomyces variotii* Bainier (isolate P1)	Thiruvananthapuram	2	Honey pots, pollen pots and propolis	Produces pale dark cream coloured floccose colonies, appear darker as it ages and give powdery appearance colonies on PDA, covers 9.0 cm in 14 days.	Conidiophores appear in dense, vertically arranged whorls or branches. Conidia are hyaline to light yellow, smooth-walled, shape varies from subglobose to ellipsoidal.	NFCCI 3618
2.	*Paecilomyces variotii* Bainier (isolate P2)		1	Honey pots, pollen pots and propolis	Produces pale dark cream coloured floccose colonies, appear darker as it ages and give powdery appearance colonies on PDA, covers 9.0 cm in 14 days.		NFCCI 3621
3.	*Aspergillus niger* Tiegh	Pathanamthitta	3	Dead bees	Produces white mycelium, conidial head dark brown-black, covers 9.0 cm in 12 days in PDA and showed a tendency to loose vigour in culture after continuous subculturing	Conidiophores were hyaline and 500- 1000 µm in length. Conidial head (vesicle) was large globose and dark brown in colour	NFCCI 3619

* Out of 100 stingless bee colonies

Plate 13. *P. variotii* infection in hive

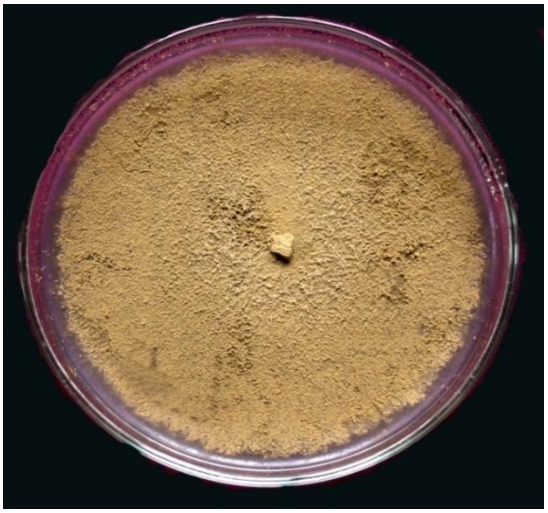


Plate 14. *P. variotii* culture in PDA

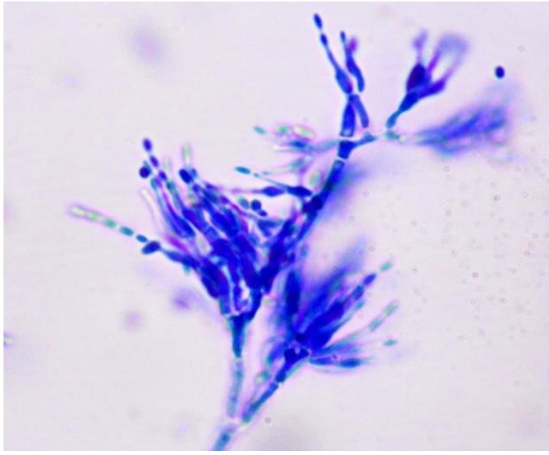


Plate 15. Microscopic characters of *P. variotii* (400 X)

Plate 16. Dead bees in the hive due to *A. niger* infection

Plate 17. Cultural characters of *A. niger* in PDA

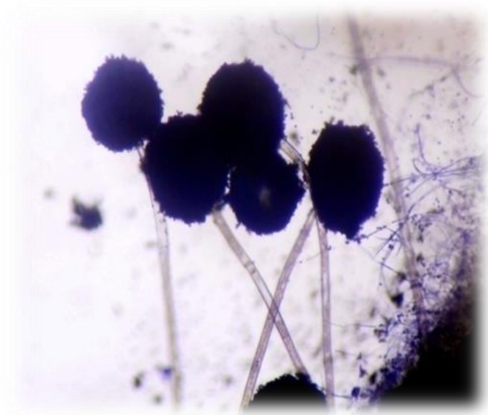


Plate 18. Conidiophores of *A. niger* (100 X)

Plate 19. Microscopic characters(400 X)

4.1.4.2.1. P. variotii

The bees were found dead after 48 hr of inoculation with spores of *P. variotii*. No visible mycelial growth was seen on the cadaver.

4.1.4.2.2. A. niger

The bees were found dead after 48 hr of artificial inoculation with spores of *A. niger*. White fungal mycelium was visible on the bee cadaver with black pinhead like conidiophores arising from the cuticle.

4. 1. 5. Predator Population (Spiders and Insect Predators) in Five Districts

The mean population of total predators (spiders and insect predators) observed at two periods (colony division - July to December 2013 and honey harvesting period - January to June 2014) from five districts are presented in Table 3.

Among the five districts surveyed during the two periods, the highest mean population of predator was observed from Thiruvananthapuram (4.31) followed by Kollam (2.62), Pathanamthitta (1.36), Idukki (1.33) and Kottayam (1.24).

The total predator population was highest during the colony division period in all the districts except Pathanamthitta. The total predator population during colony division was 4.73 in Thiruvananthapuram, 2.74 in Kollam, 1.45 in Idukki and 1.39 in Kottayam while it was 3.90, 2.51, 1.22 and 1.09 respectively during the honey harvesting period. In Pathanamthitta district, the highest predator population was recorded in honey harvesting period (1.49) followed by colony division period (1.23).

4. 1. 6. Spider Population in Five Districts

The mean population of total predatory spiders per hive observed in two periods colony division period (July- December 2013) and honey harvesting period (January- June 2014) from the study area are presented in Table 4.

Among the five districts, significant difference in the predatory spider population between the two periods was observed only in Kottayam and Idukki. Higher population was observed during the colony division period both in Kottyam (1.50) and Idukki (1.65) followed by the honey harvesting period (0.75 and 1.30 respectively).

While in the other three districts, the total predatory spider population was highest during the honey harvesting period which ranged from 1.70 to 2.30 followed by the colony division period which ranged from 1.55 to 1.65.

4.1.7. Insect Predator Population in Thiruvananthapuram and Kollam Districts

Two insect predators, *A. siva* and *Sycanus* sp. were recorded from Thiruvananthapuram while *Sycanus* sp. alone was observed from Kollam (Table 5). Insect predators were not recorded from the other three districts. Significant difference in insect predator population between the two periods was observed in the case of *A. siva* in Thiruvananthapuram and *Sycanus* sp. in Kollam. In Thiruvananthapuram, higher population of *A. siva* was observed during the colony division period (1.90) followed by honey harvesting period (0.85). In Kollam also, the same trend was observed in the case of *Sycanus* sp. where maximum population was observed in colony division (1.85) followed by honey harvesting period were (0.70).

While comparing population of *Sycanus* sp. in two districts, maximum population was observed in Thiruvananthapuam (3.10).

Table 3. District wise population of total predators (spider and insect predator) of stingless bee colonies

Periods	Total predator population				
	Thiruvananthapuram	Kollam	Pathanamthitta	Kottayam	Idukki
Colony division	4.73	2.74	1.23	1.39	1.45
Honey harvesting	3.90	2.51	1.49	1.09	1.22
Mean	4.31	2.62	1.36	1.24	1.33

Table 4. District wise population of predatory spiders in stingless bee colonies

Periods	Spider population/ hive				
	Thiruvananthapuram	Kollam	Pathanamthitta	Kottayam	Idukki
Colony division	1.55 (1.40)	1.55 (1.21)	1.65 (1.23)	1.50 (1.39)	1.65 (1.45)
Honey harvesting	1.70 (1.45)	2.20 (1.46)	2.30 (1.49)	0.75 (1.09)	1.30 (1.22)
CD (0.05)	NS	NS	NS	0.200	0.200

Table 5. Insect Predator Population in Thiruvananthapuram and Kollam Districts

Periods	Insect predator population		
	Thiruvananthapuram		Kollam
	A. siva	*Sycanus* sp.	*Sycanus* sp.
Colony division	1.90 (1.53)[a]	3.10 (1.80)	1.85 (1.52)[a]
Honey harvesting	0.85 (1.10)[b]	3.10 (1.34)	0.70 (1.04)[b]
CD (0.05)	0.300	NS	0.250

4. 2. ASSESSMENT OF THE EXTENT OF DAMAGE AND YIELD LOSS DUE TO PESTS AND DISEASES

4. 2. 1. Mode of Damage by Pests

4. 2. 1. 1. Black Soldier Fly H. illucens

The maggots of the dipteran fly, *H. illucens* fed massively on the brood cells, pollen pots, honey pots and cerumen deposits of *T. iridipennis* (Plate 20). The affected colony was completely damaged and sluggish maggots of the fly were seen entangled within a slimy mixture of pollen pots, honey pots, brood cell and cerumen. Its excreta were found inside the hive which resulted in a foul smell. The maggots were also found to inhabit the nests abandoned by stingless bee colonies after migration. The adult flies entered such hives and deposited their eggs in it.

4. 2. 1. 2. Nitidulid Beetle Aethina sp.

The grubs and adults of nitidulid beetle tunneled through pollen pot, honey pot and brood cells, feeding and defecating, causing discoloration and fermentation of the honey (Plate 21). Beetle primarily attacks weak or small colonies, causing little damage to well established colonies. More than thousand adult beetles and several hundred grubs were observed in infested hives. Apart from these, the colony was found to abscond in severely infested hives. The damaged honey was not good for human consumption.

4. 2. 2. Prey Capturing Behaviour of Insect Predators of Stingless Bees

4. 2. 2. 1. Reduviid Bug A. siva

The adults and nymphs of *A. siva* was observed around the hives, crevices and on tree trunks in the vicinity of stingless bee hives. The adults of *A. siva* mostly preyed upon the worker bees that were found around the hive entrance.

Plate 20. Maggots of *H. illucens* inside severely infested hive

Plate 21. Grubs of *Aethina* sp. on brood cells and honey pots

Both adults and nymphs preyed upon the bees by piercing its body and immobilizing them before sucking the fluid with stylet.

4. 2 .2 .2 Assassin Bug Sycanus sp.

The adults of *Sycanus* mostly preyed upon the worker bees that were found around the hive entrance while the nymphs preyed upon the forager bees when they enter or leave the hive. Feeding behavior is similar to that of *A. siva*. They often grasp their prey with their front legs, then swing their stylet up from under their bodies to insert into the bees.

4. 2. 3. Prey Capturing Behaviour of Spider Predators

4. 2. 3. 1. Cream Crab Spider T. projectus

The adult forager bees were preyed upon by *T. projectus*, found camouflaged inside the flowers, when the bees visited flowers. Mostly the female spiders were found to capture the bees.

4. 2. 3.2. White Crab Spider T. lobosus

Crab spiders were commonly found in flowers hiding behind sepals and petals. They do not build web instead they moved over the ground and climbed the flowers and plants in search of bees. The spider was also found to camouflage on flowers and attack the adult bees during foraging.

4. 2. 3. 3. Grey Sphere Spider N. nautica

Commonly seen in grasslands associated with water bodies and appears in the web during evening only. Foraging stingless bees were seen entangled in the web.

