

**BIOECOLOGY OF SMALL HIVE BEETLES AND
ASSESSMENT OF THEIR DAMAGE IN STINGLESS BEE
COLONIES**

by

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(2015-11-044)

THESIS

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DECLARATION

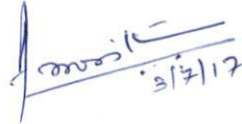
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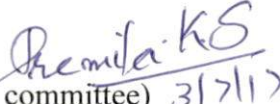
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LIST OF ABBREVIATIONS AND SYMBOLS USED

$^{\circ}\text{C}$	Degree Celsius
%	Per cent
cm	Centimetre
Cm^3	Centimetre cube
<i>et al.</i>	And others
Fig.	Figure
KAU	Kerala Agricultural University
No.	Number
Sl.	Serial
sp. or spp.	Species (Singular and Plural)
<i>viz.,</i>	Namely
Nos.	Numbers
GQ	Good quality
BQ	Bad quality
TVM	Thiruvananthapuram
KLM	Kollam
PTA	Pathanamthitta
>	greater than
<	Less than
$^{\circ}\text{E}$	Degree East
$^{\circ}\text{N}$	Degree North

P	primary
S	Secondary
N	Nectar
P	Pollen
FN	Fortnight
AICRP	All India Co-ordinated Project
PCR	Polymerase Chain Reaction
SD	Standard deviation

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Introduction

1. INTRODUCTION

The Indian stingless bee or dammer bee, *Tetragonula (Trigona) iridipennis* Smith was first described by Frederick Smith (1854) from Ceylon in Sri Lanka. They are black, tiny and smallest of honey producing bees. They lack a functional sting, hence known by the name 'stingless bees'. Their protective action against enemies is mainly through mandibles. The generic name '*Trigona*' refers to their triangle shaped abdomen and '*iridipennis*' refers to their iridescent wings. They are diverse group of social insects and exhibit complex eusocial behaviour of existence in a large perennial colony. Concealed places, rock crevices, partially exposed places and building foundations are the natural domicile of these bees. The colony consists of different castes viz., workers, drones and a queen. Among which worker caste undertake a complex division of labour.

The products from stingless bee hives are widely used as both food and medicine. Stingless bee honey is highly medicinal because of its antioxidant and antibiotic properties. This honey has been named as the 'elixir of life' considering it's nutritional and health benefits conferred to mankind. Ancient records showed that the honey is utilized to cure wound, intestinal infection, visual disorders, ulcers and kidney disorders. Stingless bee produce limited quantities of dense, darkish, sour honey up to a maximum of 100 ml colony⁻¹ (Ramanujam *et al.*, 1993). Though the honey produced from a single hive is comparatively lesser, it has great demand and fetches high price in the commercial market.

Stingless bees are ecologically important because of their role in pollination of tropical flora. They are considered as main pollinating agents in tropical crops and medicinal plants. Due to its small size, they are well adapted for visiting even very small flowers that are not visited by other bees, thus contributing more to subsistence of a well-balanced ecosystem. Globally, around 250 crop species are benefited by the vital pollination service provided by them (Roubik, 1992).

In Kerala, stingless bees are reared as a backyard beekeeping practice mainly for honey production. Rearing of stingless bees in every homestead will assist in yield enhancement and thus contribute more to food safety. The natural feral colonies found in tree and wall cavities can be domesticated in different types of hives *viz.*, wooden box, mud pots, bamboo hives and coconut shells were used by the bee keepers of the state. Presently, the bee keeping industry of the state is facing challenges due to a number of factors *viz.*, non-judicious application of pesticides, pest and diseases, climate change, habitat loss and fragmentation, mobile tower radiations and other anthropogenic activities (AICRP, 2004). Research reports on pest infestation in stingless bee colonies of Kerala were meagre. Smaller size of the bees and the concealed hive habitat might be the possible reason for it. Recently, the incidence of certain pest problems drained the momentum of stingless bee keeping in the state.

A dipteran fly *Hermetia illucens* L. and mite *Amblyseius* spp have already been reported as pests that attack the stingless bee colonies of Kerala (Nisha, 2002). Recently, a nitidulid beetle is reported to infest stingless bee colonies in Kerala where the adult female beetles lay eggs inside the hive and the grubs feed on the brood, pollen and honey in the colony thus causing complete destruction of colonies (Devanesan *et al.*, 2014). The incidence of small hive beetle also reported from stingless bee colonies in southern districts *viz.*, Kottayam and Idukki of Kerala (Jayalekshmi, 2015). Being an emerging pest, the incidence, biology, bioecology and assessment of damage by small hive beetle is still an unexplored area. Hence studies on the biology as well as the extent of damage caused by the hive beetle are imperative for its future line of research.

In this context, the present investigation was conducted with the following objectives:

- Estimation of small hive beetle incidence in southern Kerala
- Bioecology of small hive beetle
- Assessment of population and damage caused by small hive beetle

Review of Literature

2. REVIEW OF LITERATURE

The review of literature pertaining to the bioecology of small hive beetles and assessment of their damage in stingless bee colonies are presented in this chapter.

2.1. SMALL HIVE BEETLE INCIDENCE IN STINGLESS BEE COLONIES

Kerala is blessed with rich floral diversity and congenial climatic conditions, which offers immense scope for beekeeping in the state. The Western Ghats mountain range in Kerala encompasses the vast majority of bee flora (Devanesan *et al.*, 2014).

The art and science of rearing of stingless bees is known as meliponiculture. As far as Kerala's biodiversity is concerned, meliponiculture has ample scope in our state, because of its different agroclimatic conditions. In recent years, stingless bee honey production gained wide importance due to its high medicinal quality. It has antioxidant and antibiotic properties which help in healing wounds and infections and also cures digestive, respiratory and eye diseases (Vit, 1992). It is often stated that stingless bees are important pollinators of crops in tropical and subtropical parts of the world (Roubik, 1992).

2.1.1. Stingless Bee or Dammer Bee, *Tetragonula (Trigona) iridipennis* Smith

Stingless bees are black, tiny and smallest of honey producing bees. They are mainly seen in tropical and subtropical parts of the world. They naturally inhabit in concealed places, rock crevices, partially exposed places and building foundations. They exhibit complex eusocial behaviour and a colony consists of different castes *viz.*, queen, workers and drones.

The taxonomic position of stingless bees (Willie, 1983)

Super Family : Apoidea
Family : Apidae

Sub family : Meliponinae
 Tribe : Trigonini

The Indian stingless bee or dammer bee, *Tetragonula (Trigona) iridipennis* was first described by Frederick Smith (1854) from Ceylon in Sri Lanka (Smith, 1954). They possess vestigial sting and hence, their protective action against enemies is mainly through mandibles.

Stingless bees are oldest among the honey bees and are taxonomically different from other groups (Sakagami, 1978). The bumble bees (Bombinae), honey bees (Apinae) and stingless bees (Meliponinae) belong to the family Apidae. Two genera namely *Trigona* and *Melipona* were described under the tribe Meliponini. The genus *Trigona* is the biggest and most widely distributed genus with 130 species under ten sub genera. On the other hand, genus *Melipona* consists of 50 species and is mostly confined to the Neotropics. All Asian and African stingless bees belong to the tribe Trigonini. The important genera under this tribe include *Trigona*, *Plebeia*, *Tetragonula* and *Nanotrigona* (Camargo *et al.*, 1988)

Sakagami (1978) reported that the stingless bee belonging to the genera *Trigona* are most common in India. *T. iridipennis* is the most common stingless bee that are found in Kerala (Raakhee and Devanesan, 2000), Tamil Nadu and Karnataka (Swaminathan, 2000). Later on, Rasmussen and Cameron (2010) identified that genus *Trigona* are restricted to Neotropics and they are distantly related to Indo-Malayan/ Australasian groups of stingless bees. Hence stingless bees present in the Indo- Malayan/ Australasian taxa were transferred in to genus *Lepidotrigona* Smith and *Tetragonula* Moure (Michner, 2013). Thus the name of Indian stingless bee *Trigona iridipennis* was changed into *Tetragonula. iridipennis*. The genus *Tetragonula* include more than 150 stingless bee species, out of which *T. iridipennis* , *T. benghalensis* Cameron, *T. praeterita* Walker, *T. leaviceps* Smith,

T. ruficornis Smith and *Lepidotrigona arcifera* Cockerell are common in Indian subcontinent.

Stingless bees naturally inhabit in protective cavities, concealed places, rock crevices, partially exposed places and building foundations to ensure a safety condition. A typical stingless bee nest usually consists of an entrance with or without an external tube, resin dump, waste dump, brood cells, pots for storing pollen and honey, and nest envelopes involucrum (structure of nests of stingless bee formed by sheets of cerumen) and batumen (mixture of wax and resins that they used to build their nest entrance) (Pooley and Michener, 1969). The brood cells, honey pots and pollen pots are arranged separately and are made of cerumen which is a mixture of wax and resin. Worker bees at first construct pillars of wax clusters that provide a base to the brood cells. The cells of pots will be in contact with one another, otherwise connected by small pillars or connectives of soft cerumen (Nisha, 2002; AICRP, 2004).