4. 2. 3. 4. Common Wall Jumper M. bivittatus

M. bivittatus inhabits tree trunks and were seen mostly near the stingless bee feral colonies. The spider actively moved in a criss-cross pattern in search of

Table 6. Extent of damage caused by pests of stingless bees

Sl.No.	Pests	Districts	Number of infested colonies*	Mean per cent damage of			
				Brood cells	Pollen pots	Honey pots	Nest
1.	*Hermitia illucens*	Pathanamthitta	5.00	13.25	14.25	16.25	18.12
2.	*Aethina* sp.	Kottayam	4.00	17.25	18.25	19.00	24.00
		Idukki	5.00	14.75	11.75	14.00	20.54

* Out of 100 stingless bee colonies

Table 7. Loss in honey yield due to pests and disease infestation (mean of 3 samples)

District	Honey yield / colony		
	Healthy (g)	Infested (g)	Percentage reduction
Thiruvananthapuram	403.33	20.66	94.76
Kollam	-	-	-
Pathanamthitta	453.33	15.00	96.50
Kottayam	466.66	27.00	93.93
Idukki	600.00	19.00	96.76

small insects like stingless bees especially on sunny days. Both the young and old spiders were found to feed on stingless bees which alight on nest walls. They do not build a web but instead, stalked their prey before launching an attack by leaping on the victim.

4. 2. 3. 5. Common Zebra Jumper P. paykulli

Seen on walls of houses, on compound walls and tree trunks near to stingless bee hives. Males are active under sunshine. These spiders actively jump over the stingless bees in feral colonies for feeding.

4. 2. 3. 6. Two Tailed Spider H. savignyi

It lives on the tree trunks, feeding on moths, ants and small insects like stingless bees. These type of spiders exhibit colour variation according to the substrate. They capture stingless bees mainly near the hives, though they were found mostly attacking feral colonies.

4. 2. 3.7. Common House Spider H. venatoria

Common house spiders do not spin webs and are occasionally seen on tree trunks in gardens. They used to wait quietly on a vertical surface and rushed forward when bees reach within the closed range. Their exceptional agility and speed, as well as their ability to contort and squeeze through tight spaces, give them a strong advantage both in capturing prey and evading predators. They feed at night.

4. 2. 3. 8. Box Spider C. alyoni

C. alyoni is synanthropic, preferring to live near stingless bee hives. They usually build large irregular webs on sides or entrance of hives. Construct irregular webs in corners of ceilings and vibrates the web rapidly when it is touched.

4.2.4. Assessment of the Damage by Pests of Stingless Bees

The extent of damage caused by pests of stingless bee colonies is presented in Table 6.

Out of the 100 stingless bee colonies surveyed, only five colonies showed the infestation of *H. illucens* in Pathanamthitta district. Similarly, four stingless bee colonies from Kottayam district and five stingless bee colonies from Idukki district were found to be infested by *Aethina* sp.

The mean per cent damage by *H. illucens* were 13.25 in brood cells, 14.25 in pollen pots, 16.25 in honey pots and 18.12 inside the nest. The mean per cent damage by *Aethina* sp. was more in Kottayam when compared to Idukki district. The mean per cent damage of brood cells, pollen pots, honey pots and nests were 17.25, 18.25, 19.00 and 24.00 where as in Idukki the observed values were 14.75, 11.75, 14.00 and 20.54 respectively.

4.2.5. Assessment of the Loss in Yield of Stingless Bee Honey due to Pests and Diseases

The yield loss of stingless bee honey due to pests and diseases are presented in Table. 7

Pests and disease incidence was not recorded from Kollam district. The mean percentage reduction in honey yield in the other districts ranged from 93.93 to 96.76. The mean honey yield per healthy colony ranged from 403.33 g to 600 g while that of infested colonies ranged from 15.00 g to 27.00 g throughout the districts surveyed.

4. 2. 6. Assessment of Quality Parameters of Healthy and Infested Stingless Bee Honey Samples

The results of the different quality parameters of stingless bee honey (healthy and infested samples) are presented in Table 8.

4. 2. 6. 1. Colour

Colour variations in healthy/ infested honey samples (Plate 22) revealed that the colour varied from light amber to dark brown. The healthy honey samples were mostly light amber and extra light amber but the infested samples were dark brown and amber.

4.2.6. 2. Acidity

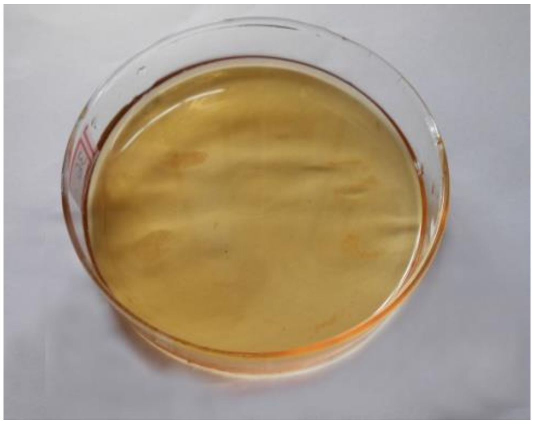
Acidity of healthy honey samples from Thiruvananthapuram, Kollam, Pathanamthitta, Kottayam, and Idukki ranged from 0.14 to 0.26, where as in the case of infested samples the mean values ranged from 0.12 to 0.46. The computed t- value of 5.92 showed that there is a significant difference in mean acidity between the healthy (0.22) and infested samples (0.35) at 0.1 per cent level of significance.

4.2.6.3. Moisture

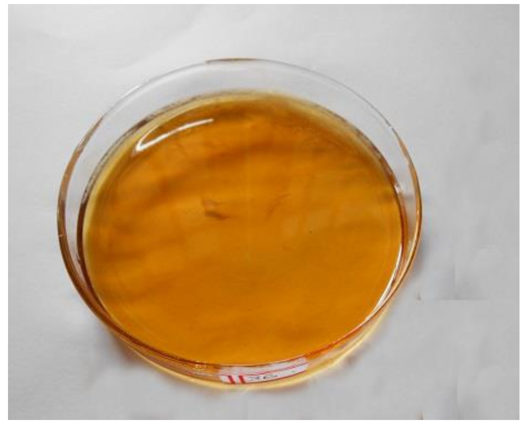
The mean per cent moisture content of healthy samples from Thiruvananthapuram, Kollam, Pathanamthitta, Kottayam and Idukki were 18.07, 20.19, 18.19, 18.75 and 19.80 respectively. In the case of infested honey samples the mean values were 15.53, 16.85, 13.40, 22.65 and 15.16 respectively. The computed t- value of 3.53 revealed that there is a significant difference in mean per cent moisture between healthy (19.02) and infested samples (16.71) at 0.1 per cent level of significance.

4.2.6.4. Electrical Conductivity

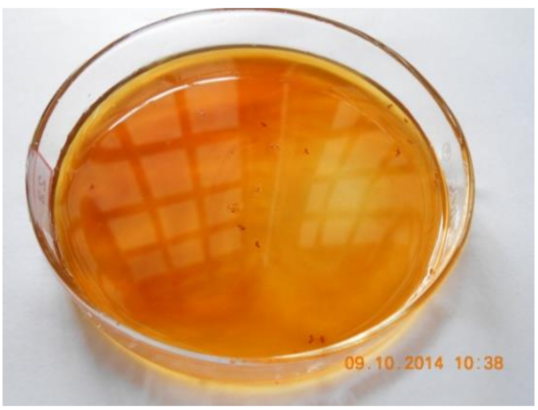
The mean EC value of healthy samples from Thiruvananthapuram, Kollam, Pathanamthitta, Kottayam and Idukki was 29.38, 28.46, 37.66 41.76 $dSm^{-1}$and 31.28 while that of infested samples from five districts was 24.95, 26.38, 51.59, 51.45 and 48.29 dSm^{-1} respectively. The computed t- value of 2.29 showed a significant difference in EC between healthy (33.70) and infested samples (40.53) at 0.1 per cent level of significance.



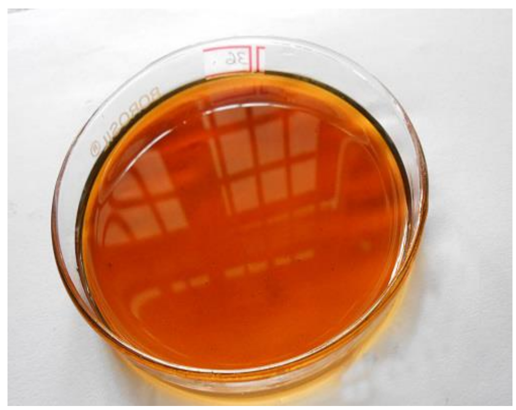
Extra light amber



Light amber



Amber



Dark amber

Plate 22. Different colours of honey

4.2.6.5. Hydroxy Methyl Furfural (HMF)

The mean HMF value of healthy samples were 4.32, 9.72, 9.77, 10.41 and 13.19 mg kg^{-1} from Thiruvananthapuram, Kollam, Pathanamthitta, Kottayam and Idukki respectively where as that of infested samples were 127.90, 189.04, 123.56, 417.19 and 138.06 mg kg^{-1} respectively. The computed t- value 9.74 revealed a significant difference in HMF between the healthy (9.48) and infested (199.15) honey samples at 0.1 per cent level of significance.

4.2.6.6. Sugars

4.2.6.6.1. Glucose

The mean per cent of glucose of healthy samples from Thiruvananthapuram, Kollam, Pathanamthitta, Kottayam and Idukki were 33.35, 37.41, 32.34, 37.74 and 37.63 respectively and that of infested samples were 29.04, 24.04, 23.46, 19.28 and 18.29 respectively. The computed t- value of (14.33) revealed that there was a significant difference in glucose content between healthy (35.73) and infested samples (22.81) at 0.1 per cent level of significance.

4.2.6.6.2. Sucrose

The mean sucrose content of healthy honey samples from Thiruvananthapuram, Kollam, Pathanamthitta, Kottayam and Idukki was 4.88, 5.89, 5.55, 2.82 and 4.36 per cent respectively. The sucrose content of infested samples were 6.43, 4.35, 3.37, 6.35 and 9.15 per cent respectively. The computed t- value of 2.94 showed a significant difference in mean per cent sucrose content between the healthy (5.93) and infested samples (4.70) at 0.1 per cent level of significance.

4.2.6.7. pH

The pH values of healthy honey samples were 3.77, 3.74, 4.03, 3.66 and 3.62 from Thiruvananthapuram, Kollam, Pathanamthitta, Kottayam and

Idukki respectively while that of infested honey samples were 3.30, 3.14, 2.59, 2.99 and 3.21 respectively. The computed t- value of 9.89 revealed that there was a significant difference in pH between the healthy (3.76) and infested samples (3.04) at 0.1 per cent level of significance.

4. 2. 6.8. Diastase Activity

The mean per cent diastase of healthy honey samples from Thiruvananthapuram, Kollam, Pathanamthitta, Kottayam and Idukki district was 1.35, 2.07, 2.05, 1.45 and 2.40 respectively where as that of the infested samples were 8.83, 8.13, 5.77, 6.44 and 6.22 respectively. The computed t- value of 15.31 showed that there is a significant difference in diastase between the healthy (1.93) and infested samples (6.94) at 0.1 per cent level of significance.

4.2.6.9. Invertase Activity

The mean invertase content of healthy samples from Thiruvananthapuram, Kollam, Pathanamthitta, Kottayam and Idukki were 81.10, 88.13, 90.66, 98.00, and 65.53 per cent respectively where as that of infested samples were 42.28, 44.53, 37.66, 44.81 and 38.71. The computed t- value of 21.73 showed a significant difference in invertase content between the healthy (84.68) and infested samples (41.60) at 0.1 per cent level of significance.