2.1.2. Pests of Stingless Bees

Pest incidence in stingless bee colonies was comparatively less when compared to other honey bee species due to its confined and concealed nest architecture. The following groups of pests are reported from different parts of the world.

2.1.2.1. Coleopteran pests

Leonard (1983) reported infestation of coleopteran pest such as *Cetonia apataca* L. and *Cetonia morio* L. in Maryland, USA. The larvae of the beetle enter the hive and feed the honey and thus damage the hive through tunneling the wax. Similarly, larvae and adults of a few *Brachypeplus* spp. (F. Nitidulidae) infested the *Tetragonula* species of stingless bees in Australia by feeding wax and honey in the

colony (Hebeck, 2002). Apart from this, a number of nitidulid beetles such as *B. auritus* Murray, *B. basalis* Erichson infested the hives of stingless bee species viz., *Meliponula bocandei* Cockerell, *M. ferruginea* Cockerell (reddish brown), *M. ferruginea* Cockerell (black) and *M. lendliana* Friese. He also documented *Rhizoplatys mucronatus* Beauvois (Coleoptera: Scarabaeidae) and *Tenebroides mauritanicus* Linne (Coleoptera: Tenebroididae) from the colonies of stingless bee species *M. bocandei* and *M. ferruginea* respectively from Kenya forest.

Krishnan *et al.* (2015) reported the infestation of nitidulid beetle *Epuraea (Haptoncus) luteolus* Erichson in multiple colonies of stingless bee species, *Trigona thoracica* Smith, *Heterotrigona itama* Smith and *Tetragonula laeviceps* Smith in Malaysia. The beetle complete its entire life cycle within the nesting material by consuming brood, honey and pollen pots.

Recently, a nitidulid beetle was reported to infest stingless bee (*T. iridipennis*) colonies in Kerala, the adult female beetles lay eggs inside the hive and the grubs feed on the brood, pollen and honey in the colony thus causing complete destruction of colonies (Devanesan *et al.*, 2014). Jayalekshmi (2015) also reported the incidence of a small hive beetle which infests stingless bee colonies in Kottayam and Idukki districts of Kerala.

2.1.2.1. 1.Small hive beetle

The small hive beetle, *Aethina tumida* Murray was first described by Murray in 1867 from Sub Saharan Africa (Murray, 1867). It belongs to the coleopteran family Nitidulidae, which comprises of about 2,800 described species in 172 genera all over the world. The important characters of hive beetle of family nitidulidae are three-segmented antennal club, small fourth tarsi, grooved metacoxae, dilated tarsal segments, and transverse procoxal cavities (Habeck, 2002). It is called by the name

small hive beetle to distinguish from other big beetle (*Hyplostoma fuliginous* Walker), which infested the honeybee colonies of South Africa.

Global trade of honey bee and other hive products transported small hive beetle from Africa to many parts of the world. Since then small hive beetle devastated many *Apis mellifera* colonies in Canada, Australia, USA, Egypt and UK. In its native range of Africa, *A. tumida* was reported as a colony scavenger. European subspecies of honey bee was reported to be more susceptible to small hive beetle than African sub species (Neumann and Ellis, 2008).

2.1.2.1.1.1. Host range

The beetles of family nitidulidae were reported as predators, herbivores and scavengers. As they feed on fresh, rotten and dried fruits, fermenting plant juices, honeybee colonies and rarely seen on flowers (Lin *et al.*, 1992; Fadamiro *et al.*, 1998; Hepburn and Radloff, 1998; Smart and Blight, 2000; Wolff *et al.*, 2001). Therefore, they are often accounted by the name 'sap beetles'. Some members of these nitidulids are seen in close association with honey bee colonies, especially in the weak colonies.