4.3. NEST ARCHITECTURE AND DEFENSE MECHANISM

The nests of stingless bees were attracted by several natural enemies because they were rich in food resources and provided many sites for reproduction. Stingless bees lack defensive organs; they do not have stings thereby increasing the risk of invasion by natural enemies. Hence the following parameters of stingless bee hive were studied.

Table 8. District wise physio- chemical characters of healthy/ infested honey samples

Sl. No.	Parameters	Quality of Sample	Districts					Mean	Standard deviation	t- value
			Thiruvananthapuram	Kollam	Pathanamthitta	Kottayam	Idukki			
1.	Acidity (%)	Healthy	0.14	0.21	0.26	0.23	0.26	0.22	0.05	5.92
		Infested	0.34	0.12	0.46	0.45	0.38	0.35	0.14	
2.	Moisture (%)	Healthy	18.07	20.19	18.19	18.75	19.80	19.02	1.93	3.53
		Infested	15.53	16.85	13.40	22.65	15.16	16.71	3.93	
3.	Electrical conductivity(EC) (dSm^{-1})	Healthy	29.38	28.46	37.66	41.72	31.28	33.70	12.26	2.29
		Infested	24.95	26.38	51.59	51.45	48.29	40.53	15.71	
4.	Hydroxy methyl furfural(HMF)	Healthy	4.32	9.72	9.77	10.41	13.19	9.48	4.05	9.74
		Infested	127.90	189.04	123.56	417.19	138.06	199.15	130.51	
5.	Glucose (%)	Healthy	33.35	37.41	32.34	37.74	37.63	35.73	4.41	14.33
		Infested	29.04	24.04	23.46	19.20	18.29	22.85	4.11	
6.	Sucrose (%)	Healthy	4.88	5.89	5.55	2.82	4.36	4.70	1.36	2.94
		Infested	6.43	4.35	3.37	6.35	9.15	5.93	2.44	
7.	pH	Healthy	3.77	3.74	4.03	3.66	3.62	3.76	0.24	9.89
		Infested	3.30	3.14	2.59	2.99	3.21	3.04	0.42	
8.	Diastase activity	Healthy	1.35	2.07	2.05	1.45	2.45	1.93	0.63	15.31
		Infested	8.83	8.13	5.77	6.44	6.22	6.94	2.10	
9.	Invertase activity	Healthy	81.10	88.13	90.66	98.00	65.53	84.68	12.54	21.73
		Infested	42.28	44.52	37.66	44.81	38.71	41.60	4.42	

4.3.1. Type of Hives

The results of the survey conducted to find out the type of hives used for domestication of stingless bees in the five Southern districts are given in Table 9. Majority of the beekeepers were maintaining stingless bee colonies in wooden boxes (70.20 %) and mud pots (18.60 %). Others used hollow bamboo bits (9.20 %) and PVC pipes (2.00 %) (Plate 23 to 27).

Considering the type of hives, wooden box was widely used in Thiruvananthapuram district (86 nos.) followed by Kollam (77 nos.), Idukki (66 nos.), Pathanamthitta (63 nos.) and Kottayam (59 nos.). Throughout the districts, 30 beekeepers of Kottayam district, 22 of Idukki, 21 of Pathanamthitta, 13 of Kollam and 7 of Thiruvananthapuram used mud pots for domesticating stingless bees. Bamboo bits were used by 11 beekeepers of Pathanamthitta, 10 each of Kollam and Idukki, 8 of Kottayam and 7 of Thiruvananthapuam for domesticating stingless bees; whereas PVC pipes were used only in three districts, *viz*., Pathanamthitta (5 nos.), Kottayam (3 nos.) and Idukki (2 nos.).

The per cent damage of stingless bee hives due to pests and diseases recorded from each district varied with the type of hive used. In the case of wooden boxes, 7.93 per cent were damaged by pests and diseases in Pathanamthitta district. It was followed by Idukki (7.57 %), Kottayam (6.77 %) and Thiruvananthapuram (3.48 %). Regarding mud pots and PVC pipes, the pests and disease damage was observed only in Pathanamthitta district (9.52 and 20.00 % respectively). No pests and diseases incidence was recorded from bamboo bits throughout the districts.

4.3.2. Design of Hive Entrance

Various types of hive entrances were observed in different hives during the survey (Table 10). Four types of hive entrances such as long tubular, short tubular, round and cryptic (Plates 28 to 35) were observed.

Table 9. Types of stingless bee hives used by beekeepers and per cent damage of hives by pests and diseases

Districts	Types of hives used by beekeepers (no.)*				Per cent damage of hives by pests and diseases			
	Wooden box	Mud pot	Hollow bamboo bits	PVC pipe	Wooden box	Mud pot	Hollow bamboo bits	PVC pipe
Thiruvananthapuram	86	7	7	-	3.48	-	-	-
Kollam	77	13	10	-	-	-	-	-
Pathanamthitta	63	21	11	5	7.93	9.52	-	20
Kottayam	59	30	8	3	6.77	-	-	-
Idukki	66	22	10	2	7.57	-	-	-
Mean	70.20	18.60	9.20	2.00				

*Out of 100 hives


Plate 23. Wooden hives


Plate 24. Mud pots

Plate 25. Hollow bamboo bits

Plate 26. PVC Pipes

Plate 27. Coconut shells

In the case of wooden hives, the number of short tubular hive entrances was dominant in all the districts which ranged from 18 to 28 except Pathanamthitta (14). The next dominant type was cryptic; 22 nos. in Thiruvananthapuram 18 nos. each in Kollam and Pathanamthitta and 16 nos. in Idukki and 13 nos. in Kottayam. Round type was dominant in Pathanamthitta (22 nos.) while it ranged from 12 to 21 in the other districts. Long tubular was more in Thiruvananthapuram (17 nos.) followed by Kottayam and Idukki (16 nos. each), Kollam (14 nos.) and Pathanamthitta (9 nos.).

Considering mud pots, the number of short tubular hive entrances was dominant in Kottayam (13) and in other districts it ranged from four to eight. Five numbers each of round type of hive entrance was recorded from Pathanamthitta and Kottayam districts and four numbers each were recorded from Kollam and Idukki whereas Thiruvananthapuram recorded only one. The number of long tubular hive entrances ranged from two to six in all the districts except Kollam. Similarly the number of cryptic hive entrances ranged from three to eight in the districts surveyed except Thiruvananthapuram.

In the case of bamboo hives the number of short tubular hive entrances was recorded from all the districts and was more in Kollam (five). The number of round and cryptic hive entrances ranged from two to five and that of long tubular type ranged from one to two.

Regarding PVC pipes three types of hive entrances *viz*., short tubular, round and cryptic entrances were recorded from Pathanamthitta, Kottayam and Idukki districts where it ranged from one to three.

4.3.3. Length, Width and Number of Guard Bees of Entrance Tube

The hive parameters such as length, width of entrance tube and number of guard bees observed during the survey (Plate 36) in the five Southern districts of Kerala are presented in Table 11.

Table 10. Design of hive entrance in wooden box, mud pot, bamboo hives and PVC pipe from five Southern districts

Districts	Wooden box (no.)				Mud pot (no.)				Bamboo hives (no.)				PVC Pipes (no.)			
	Long tubular	Short tubular	Round	Cryptic	Long tubular	Short tubular	Round	Cryptic	Long tubular	Short tubular	Round	Cryptic	Long tubular	Short tubular	Round	Cryptic
Thiruvananthapuram	17	28	19	22	2	4	1	-	1	2	2	2	-	-	-	-
Kollam	14	24	21	18	-	6	4	3	-	5	-	5	-	-	-	-
Pathanamthitta	9	14	22	18	2	8	5	6	-	3	4	4	-	1	3	1
Kottayam	16	18	12	13	6	13	5	6	-	3	5	-	-	-	1	2
Idukki	16	20	14	16	2	6	4	8	2	1	5	2	-	-	-	2

4.3.3.1. Wooden Hives

In the case of wooden hives, the mean length of entrance tube in long tubular was highest (7.08 cm) in Thiruvananthapuram followed by Pathanamthitta (6.83 cm), Idukki (6.32 cm), Kottayam (6.21 cm) and Kollam (6.12 cm). The width of the long tubular hive entrance was more in Thiruvananthapuram (2.24 cm) followed by Idukki (2.06 cm) and it ranged from 1.53 to 1.95 cm in other districts. The number of guard bees were more in Idukki (8.06 nos.) followed by other districts where it ranged from 6.88 to 7.78 nos.

Considering the short tubular hive entrance of wooden hives, the length, width and number of guard bees recorded were highest in Kottayam (4.63 cm, 1.93 cm and 9.00 nos. respectively). The length, width and number of guard bees of short tubular hive entrance in wooden boxes ranged from 3.74 to 4.02 cm, 1.49 to 1.88 cm and 6.91 to 8.50 nos. respectively in other districts.

In the case of round hive entrance of wooden hives, the length was more in Pathanamthitta (2.24 cm) width was more in Idukki (2.30 cm) and the number of guard bees recorded was more in Thiruvananthapuram (8.15 nos.). The length ranged from 1.00 to 1.60 cm, width ranged from 1.67 to 2.16 cm and the number of guard bees ranged from 6.58 to 7.90 in the other districts surveyed.

With regard to the cryptic hive entrance of wooden hives, only the number of guard bees were recorded in which more numbers were recorded from Kottayam (7.76 nos.) followed by Pathanamthitta (7.61 nos.), Thiruvananthapuram (6.54 nos.), Kollam (5.61 nos.) and Idukki (5.43 nos.) districts.

4.3.3.2. Mud Pots

The architectural features of stingless bees in mud pots showed that the length, width and number of guard bees of long tubular type of hive entrance were highest in Pathanamthitta (6.50 cm, 1.70 cm and 7.00 nos. respectively). The length, width and number of guard bees of long tubular hive entrance of mud pots ranged from 5.60 to 6.30 cm, 1.50 to 1.61 cm and 6.00 to 7.00 nos. respectively in the other districts surveyed.

The maximum length (4.65 cm), width (1.80 cm) and number of guard bees (7.66 nos.) were recorded from short tubular hive entrance of mud pots in Kollam district while it ranged from 3.50 to 4.13 cm, 1.60 to 1.68 cm and 6.50 to 7.53 nos. respectively in the other districts.

Considering the round hive entrance of mud pots, the length was highest in Thiruvananthapuram (5.30 cm) and it ranged from 0.72 to 1.87 cm in the other districts. The width of entrance tube was maximum in Idukki (2.30 cm) and it ranged from 1.40 to 2.22 cm and the number of guard bees was highest in Kollam (8.00 nos.) and it ranged from 6.00 to 6.75 nos. in the other districts.

Number of guard bees was only recorded from the cryptic hive entrance of mud pots, where maximum number was recorded from Kollam (9.00 nos.). Guard bees were not observed from Thiruvananthapuram district while it ranged from 4.66 to 7.25 nos. in the other districts surveyed.

4.3.3.3. Bamboo Hives

In view of the bamboo hives, the length, width and guard bee numbers of short tubular hive entrance was recorded from Thiruvananthapuram and Idukki districts only. The number of guard bees were maximum in Thiruvananthapuram (8.00 nos.) and that of Idukki (7.00 nos.). The length and width of long tubular type of hive entrance tube was 5.30 and 1.40 cm in Thiruvananthapuram and 5.50 and 1.50 cm in Idukki district.