2.1.2.1.1.2. Nitidulid beetles associated with stored grains and fruits

Nitidulid beetles of the genus *Carpophilus* has been reported as a pest of dried fruits and stored grains. Dowd and Bartlet (1991) described nitidulid beetle *Carpophilus hemipterus* (L.) (dried fruit beetle) which is a cosmopolitan pest of fresh and dried fruit, as well as seeds, drugs, spice and stored grains. *C. dimidiatus* (corn sap beetle) is another member of this genus and is reported as a common pest of stored grain in China (Dunkel *et al.*, 1983). Mostafa and Williams (2002) stated that the beetle (*A. tumida*) is associated with a number of fruits such as cantaloupe melon, grape fruit, avocado, strawberry, papaya, apple, mango, orange and pineapple and it completes its life cycle in these fruits. Ellis (2004) asserted that the beetle has opportunistically transferred to honey bee colonies in the absence of decaying fruits.

2.1.2.1.1.3. Nitidulid beetles associated with the stingless bees

Small hive beetle (*A. tumida*) was reported to infest Australian stingless bee (*Austroplebeia* and *Trigona*) colonies and stingless bees (*Dactylurina staudingerii* Gribodo) in West Africa (Mutsaers, 2006). Recently, Pena *et al.* (2014) reported the infestation of *A. tumida* from wild and managed stingless bee *Melipona beecheii* Bennett colonies in Cuba. They could identify that seven hives were infested with small hive beetle out of the 258 surveyed. From Korea, Park *et al.* (2014) reported mycetophagous nitidulids (*Aethina suturalis*), which feed on fungi. Jayalekshmi (2015) reported the incidence of small hive beetle, *Aethina* spp from the stingless bee colonies of Kerala.

2.1.2.1.1.4. Nitidulid beetles associated with the genus *Apis*

Other than *A. tumida*, nitidulid beetles of different genus are often associated with honey bee colonies as harmful parasites or harmless associates. Lundie (1940) reported nitidulid beetle, *A. tumida* as a parasite and scavenger of *A. mellifera scutellata* colonies of Sub Saharan Africa. Hinton (1945) reported another nitidulid beetle *Carpophilus dimidiatus* seen in pollen cells of *A. mellifera* colonies. Torto *et al.* (2007) asserted that *A. tumida* infected colonies attract other nitidulid beetles as secondary infestation in fermenting hive products.

Neumann and Ellis (2008) reported the infestation of nitidulid beetle *Cychramus luteus* Fabricius from *Apis mellifera* colonies in Germany. Moreover, a number of nitidulids are also being reported from Central America by Ellis and Delaplane (2008). *Lobiopa insularis* Laporte, *Epuraea corticina* Erichson, *Glischrochilus fasciatus* Olivier and *C. dimidiatus* are those nitidulids reported by them. Adults of *G. fasciatus* documented from colonies already infested with *A. tumida* in Georgia, USA. The beetle was capable to reproduce in pollen, brood and honey in the colony. The larvae of the beetle were mycetophagous and were never associated with honey bees.

Dasgupta *et al.* (2016) asserted that *L. insularis* and *Epuraea corticina* were found less frequently in bee colonies. High incidence of *L. insularis* is reported from an apiary in South Carolina, USA, which was very near to a tomato field, whereas *E. corticina* seen in pollen patties (sugar/water +soy-based product used as a bee food) which was placed under the lids of hives, never on the colony debris. They could gather the specimens of *E. corticina* also from fermenting brown sugar and fruit traps that were placed in the apiaries of South Eastern USA.

2.1.2.1.1.5. Diagnosis of small hive beetle incidence in the apiaries

Certain methods have been identified for the effective diagnosis of small hive beetle, since it became an invasive pest. According to Spiewok *et al.* (2007), visual inspection of colonies for the presence of hive beetle was a primary effective method for diagnosis. Typical rotten smell due to fermentation of honey was also a characteristic feature of small hive beetle infested apiaries (Schafer and Ritter, 2014).

2.1.2.1.1.6. Seasonal incidence of hive beetle

Literatures indicated that the seasonal variation plays an important role in hive beetle reproduction. According to Rhodes and McCorkell (2007), the peak period of small hive beetle was in October. From USA, Arbogast *et al.* (2010) reported that the peak period of infestation of small hive beetle (*A. tumida*) was during May and June (spring and summer months). A dissimilar observation was made by Torto *et al.* (2010) from Kenya, where they could trap small hive beetles abundantly during the rainy season.

2.1.2.2. Dipteran pests

An endoparasitic phorid fly *Mealonctia sinistia* Borgmeier (Diptera, Phoridae) is described as a pest from the stingless bee (*Nannatrigona (Scaptotrigona) postica* Latreille) nest in Brazil (Simoes *et al.*, 1980). Phorid fly *Megaselia scalaris*

Leow is reported as a pest from all the five stingless bee colonies in Kakamega forest, Kenya (Nkoba, 2012).