In the case of short tubular entrance of bamboo hives, the length, width and number of guard bees recorded were highest in Kollam (5.48 cm, 2.08 cm and 7.60 nos. respectively). The length, width and number of guard bees of short tubular entrance of bamboo hives ranged from1.56 to 4.90 cm, 1.20 to 1.96 cm and 6.00 to 7.00 nos. respectively in the other districts surveyed.

The observations taken from the round entrance of bamboo hives showed that the length, width and number of guard bees were maximum in Thiruvananthapuram district (1.42 cm, 2.72 cm and 10.00 nos. respectively) while it was not observed from Kollam. In the other districts surveyed it ranged from 0.82 to 1.32 cm, 2.20 to 2.30 cm and 5.20 to 7.55 nos. respectively.

Maximum number of guard bees was recorded in the cryptic entrance of bamboo hives in Kollam district while it was not observed from Kottayam district. In other districts, it ranged from 4.50 to 7.00 nos.

4.3.3.4. PVC Pipes

Considering the PVC pipes, long tubular hive entrance was not observed in any of the districts surveyed. Short tubular entrance was observed only from Pathanamthitta where the length, width and number of guard bees were 3.20 cm, 1.10 cm and 8.00 nos. respectively. The length, width and number of guard bees in the round type of entrance were observed from Pathanamthitta (1.23 cm, 2.86 cm and 7.00 nos.) and Kottayam (1.20 cm, 1.90 cm and 6.00 nos.) districts only. The guard bees in cryptic entrance were observed only from Pathanamthitta (6.00 nos.), Kottayam and Idukki (5.50 nos. each).

4. 3. 5. Materials Used for Constructing the Hive Entrance

The materials used for construction of hive entrances in five districts are presented in Table 12. Generally the 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 (Plate 37). The observations taken from the five Southern districts revealed that majority of stingless bees collected waxes and resins from native trees *viz.*, *Mangifera indica* L. (Mango), *Artocarpus heterophyllus* Lam. (Jack

Table 11. District wise length, width and number of guard bees in different entrances of wooden hives, mud pots, bamboo hives and PVC pipes

Districts	Long tubular			Short tubular			Round			Cryptic
	Length (cm)	Width (cm)	Guard bees (no.)	Length (cm)	Width (cm)	Guard bees (no.)	Length (cm)	Width (cm)	Guard bees (no.)	Guard bees (no.)
Wooden box										
TVM	7.08	2.24	6.88	3.74	1.67	6.92	1.37	1.67	8.15	6.54
KLM	6.12	1.95	7.78	3.79	1.88	6.91	1.60	2.07	7.09	5.61
PTA	6.83	1.60	6.88	4.02	1.49	8.50	2.24	1.87	7.90	7.61
KTYM	6.21	1.53	7.75	4.63	1.93	9.00	1.00	2.16	6.58	7.76
IDK	6.32	2.06	8.06	3.83	1.68	7.85	1.55	2.30	7.21	5.43
Mud pot										
TVM	5.75	1.55	6.00	3.50	1.62	7.25	5.30	1.40	6.00	-
KLM	-	-	-	4.65	1.80	7.66	1.87	1.52	8.00	9.00
PTA	6.50	1.70	7.00	3.42	1.68	7.12	1.80	2.00	6.20	4.66
KTYM	5.60	1.61	6.83	4.13	1.60	7.53	0.72	2.22	6.60	7.00
IDK	6.30	1.50	7.00	3.83	1.68	6.50	1.55	2.30	6.75	7.25
Bamboo hives										
TVM	5.30	1.40	8.00	4.90	1.50	6.52	1.42	2.72	10.00	4.50
KLM	-	-	-	5.48	2.08	7.60	-	-	-	7.60
PTA	-	-	-	1.56	1.50	7.00	1.32	2.30	7.55	7.00
KTYM	-	-	-	3.43	1.96	7.00	0.82	2.20	7.00	-
IDK	5.50	1.50	7.00	3.40	1.20	6.00	1.32	2.30	5.20	5.50
PVC pipes										
TVM	-	-	-	-	-	-	-	-	-	-
KLM	-	-	-	-	-	-	-	-	-	-
PTA	-	-	-	3.20	1.10	8.00	1.23	2.86	7.00	6.00
KTYM	-	-	-	-	-	-	1.20	1.90	6.00	5.50
IDK	-	-	-	-	-	-	-	-	-	5.50

TVM- Thiruvananthapuram KLM-Kollam PTA-Pathanamthitta KTYM- Kottayam IDK- Idukki

Table 12. Materials used by stingless bees for construction of hive entrance in five Southern districts

Sl. No.	Districts	Materials used
1	Thiruvananthapuram	Sand, plant resins of mango tree and jack tree, cerumen bits, fragments, wood chips.
2	Kollam	Plant resins of jack, wood chips, sand, plant wax, cerumen, dried bark of trees, leaves and vegetative parts.
3	Pathanamthitta	Sand, plant resins and waxes of maruthu, punna and mango tree, saw dust, tar, cerumen.
4	Kottayam	Plant wax and resins of anjilli, mango tree, cashew and coconut tree, wood chips, sand and cerumen.
5	Idukki	Cerumen, plant resins and waxes of kudam pulli, cashew, mango, sand, wood chips, saw dust

Plate 28. Long tubular entrance in wooden hive

Plate 29. Long tubular entrance in mud pot

Plate 30. Short tubular entrance in wooden hive

Plate 31. Round entrance in mud pot

Plate 32. Round entrance in wooden hive

Plate 33. Round entrance in mud pot

Plate 34. Cryptic entrance in wooden hive

Plate 35.Cryptic entrance in mud pot

Plate 36. Number of guard bees in entrance

Plate 37. Materials used for construction of hive entrance

fruit tree), *Anacardium occidentale* Linn. (Cashew), *Terminalia* spp. (Maruthu), *Cocos nucifera* L. (Coconut), *Calophyllum inophyllum* L. (Punna tree), *Garcinia gummi-gutta* L. (Kudam puli), *Artocarpus hirsutus* Lam. (Anjili).

4. 3. 6. Entrance Closure

Observations on the behavior of entrance closure of hive showed that the stingless bees mostly close their entrance after the dusk. This behaviour was mostly found in strong and healthy colonies with enough number of worker bees.

The entrance is closed using sticky materials such as resins and gums of plants to prevent the entry of predators and pests into the hive during night time. The entrance tube remained completely closed during the night time and was opened during the early morning hours before the foraging commenced.

4. 3. 7. Height of Stingless Bee Colonies from the Ground Level (%)

The mean per cent stingless colonies maintained at different heights from the ground level (3-5 ft, 6-8 ft, 9-11 ft and >12 ft) in the five Southern districts of Kerala are presented in Table 13.

The results of the survey showed that 36 per cent of colonies in Thiruvananthapuram district were maintained at 9-11 ft from the ground level while 33 per cent at 6-8 ft and 29 per cent at 3-5 ft from the ground level. Only two per cent of colonies, suspended from the sunshade, were kept at >12 ft from the ground level.

In Kollam district, majority of the colonies (46 %) were maintained at >12 ft above the ground level. Thirty per cent colonies were at 6-8 ft and 24 per cent colonies were maintained at 9-11 ft above the ground level. None of the colonies observed were maintained at 3-5 ft height from the ground level.

In Pathanamthitta district, majority of the colonies (52 %) of colonies were maintained at the height 6-8 ft, 33 per cent of colonies were maintained at the

Table 13. Height of stingless bee colonies from the ground level (%) (Mean of 100 hives)

Sl. No.	Height from the ground (ft)	Thiruvananthapuram	Kollam	Pathanamthitta	Kottayam	Idukki
1.	3 - 5	29	-	-	-	-
2.	6 - 8	33	30	52	20	11
3.	9 - 11	36	24	33	45	24
4.	> 12	2	46	15	35	65

height of 9-11 ft and 15 per cent of colonies were maintained at >12 ft above the ground level. No colonies were maintained at 3-5 ft height from the ground level.

The observations from Kottayam district revealed that 45 per cent of colonies were maintained at 9-11 ft, 35 per cent of colonies at >12 ft and 20 per cent of colonies at 6-8 ft above from the ground level. None of the colonies observed were maintained at 3-5 ft height from the ground level.

Sixty five per cent of the colonies in Idukki district were maintained at >12 ft height above the ground level; while twenty four per cent of colonies were maintained at 9-11 ft height and 11 per cent of colonies were maintained at 6-8 ft height from the ground level.

4. 3. 8. Incidence of Pests/Predators and Diseases in Stingless Bee Colonies at Different Height from the Ground Level

The data pertaining to the incidence of pests/predators and diseases in stingless bee colonies maintained at different heights from the ground level (3-5 ft, 6-8 ft, 9-11 ft and > 12 ft) are presented in Fig. 1.

None of the colonies maintained at 3-5 ft height from the ground level had pests/predator or disease incidence. Whereas in colonies maintained at 6-8 ft height, 4.69 per cent of colonies had insect pest incidence, 7.03 per cent of colonies had predator incidence and 2.54 per cent of colonies had disease. The colonies maintained at 9-11 ft height from the ground level recorded a pest incidence of 5.10 per cent and predator incidence of 19.96 per cent. A predator incidence of 9.21 per cent was recorded in colonies maintained at 12 ft height from the ground level.

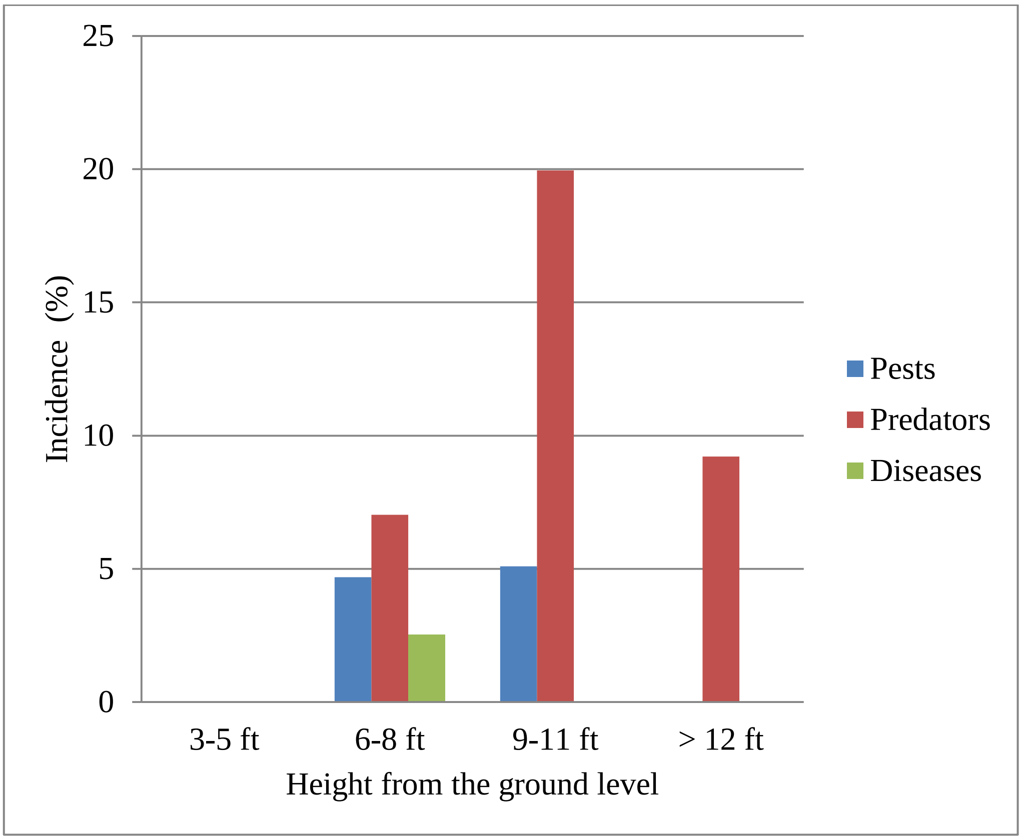


Fig.1. Incidence of pests/ predators and diseases in stingless colonies at different height from the ground level

5. DISCUSSION

5. DISCUSSION

The stingless bees Tetragonula (*Trigona*) *iridipennis* are eusocial group of insects belonging to the order Hymenoptera and family Apidae. In India, they are usually observed in the tropical peninsular states of Kerala, Tamil Nadu and Karnataka. The feral bee colonies reside in tree cavities, dead trees, among boulders, foundations and walls of old buildings. Stingless bee honey fetches high price as well as possess medicinal value. They are also pollinators of tropical flora. In Kerala the stingless bees are domesticated by rearing them in wooden boxes, mud pots, bamboo hollows, coconut shells, etc. The improper hive management practices especially during honey harvest results in the incidence of pests and diseases which in turn adversely affects the productivity as well as quality of the stingless bee honey. However, information of the pests and diseases affecting stingless bees in Kerala is limited. Knowledge on the above as well as nest architecture and defense mechanisms will be invaluable in scientific meliponiculture

In this context, studies were undertaken to document the pests and diseases, damage and yield loss caused by them, nest architecture and defense mechanism of stingless bees. The findings of the study are discussed below.

5.1. DOCUMENTATION OF THE PESTS AND DISEASES OF *T.IRIDIPENNIS*

A survey was conducted in five districts (Thiruvananthapuram, Kollam, Patahnamthitta, Kottayam and Idukki) of Kerala in 2013. A total of 500 colonies were observed for pests and diseases in the five districts.

In the present study, insect pest incidence was recorded only from *three* districts *viz*., Pathanamthitta, Kottayam and Idukki. The dipteran fly *Hermitia illucens* was recorded from Pathanamthitta while the nitidulid beetle, *Aethina* sp. was observed only from Kottayam and Idukki districts. *H. illucens* has already been reported as a pest of stingless bee colonies in Kerala by Nisha (2002) and AICRP (2004). Studies on the mode of infestation as well as the insect characters revealed that both the maggots and adults of *H. illucens* were observed from within stingless bee hives and the larval stage was found to feed on the brood, pollen and honey pots. The adult fly was smoky black winged, easily recognized by the presence of two translucent spots on first abdominal segment and the maggots were dull, whitish color, plump, firm and leathery with tiny yellowish black head. These were in accordance with the findings of Nisha (2002) and AICRP (2004). The nitidulid beetle, *Aethina* sp. is not reported as a pest of stingless bee in Kerala hitherto. The grubs and beetles of *Aethina* sp. observed during the present study were found on decaying hive materials. The adult beetle was broad, flattened and oval in shape with clubbed antennae and shortened elytra. Though Kiatoko (2012) has also reported *Aethina* sp. as a brood pest in other stingless bee species; *M. bocandai*, *M. ferruginea* (reddish brown), *M. ferruginea* (black), *M. lendliana* and *H. gribodoi* in Kakamega forest, Kenya, studies on the insect characters were not much detailed.

Two insect predators were recorded from stingless bee hives of Thiruvananthapuram and Kollam districts. The incidence of reduviid bug *Acanthaspis siva* and assassin bug *Sycanus* sp. were observed from Thiruvananthapuram while *Sycanus* sp. alone was recorded from Kollam district. Observations on the adult characters of *A. siva* revealed that they are black or brown coloured with elongated head while the nymphs camouflaged themselves by attaching bits of bee cadaver onto their body. Similar observation have been reported by Premila *et al.* (2013) where *A.siva* was recorded as a predator of stingless bees and honey bees in Kerala. The assassin bug *Sycanus* sp. is observed as a predator of *T. iridipennis* for the first time in Kerala. The adults and nymphs of *Sycanus* sp. were observed around the hives, crevices and on tree trunks in the vicinity of stingless bee hives during the period of study. Adult insects were orange or black in colour and the stylets were at least twice as thick as their antennae. Earlier reports on *Sycanus* sp. was few in abroad where Silva and Gl-Santana (2004) reported an assassin bug *Apiomerus pillipes* as a predator of *Melipona* species in Brazil and Wattena and Jongjitvimol (2007) reported

Pahabengkakia piliceps as a predator of the stingless bee *Trigona collina* in Thailand.

Eight different predatory spiders, *Thomisus projectus, Thomisus lobosus, Neoscona nautica, Menemerus bivittatus, Plexippus paykulli*, *Hersilia savignyi*, *Heteropoda venatoria* and *Crossopriza lyoni* were recorded during the survey from different districts. Of these, *Thomisus* sp. has already been reported as a predator of *T.iridipennis* in Kerala by Devanesan *et al.* (2009). Craig (1994) reported *Nephila clavipes*, a large orb spinning spider as a predator of *Trigona fluviventris* while *Nephila maculate*, a golden orb web spider and *Argiope* sp. a golden garden spider were reported as predators of *T. collina* nests in Thailand (Wattena and Jongjitvimol, 2007).

During the course of the investigation, two fungal isolates of *Paecilomyces variotii* were obtained from Thiruvananthapuram district while a single isolate of *Aspergillus niger* was observed from Pathanamthitta district. *P. variotii* showed the symptoms of fungal growth in abandoned hives, pollen pots, honey pots, propolis and on the walls of the hive. The cultural and microscopic characteristics of *P. variotii* such as conidia, chlamydospores and phialides were observed under Motic BA 210 compound microscope under 400X objective magnification. Fungus covered pale cream coloured colonies 9.0 cm petri plate in 14 days. Conidiophores were dense, vertically arranged whorls or branches, phialides appeared solitary or in whorls, length (10-25 x 2.0-5.0 µm). Chlamydospores were 4.0-8.0 µm in diameter singly or in short chains globose and thick-walled. In the case of *A. niger* covered 9 cm petri plate in 12 days on PSA. Conidiophores were hyaline and 500- 1000 µm in length. Conidia were dark brown in colour (up to 75 µm in length). Conidia were globose to subglobose 3-4 µm with rough surface. The incidence of fungal attack is reported for the first time in stingless bees in Kerala where as Anderson and Gibbs (1982) has recorded the infection of *Trigona* pupae by Kashmir bee virus. Iaponte (1996) reported five *Bacillus* species from the colonies of *Trigona hypogea.* Apart from these, infection by yeast in the hives of three species of stingless bees, *M.*

ferruginea (black), *M. ferruginea* (reddish) and *M. lendliana* was reported by Kiatoko,(2012) in Kenya.

The population of total predators (spiders and insect predators) observed at two periods (colony division - July to December 2013 and honey harvesting period - January to June 2014) are given in Fig. 2. The results showed that the mean population of total predator was highest during the colony division period (2.31) than honey harvesting period (2.04). Literature on the population of total predators during the colony division and honey harvesting period is meagre.

An overall view of the documentation of the study in five districts showed that the pests likes *H.illucens* and *Aethina* sp. is in the verge of becoming a serious threat to the domestication of stingless bees. At present these are minor/ scavenger pests. The study revealed that two insect predators and eight spider predators were present at two seasons. The insect predators like *A. siva*, *Sycanus* sp. and eight different spiders have not attacked the hive materials, but they captured the stingless bees while foraging or at nest entrance resulting in reduction of stingless bee population. Two fungal isolates of *P. variotii* and a single isolate of *A. niger* obtained during the survey caused symptoms of fungal growth on the bee cadavers, inside the hives, pollen pots, honey pots, propolis and on the walls of the hive resulting in total loss of the colonies.

5.2. ASSESSMENT OF EXTENT OF DAMAGE AND YIELD LOSS

Information on the extent of damage a pest is capable of inflicting in stingless bee colonies is highly essential for estimating the yield loss. Regarding the mode of damage by the maggots of *H. illucens* which fed massively on the brood cells, pollen pots, honey pots and cerumen deposits of *T. iridipennis,* the affected colony was found completely damaged and sluggish maggots of the fly were seen entangled within a slimy mixture of hive materials. These findings are supported by the earlier reports of Nisha (2002) and AICRP (2004) from Kerala. The grubs and adults of nitidulid beetle, *Aethina* sp. which tunnelled through pollen pot, honey pot and brood cells, feeding and defecating,

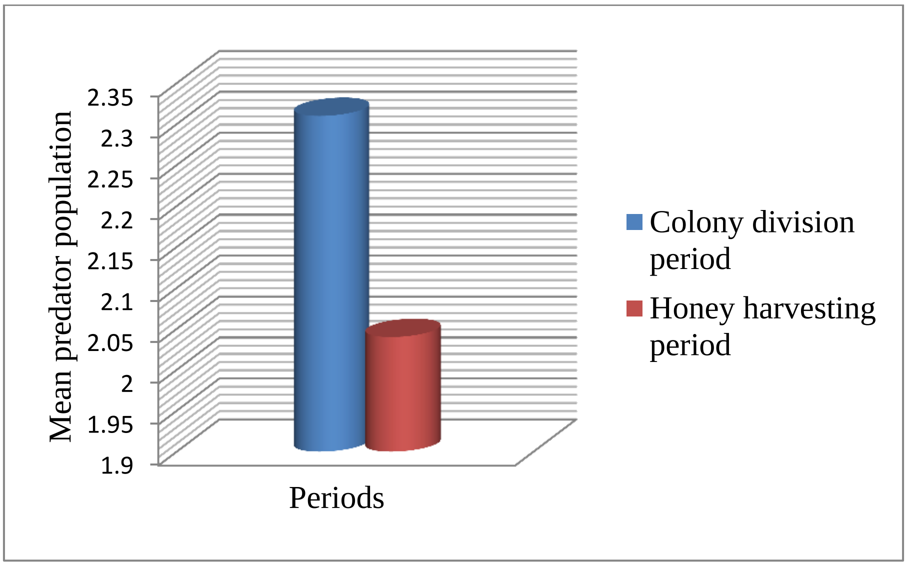


Fig. 2. Population of total predators

causing discoloration and fermentation of the honey, thus become unfit for human consumption. These findings are endorsed by the earlier reports of Kiatoko (2012) where he discussed the impact of infestation by *A. tumida* which was severe as it resulted in rotting of the whole nests, which dried up over time.

The adults of *A. siva* preyed upon the worker bees at the hive entrance while the nymphs camouflaged themselves at the entrance by attaching bits and debris of bees on their bodies. Both adults and nymphs predate upon the bees by capturing, immobilizing and feeding on the bee with its stylets. The observations on the mechanism of camouflaging and the mode of attack conform to that of Premila *et al.* (2013). However the adults of *Sycanus* sp., though not reported earlier, was similar to that of *A. siva* in their prey capturing behaviour but do not exhibit camouflaging. They often grasp their prey with their front legs, then swing their stylets up from under their bodies to insert into the bees.

Based on the observations on the prey capturing behaviour of eight spiders, most of them are camouflaged on the flowers and plants and attacked the adult bees while foraging, either by direct predation or entangled in the web. Some species stalked the bees before launching and attacking by leaping on the victim while others actively jumped on the stingless bees in feral colonies for feeding. Some spiders constructed irregular webs and vibrated the web rapidly when it was touched by bees. Literature on the prey capturing behavior of spider predators is meagre. However Rao *et al*. (2008) reported that spiders constructed their webs towards sources of abundant prey. They also mentioned that *Argiope* spiders generally build their webs in stingless bee habitat which increased the chance of encounter with the spider web whilst foraging and returning to the hive. Devanesan *et al.* (2009) observed that *Thomisus* spiders were attacking the stingless bees at the hive entrance as well as from their foraging sites. The results of the present study are in conformity with these earlier reports.

The insect pest, *H. illucens* which was only recorded from Pathanamthitta district caused a mean per cent damage of 13.25 in brood cells, 14.25 in pollen pots, 16.25 in honey pots and 18.12 inside the nest. While *Aethina* sp. recorded from Kottayam and Idukki; produced a mean per cent damage of 17.25, 18.25, 19.00 and 24.00 in Kottayam and 14.75, 11.75, 14.00 and 20.54 respectively in Idukki. Nisha (2002) reported that the larvae of dipteran fly, *H.illucens* massively fed on the pollen, honey, brood and cerumen deposits of *T. iridipennis*. Similar studies on the extent of damage by *A. tumida* in the split and unsplit nests of *M. bocandei* showed that there was 100 per cent nest damage (Kiatoko, 2012) in Kenya. The mean percentage reduction in honey yield in the different districts ranged from 93.93 to 96.76. The mean honey yield per healthy colony ranged from 403.33 g to 600.00 g while that of infested colonies ranged from 15.00 g to 27.00 g throughout the districts surveyed. Literature on the loss in yield by pests and diseases of *T. iridipennis* is lacking.

Considering the quality parameters of healthy and infested stingless honey samples, the color of honey collected from different locations of five districts, varied in different shades such as extra light amber, light amber, amber and dark brown color. Most of the healthy honey samples were light amber, extra light amber and amber in color whereas infested samples were mostly amber and dark brown in color. White *et al*. (1962) reported that colors of honey form a continuous range from very pale yellow through ambers to a darkish red amber to nearly black. The variations are almost entirely due to the plant source of the honey, although climate may modify the color somewhat through the darkening action of heat. Nisha (2002) reported that the *Trigona* honey from different tracts of Kerala varied widely in color ranging from light yellow to dark amber color with intermediate shades. The healthy honey samples collected from Pakistan were found to be darker in colour (Shafiq *et al*., 2014). The present investigation is supported by the above findings.

Acidity of healthy honey samples from five districts ranged from 0.14 to 0.26 per cent, where as in the case of infested samples it ranged from 0.12 to 0.46. Higher value of acidity in stingless bee honey was reported by Vit (1992). Premila *et al.* (2007) also observed that the acidity of stingless bee honey ranged from 0.178-0.298 in Kerala, thus in the present study the acidity of healthy honey matches with the earlier reports. Moisture content of healthy samples from Thiruvananthapuram, Kollam, Pathanamthitta, Kottayam and Idukki ranged from 18.07 to 19.80 per cent. The moisture content of infested honey samples from five districts varied from 15.53 to 22.65 per cent. Mishra (1995) reported that the moisture content may even change during storage because honey is hygroscopic. He also mentioned that the honey can be saved from absorbing moisture leading to fermentation by proper storage at humid areas. Similar studies conducted by Bijlsma *et al.* (2006) showed that the moisture content of stingless bee honey collected from Trinidad and Tobago varied between 28.40 per cent (*Melipona favosa*) and 42.00 per cent (*Plebeia tobagoensis*) depending on the area where it is produced. In the present investigation, the moisture content of healthy and infested honey of the stingless bees varied and it may be due to the reasons suggested by the above researchers and also due to fermentation.

The electrical conductivity of healthy samples from Thiruvananthapuram, Kollam, Pathanamthitta, Kottayam and Idukki ranged from 28.46 to 41.76 while that of infested samples from five districts varied from 24.95 to 51.59. Contrarily the literature showed that the electrical conductivity of stingless honey from Nigeria ranged from 0.05 to 0.41 and that of stingless honey from India varied from 152.33 to 371.66 (Buba *et al*., 2013 and Kumar *et al*., 2013) respectively. The mean Hydroxy methyl furfural (HMF) value of healthy samples and infested samples ranged from 4.32 to 13.19 and 123.56 to 417.19 mg kg^{-1} respectively. According to Mishra (1995) HMF was responsible for the aroma which was produced during degradation of honey sugars while fructose/ glucose ratio gives an indication of rapidness with which it granulates. The observation on the HMF value of healthy samples of stingless honey conform to that of Subramanian *et al.* (2007) who reported that HMF content of fresh honey was less than 15 mg kg^{-1} and that of infested honey samples matches with that of thermally stressed honey in which the HMF was 330 mg kg^{-1}.

The glucose and sucrose content of healthy samples from five districts varied from 32.34 to 37.74 and 2.82 to 5.89 per cent whereas in the case of infested samples it ranged from 18.29 to 29.04 and 3.37 to 9.15 per cent respectively. The observations made conform to that of Nisha (2002) who reported that the glucose and sucrose content of stingless honey ranged from 33.53 to 35.92 and 1.23 to 1.81 per cent respectively.

The pH value of healthy and infested samples from Thiruvananthapuram, Kollam, Pathanamthitta, Kottayam and Idukki ranged from 3.62 to 4.03 and 2.59 to 3.30 respectively. Similar observations were recorded by Nisha (2002) and Premila *et al*. (2007) who reported that the pH of *Trigona* honey ranged from 3.76 to 4.40 in Kerala. Kumar *et al.* (2013) also reported that the pH of stingless honey ranged from 3.30- 4.13 in the samples collected from different locations of India.

The mean per cent diastase and invertase activity of healthy honey samples from five districts varied from 1.35 to 2.40 and 65.53 to 98.00 where as that of the infested samples ranged from 5.77 to 8.83 and 37.66 to 44.81 per cent respectively. The lower diastase activity of healthy honey conform to the reports of Oddo *et al*. (1990) who revealed that unifloral honey had very low diastase values. According to White (1994) the variation in honey enzyme activity has been shown to occur due to amount of sucrose in food sources, rate of nectar flow and even age of bees whereas long storage at moderate temperatures and exposure to high temperatures will inactivate diastase in honey (Ropa, 2010).

The extent of damage by pests and prey capturing behaviour of predators showed that they were found to inhabit the weak colonies of stingless bees, most often after migration. The pests caused complete damage in colonies. *A.siva* and *Sycanus* sp. are specialized predators which showed a variety of morphological and behavioural adaptations that apparently increased its ability to prey stingless bees. Insect predators and spider predators showed a behaviour pattern of predation that involves the capture, immobilization, death and manipulation of the prey resulting in decline of worker population and in the colonies. Regarding the assessment of quality parameters between healthy and infested stingless bee honey samples, wide variations were observed in HMF, diastase and invertase activity. This may be due to the differences in their habitat, resources (nectar and pollen) and weather conditions.

5.3. NEST ARCHITECTURE AND DEFENSE MECHANISM

Regarding the type of hives, majority of the beekeepers are maintaining stingless bee colonies in wooden boxes (70.20 %) and mud pots (18.60 %). Others used hollow bamboo bits (9.20 %) and PVC pipes (2.00 %). The size and shape of wooden boxes varied in different location within the district but the boxes were mostly made from timber of soft wood trees such as *Acacia manjeum* (Manjeum), *Artocarpus heterophyllus* (Jack), *Termainalia* spp. (Maruthu), *Tectona grandis* (Teak). Central Bee Research and Training Institute in Pune, recommended wooden boxes of 10× 12.5× 10 cm size with a glass top covered by a wooden lid (Percy,1989), wooden hives of size 3×14×15 cm were found superior to bamboo hive and earthen pots (Raakhee, 2000) for rearing *T.iridipennis*. A wooden hive with a volume of 1960 cc was recorded as the most suitable size for hiving and division of stingless bee colonies in Kerala (AICRP, 2010). Contrarily Sureshkumar *et al*. (2012) recommended a bamboo pole bee hive developed by Kani tribes to keep the stingless bee, *T. iridipennis* in Tamil Nadu.

Observation on the per cent damage of stingless bee hives due to pests and diseases recorded from each district varied with the type of hive used. Considering the wooden boxes, the highest incidence of pests and diseases (7.93%) was recorded from Pathanamthitta district. Regarding mud pots and PVC pipes, the pests and disease damage was observed only in Pathanamthitta district (9.52 and 20.00 % respectively). No pests and diseases incidence was recorded from bamboo bits throughout the districts. This was the first attempt to record per cent damage of stingless bee hives due to pests and diseases hence no other work has been found so far in this regard.

Various designs of hive entrances were observed in different hives (wooden, mud pots, bamboo bits and PVC pipes) during the survey. Four types of hive entrances such as long tubular, short tubular, round and cryptic were observed. Majority of stingless beekeepers used wooden hives, hence the wooden hives are mainly considered. The number of short tubular entrance was dominant in all the districts which ranged from 18 to 28 except Pathanamthitta (14). The next dominant type was cryptic (22 nos) in Thiruvananthapuram district. The round type was dominant in Pathanamthitta (22 nos) while it ranged from 12 to 21 in other districts. Long tubular was more in Thiruvananthapuram (17) which is followed by Kottaym and Idukki (16 each), Kollam (14) and Pathanamthitta (9 nos). Roubik (2006) reported that the largest entrances were those made by certain *Trigona*, *Tetragona, Scaptotrigona*, *Homotrigona* and *Geotrigona* in Asia and the individual variation in nest entrance was due to environment factors, predators and parasites. The size of the hive entrance is the most fundamental feature since it determines the intruders, the entrance tube should be smaller or even closed to be more defensible against predators and robbers (Couvillon *et al*., 2008). The results of the present study conform to that of Lima *et al*. (2013) who observed different types of entrance tubes in feral colonies of stingless bees which were categorized into round, elliptical, slot shaped or irregular, short tube entrance, long tube entrance, camouflage or cryptic entrance (no tube) and toad mouth.

When length, width and number of guard bees were considered, short tubular hive entrances were dominant in all the four types of hives, the length and width of entrance tube in wooden hives were highest in Kottayam (4.63 cm and1.93 cm) followed by Pathanamthitta (4.02 and 1.49 cm). The number of guard bees (Fig. 3) was also highest in wooden hives of Kottayam (9.00 nos.) and Idukki (8.50 nos.). In the other three types of hives the length and width varied from 1.56 to 5.48 cm, 1.10 to 2.08 cm and the number of guard bees ranged from 6.00 to 8.00. Similar studies on the length and width of entrance tube of stingless bees *Trigona fuscobalteata* and *Trigona sapiens* from Philippines by Starr and

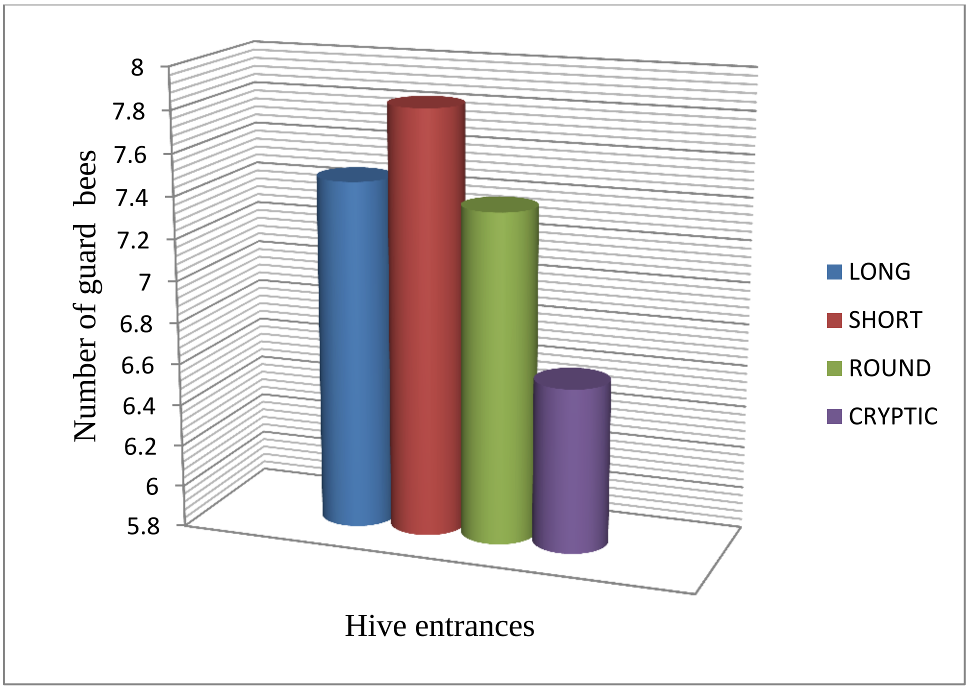


Fig.3. Number of guard bees in different types of hive entrances

Sakagami (1987) revealed that the tubes were simple and constructed with dark cerumen. The entrance tubes were of the range 1.10 and 1.30 cm in length 1.50 and 2.00 cm in width respectively. Crane (1992) reported that the flight entrance was lined with cerumen or propolis and the entrance tube was 6.25 mm length in *Trigona gribodi.* The length and width of entrance tube of stingless bee colonies was 96.55 and 3.32 mm in wall cavities and 108.80 and 5.08 mm in tree cavities (Danaraddi, 2007). He also reported that the length of entrance tube appeared to be species specific character and also may depend on the type of nesting site. The colonies of *T. iridipennis* kept in earthen pot in this study had shorter (50 mm) entrance tube. These earlier reports are in agreement with the results of the present study. Neotropical bee species of the genus *Nannotrigona*, *Tetragonisca*, *Scaptotrigona*, *Tetragona* and *Trigona* post six or more guard bees at their nest entrance. Positive correlation existed between the size of the entrance tube of the nest and the number of bee traffic into the nests had also been reported in Neotropical stingless bee species (Couvillon *et al*., 2008). Positioning of the guard bees inside the funnel around the inner entrance and the number of guard bees posted at the nest entrance are one of the mechanical defensive behaviour which was utilized by the *Meliponine* bees in the Kakamega forest of Kenya, to defend their nests from intruders (Kiatoko, 2012).

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 were generally used by stingless bees to construct different types of hive entrances. The observations taken from the five Southern districts revealed that majority of stingless bees collected waxes and resins from native trees *viz.*, *Mangifera indica* (Mango), *Artocarpus heterophyllus* (Jack fruit tree), *Anacardium occidentale* (Cashew), *Terminalia* spp. (Maruthu), *Cocos nucifera* (Coconut), *Calophyllum inophyllum* (Punna tree), *Garcinia gummi-gutta* (Kudam puli), *Artocarpus hirsutus* (Anjili). Bees of the genus *Plebia* were also known to use resin in their defense system which was reported by Patricia (2002). Similar findings by Roubik (2006) and Devanesan *et al*. (2009) reported that nest entrance may be constructed of wax or cerumen, other materials such as geopropolis, faeces and clay. The present study can be confirmed with that of Sureshkumar *et al*. (2012) who reported that stingless bees mix the plant resin (from Kongu, nangu, mango tree, jack tree) with wax to construct the entrance of the nest and also coat the resins over the hive to protect it from their enemies like ants and wasps in Tamil Nadu. It is also supported by Pavithra *et al*. (2013) who reported that the stingless bees used nest entrance closure materials such as resins, wood pieces, mud, stones and sand.

Observations on the behavior of entrance closure of hive showed that the stingless bees mostly close their entrance after the dusk. The entrance is closed using sticky materials such as resins and gums of plants to prevent the entry of predators and pests into the hive during night time. These findings are supported by Roubik (1989) and Nogueira- Neto (1997). Similar results were reported by Kiatoko (2012) where the presence of a protective barrier at the open entrance of the nest to guard against intrusion during the night was found in three stingless bee species *viz*., *M.boandei*, *M. lendliana* and *M. ferruginea* (reddish brown).

In the present study, the stingless bee hives were maintained at different heights from the ground level (3-5 ft, 6-8 ft, 9-11 ft and >12 ft) by the beekeepers. Majority of the hives were maintained at 9-11ft in Thiruvananthapuram (36%) and Kottayam (45%), whereas in Kollam (46%) and Idukki (65%) the hives were maintained at >12 ft. The observations made in the present study are in agreement with the findings of Khan and Srivastava (2013) who showed that the *Tetragonula laeviceps* mostly constructed the colonies on terrestrial shelters at height between 10 to 20 ft (52.30 colonies), 77 per cent were recorded in main trunk below 10 ft height from the ground level and nine per cent colonies were located at 20 to 30 ft height from the ground level in Pantnagar. Pavithra *et al*. (2013) revealed that stingless bees mostly preferred middle elevation range of 11 to 15 feet for nest building in Karnataka.

Considering the incidence of pests/ predators and diseases of stingless bee colonies maintained at different height from the ground level, none of the colonies recorded pests/predator or disease incidence at 3-5 ft height from the ground level; whereas in colonies maintained at 6-8 ft height, 4.69 per cent of colonies had insect pest incidence, 7.03 per cent of colonies had predator incidence and 2.54 per cent of colonies had disease incidence. The colonies maintained at 9-11 ft height from the ground level recorded a pest incidence of 5.10 per cent and predator incidence of 19.96 per cent. A predator incidence of 9.21 per cent was recorded in colonies maintained at 12 ft height from the ground level. Kajobe and Roubik (2006) studied nearly 200 feral stingless bee nests in trees in Uganda, in which mostly the nest predators (chimpanzees and humans) affected colonies were under seven meters height. Stingless bees nest in ground approximately from 20 to 200 cm, which had large colonies and resources for predators to consume (Roubik, 2006). He also stressed that the specific nests found to different faces of habitats at particular elevations provided protection against predators, wind, sun, parasites and symbionts.

Observations on the nest architecture and defense mechanism showed that the incidence of pests and diseases was meagre in bamboo hives. Even though bamboo hives were less prone to the attack of pests and diseases, majority of the beekeepers are using wooden hives for domesticating stingless bees since it is easier to handle during colony division, honey harvesting and periodical observations on need basis. Nisha (2002) endrosed the present study that the bamboo hives were more suitable for hiving *T. iridipennis* since the brood development, pollen storage, population build up and honey storage were superior in this type of hive. But the bamboo bits are often unavailable in many areas. Considering the height from the ground level, no incidence of pests, predators and diseases were recorded in the colonies maintained at 3-5 feet.

6. SUMMARY

6. SUMMARY

An investigation on 'Pests and diseases of stingless bees *Tetragonula* (*Trigona*) *iridipennis* Smith (Meliponinae: Apidae)' was conducted during the period 2012-14 under the aegis of the All India Co-ordinated Research Project (AICRP) on Honey bees and Pollinators, Department of Agricultural Entomology, College of Agriculture, Vellayani. The results of the study are summarized below.

A survey was conducted in five southern districts of Kerala *viz*., Thiruvananthapuram, Kollam, Pathanamthitta, Kottayam and Idukki. Two pests were recorded during the survey *viz*., dipteran fly *Hermitia illucens* from Pathanamthitta district and nitidulid beetle *Aethina* sp. from Kottayam and Idukki districts. The two predators recorded were reduviid bug *Acanthaspis siva* and assassin bug *Sycanus* sp. from Thiruvananthapuram and *Sycanus* sp. alone from Kollam district. Eight different predatory spiders were observed. They included cream crab spider *Thomisus projectus* which was recorded from all the five districts but the white crab spider *Thomisus lobosus* was recorded from Thiruvananthapuram and Pathanamthitta. Grey sphere spider *Neoscona nautica* was documented only from Pathanamthitta and Idukki whereas common wall jumper *Menemerus bivittatus* was observed from all the districts except Kollam. The common zebra jumper *Plexippus paykulli* was observed from Kottayam and Idukki. The two tailed spider *Hersilia savignyi* was recorded form Kollam and Idukki. The common house spider *Heteropoda venatoria* was recorded from Thiruvananthapuram, Pathanamthitta and Idukki. Box spider *Crossopriza lyoni* was documented from all the five districts.

The adult black soldier fly, *H. illucens* fly which thrived on decaying organic matter occasionally found entry into the stingless bee hives through the entrance tube, especially on weak colonies. The adult fly did not feed on the brood, pollen and honey pots but the larval stage of the pest damaged the colonies. The nitidulid beetle *Aethina* sp. damaged brood cells, pollen and honey pots.

Both the grubs and adults of beetle were observed in the rear portion of the brood, pollen, honey pots of decaying hive materials. They completed their life cycle inside the damaged hives.

The nymphs of the predator, reduviid bug *A. siva* camouflaged themselves by attaching bits of bee cadaver on to their body and remained close to the entrance tube of the colonies. The adults and nymphs of predator assassin bug *Sycanus* sp. were observed around the hives, crevices and on tree trunks in the vicinity of stingless bee hives. Eight different types of predatory spiders were observed from the hive entrance and foraging plants of stingless bee colonies.

Two fungal isolates of *Paecilomyces variotii* were obtained from Thiruvananthapuram district while a single isolate of *Aspergillus niger* was recorded from Pathanamthitta district. *P. variotii* was isolated from pollen pots, honey pots, propolis and on the walls of the affected hives. No dead bees were found in the hives from which *P. variotii* was isolated. The fungus was cultured on PDA and produced pale cream coloured colonies which appeared floccose and powdery due to dense production of conidiophores. The microscopic characters of *P. varotii* revealed dense and vertically arranged whorls or branches of conidiophores. Each conidiophore bears single to many hyaline to light yellow phialides, 30-100 µm in length. The fungus *A. niger* was isolated from dead bees lying inside the hive floor but it did not infect or contaminate the hive or its components. The fungus produced white mycelium on PDA, covered 9.0 cm in 12 days, conidiophores were hyaline and 500- 1000 µm in length. Thus the microscopic characters showed that the fungus possessed large globose conidial head (vesicle) and was dark brown in colour. Conidia were globose to subglobose 3-4 µm with rough surface.

The fungus isolated, after morphological characterization, was subjected to pathogenicity studies. The bees were found to be dead after 48 hr of inoculation with spores of *P. variotii* and *A. niger*. No visible mycelial growth was seen on the cadaver infected with *P. variotii*, whereas white fungal mycelium was visible on the bee cadaver with black pinhead like conidiophores arising from the cuticle in the case of bees affected with *A. niger*.

The population of total predators (spiders and insect predators) were observed at two periods (colony division - July to December 2013 and honey harvesting period - January to June 2014). The results showed that the mean population of total predator was highest during the colony division period (2.31) than honey harvesting period (2.04).

The study on the extent of damage of stingless bee hives showed that among 100 colonies surveyed only five colonies were damaged by *H. illucens* in Pathanamthitta district. Four colonies from Kottayam district and five colonies from Idukki district were found to be infested by *Aethina* sp.

The average honey yield from healthy stingless bee colony ranged from 403.33 g to 600.00 g while that of infested colonies ranged from 15.00 g to 27.00 g throughout the districts surveyed. Pests and disease incidence was not recorded from Kollam district hence the per cent reduction in honey was nil. The mean percentage reduction in honey yield in the other districts ranged from 93.93 to 96.76.

Nine different quality parameters of stingless bee honey (healthy and infested samples) such as colour, acidity, moisture, electrical conductivity (EC), hydroxyl methyl furfural (HMF), sugars (sucrose and glucose), p^H, Diastase and invertase activity were analysed. The colour of honey samples showed different shades and variations from extra light amber to dark brown. All other quality parameters showed a significant difference between healthy and infested samples when analysed using t- test. The parameters such as HMF, diastase, invertase showed wide variations between healthy and infested samples.

The type of hives used by bee keepers to maintain stingless bees were studied and majority of the beekeepers maintained stingless bee colonies in wooden boxes (70.20 %) and mud pots (18.60 %). Others used hollow bamboo bits (9.20 %) and PVC pipes (2.00 %).

The per cent damage of stingless bee hives due to pests and diseases recorded from each district varied with the type of hive used. In the case of wooden boxes, 7.93 per cent were damaged by pests and diseases in Pathanamthitta district. It was followed by Idukki (7.57 %), Kottayam (6.77 %) and Thiruvananthapuram (3.48 %). Regarding mud pots and PVC pipes, the pests and disease damage was observed only in Pathanamthitta district (9.52 and 20.00 % respectively). No pests and diseases incidence was recorded from bamboo bits from anywhere in the five districts.

Four types of hive entrances were observed in different hives during the survey *viz.*, long tubular, short tubular, round and cryptic. Short tubular type of hive entrance was the dominant type of hive entrance in the case of wooden hives, mud pots and bamboo hives and its range varied in each district.

The length and width of entrance tube in wooden hives with short tubular entrance were highest in Kottayam 4.63 cm and 1.93 cm followed by Pathanamthitta 4.02 and 1.49 cm. The number of guard bees was also highest in Kottayam (9.00 nos.) and Idukki (8.50 nos.). In mud pots, the maximum length (4.65 cm) and width (1.80 cm) of short tubular entrance tube were recorded from Kollam (4.65 and 1.80 cm) followed by Kottayam (4.13 and 1.60 cm). The highest number of guard bees were also recorded form Kollam (7.66) and Kottayam (7.53). In bamboo hives, the length and width of short tubular type of hive entrance tube was the highest in Idukki (5.50 and 1.50 cm) and Thiruvananthapuram (5.30 and 1.40 cm). The maximum number of guard bees were also recorded from Thiruvananthapuram (8.00 nos.) and Idukki (7.00 nos.). Considering PVC pipes, short tubular entrance was observed only from Pathanamthitta where the length, width and number of guard bees were 3.20 cm, 1.10 cm and 8.00 nos. respectively.

The stingless bees constructed the 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 common trees such as *Mangifera indica* (Mango), *Artocarpus heterophyllus* (Jack fruit tree), *Anacardium occidentale* (Cashew), *Terminalia* sp. (Maruthu), *Cocos nucifera* (Coconut), *Calophyllum inophyllum* (Punna tree), *Garcinia gummi-gutta* (Kudam puli), *Artocarpus hirsutus* (Anjili).

Hive entrance closure was observed in strong and healthy colonies with enough number of worker bees. The entrances were closed during dusk and opened during dawn using sticky materials such as resins and gums of plants to prevent the entry of predators and pests into the hive during the night time.

The stingless bee hives were maintained at different heights from the ground level (3-5 ft, 6-8 ft, 9-11 ft and >12 ft) by the beekeepers. Majority of the hives were maintained at 9-11ft in Thiruvananthapuram (36%) and Kottayam (45 %), whereas in Kollam (46 %) and Idukki (65 %) the hives were maintained at >12 ft. Observation on the incidence of pests/predators and diseases in stingless bee colonies at different height from the ground level showed that, the highest incidence of pests (5.10 %) and predators (19.96 %) were observed at 9-11 ft above ground level whereas disease incidence was highest in boxes at 6-8 ft. None of the colonies maintained at 3-5 ft height from the ground level had pests/predator or disease incidence.

Based on the results of the study, two pests, two pathogens, two insect predators and eight spider predators were observed to cause damage to stingless bees. Observations on the nest architecture and defense mechanism showed that the incidence of pests and diseases was meagre in bamboo hives. Even though bamboo hives were less prone to the attack of pests and diseases, majority of the beekeepers used wooden hives for domesticating stingless bees since it is easier to handle during colony division, honey harvesting and periodical observations on need basis. Considering the height from the ground level, no incidence of pests, predators and diseases were recorded in the colonies maintained at 3-5 feet. Thus, for scientific meliponiculture, the colonies can be domesticated in wooden hives, it is to be placed at 3-5 feet above ground level which facilitate easy handling with least incidence of pests, predators and diseases which in turn will help in the appreciable yield of honey and augment pollination of flora.

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APPENDIX-I

KERALA AGRICULTURAL UNIVERSITY

COLLEGE OF AGRICULTURE

Vellayani, Thiruvananthapuram

SURVEY SHEET

Topic : Pests and Diseases of Stingless Bees *Trigona iridipennis* Smith (Meliponinae: Apidae)

1. Name of the bee keeper :

2. Full address and contact no. :

3. Experience in beekeeping (Years) :

4. No. of hives maintained :

5. Location at which hives are kept (Farm, Homestead, Orchard etc.) :

6. Type of hives/materials used for construction of hives (Wood, Mud pot, Bamboo etc.) :

7. Shape and size of hive :

8. Type of hive entrance tube :

9. Length and width of hive entrance tube :

10. Height from ground level at which the hives are maintained :

11. No. of pest/disease infected hives
(Explain in detail about the infection) :

12. Strength of colony before and after infection :

13. Season in which pest/disease infection is
severe (honey flow/lean season) :

14. Characters of honey from infected hives :

15. Other details regarding the hives
(if necessary) :

Place :

Date : Signature of the Beekeeper

APPENDIX-II

COMPOSITION OF MEDIA USED

1. Potato Dextrose Agar (PDA)

Peeled and sliced potatoes	- 200 g
Dextrose ($C_6H_{12}O_6$)	- 20 g
Agar-agar	- 20 g
Distilled water	- 1000 ml

Potatoes were boiled in 500 ml of distilled water and the extract was collected by filtering through a muslin cloth. Agar-agar was dissolved separately in 500 ml of distilled water. The potato extract was mixed in the molten agar and 20 g of dextrose was dissolved in to the mixture. The volume was made up to 1000 ml with distilled water and medium was sterilized at 15 psi and 121 ºC for 15 min.

2. Potato Sucrose Agar (PSA)

Peeled and sliced potatoes	- 200 g
Sucrose	- 20 g
Agar-agar	- 20 g
Distilled water	- 1000 ml
Agar-agar	- 16 g
Distilled water	- 1000 ml

PESTS AND DISEASES OF STINGLESS BEES

***Trigona iridipennis* Smith (Meliponinae: Apidae)**

JAYALEKSHMI. C. R

(2012-11-172)

Abstract of the thesis

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Faculty of Agriculture

Kerala Agricultural University

Department of Agricultural Entomology

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ABSTRACT

The study entitled "Pests and diseases of stingless bee *Trigona iridipennis* Smith (Meliponinae: Apidae)" was conducted with the objective to study the pests and diseases associated with the stingless bees, their documentation, assessment of their damage and yield loss during the period 2013-14 at the AICRP on Honey bees and Pollinators, Department of Agricultural Entomology, College of Agriculture, Vellayani.

A survey was conducted in five Southern districts of Kerala *viz*., Thiruvananthapuram, Kollam, Pathanamthitta, Kottayam and Idukki. The pests of stingless bees documented were *Hermitia illucens* (Pathanamthitta) and *Aethina* sp. (Kottayam and Idukki). Predators recorded were *Acanthaspis siva* (Thiruvananthapuram) and *Sycanus* sp. (Kollam). Eight different predatory spiders, *Thomisus projectus, Thomisus lobosus, Neoscona nautica, Menemerus bivittatus, Plexippus paykulli*, *Hersilia savignyi*, *Heteropoda venatoria* and *Crossopriza lyoni* were recorded. Two pathogens *viz.*, *Paecilomyces variotii* and *Aspergillus niger* were also observed.

Out of the 100 stingless bee colonies surveyed, only five colonies were infested with *H. illucens* in Pathanamthitta district whereas *Aethina* sp. was observed in four and five stingless bee colonies each from Kottayam and Idukki districts. The reduction in honey yield due to pests and diseases ranged from 93.93 to 96.76 per cent. Nine physiochemical parameters of stingless bee honey were observed, among which wide variation was observed in hydroxy methyl furfural and diastase activity between healthy and infested samples.

Regarding the nest architecture and defense mechanism of stingless bees, out of the four types of hives, majority of the beekeepers maintained stingless bee colonies in wooden hives (70.20 %) and mud pots (18.60 %). The incidence of pests/predators and diseases in stingless bee colonies showed that bamboo bits were free from pests and diseases incidence in all districts. In the case of wooden hives, short tubular hive entrance was dominant in all the districts. The length and width of entrance tube and number of guard bees in the short tubular type of

entrance ranged from 3.74 to 4.63 cm, 1.49 to 1.93 cm and 6.91 to 9.00 nos. respectively. The materials used for constructing the hive entrances included natural materials *viz.*, resins, waxes, tiny chips of wood, saw dust, sand, bee cadavers, leaves and vegetative parts, cerumen, wood and bark of trees. The hive entrance was closed after the dusk to prevent the entry of predators and pests into the hive during night time. The stingless bee hives were maintained at different heights from the ground level (3-5 ft, 6-8 ft, 9-11 ft and >12 ft) by the beekeepers. The hives maintained at 9-11ft above the ground level recorded the highest incidence of pests (5.10%) and predators (19.96%) whereas disease incidence was highest in boxes at 6-8 ft. None of the colonies maintained at 3-5 ft height from the ground level had pests/predator or disease incidence.

Based on the results of the study, two pests, two pathogens, two insect predators and eight spider predators were observed to cause damage to stingless bees. Observations on the nest architecture and defense mechanism showed that the incidence of pests and diseases was meagre in bamboo hives. Even though bamboo hives were less prone to the attack of pests and diseases, majority of the beekeepers used wooden hives for domesticating stingless bees since it is easier to handle during colony division, honey harvesting and periodical observations on need basis. Considering the height from the ground level, no incidence of pests, predators and diseases were recorded in the colonies maintained at 3-5 feet.