It was asserted by Disney and Bartareau (1995) that phorid fly *Dohrnipora* associated with stingless bee *T. carbonaria* in Queensland feed on pollen storage within the hive. Studies conducted by Robroek *et al.* (2003) in El Salvador and Costa Rica reported the infestation of a phorid fly *Pseudohypocera kerteszi* in the nest of stingless bee *Melipona beecheii* Bennett. Adult parasitic phorid fly enter the stingless bee nests and reproduce inside the hives, thus causing complete damage of the colony. Hence it was considered as the major pest of stingless bee in Central America

A scavenger fly (Black soldier fly) *Hermetia illucens* Linnaeus recognized as a potent pest of stingless bee (*T. irridipennis*) colonies in Kerala. Adult fly enter the hives and deposit their eggs in it, emerging sluggish larvae feed massively on the brood cell, pollen pots, honey pots and cerumen deposits (Nisha, 2002; Jayalekshmi, 2015).

2.1.2.3. Mite pests

Delfinado- Baker and Beker (1985) documented a new species of mite from stingless bee hive in Malaysia, the mite belong to the genus *Neocypholaelaps* (Ameroseiidae) which mainly infested the colony as a pollen feeder. They also described and illustrated three mite species, *Eumellitiphis inouei* Turk, *Eumellitiphis philippinensis* Turk, and *Eumellitiphis mellitus* Turk from *Trigona* nest in Sumatra, Malaysia and Philippines.

Studies conducted by Nisha (2002) documented the infestation of a predatory mite *Amblyseius* in bamboo hives of stingless bees in Kerala. Vijayakumar and Jayaraj (2013) reported the infestation of parasitic mite *Pymotes* spp on *T. iridipennis* colonies from Tamil Nadu. The parasitic mites which survive on the intersegments of queen bee as well as brood cells, weakened the immune system and thus resulted in a

queenless condition inside the stingless bee hive. Similarly, other mite species *Carpoglyphus lactis* Linnaeus was also reported from stingless bee colonies of Tamil Nadu. The mite mainly infested the pollen store and successively spreaded over the brood cells. The pollen pots were damaged and completely powdered by mite infestation. Within a period of one month complete destruction of the colony was noted (Vijayakumar *et al.*, 2013).

2.2. BIOECOLOGY OF SMALL HIVE BEETLE

The life cycle of *A. tumida* may also occur in stored bee products (Lundie, 1940), alternate hosts and on alternate food sources such as fruits (Ellis *et al.*, 2002). The small hive beetle undergoes complete metamorphosis by passing through different life stages *viz.*, egg, larva, pupa and adult.

2.2.1. Eggs

The eggs of *A. tumida* are pearly white or creamy white in colour, arcuate, approximately 1.4 mm long and 0.26 mm wide. The eggs were found smaller than honey bee eggs, although it is similar in appearance with honey bee eggs (Lundi, 1940). He stated that the egg period of *A. tumida* is generally 3 days, ranges up to 6 days, whereas Schmolke (1974) reported that no more larvae emerged after 52 h even if incubation was continued for some more period. Somerville (2003) investigated the egg laying capacity of adult female and concluded that they were capable of laying 1000-2000 eggs during its life time. Jayalekshmi (2015) reported that egg period of *Aethina* spp as 2-3 days.

2.2.2. Larvae

The larvae of *A. tumida* were creamy white, oiligopodous and emerge through longitudinal slits made at the anterior part of the egg and reported as the main damaging stage in honey bee colonies. They were grown up to 10-11 mm in length after the completion of four recognizable larval instars. The chief distinguishing

character of small hive beetle larvae was the presence of six anterior legs and numerous dorsal rows of spines on the body. This feature safeguards them from being smothered in honey. On the rear end of dorsum of larvae, two paired spines were present, which distinguish them from wax moth larvae *Galleria mellonella* Linnaeus (Jayalekshmi, 2015).

The distinct feeding phase might last up to 29 days in some cases as reported by Schmolke (1974). The larval stage reported to have distinct feeding and post feeding phase (wandering stage). Hatching larvae soon after emergence begins to feed the available food sources (honey, pollen and brood) Once the feeding phase is completed by the larvae, post feeding phase (wandering stage) starts, where they leave the food source and migrated out of the colony for pupation in soil. The larvae can sustain in a post feeding phase for a period of 48 days without food and water (Hood, 2000).

2.2.3. Pupal Stage

The pupae were pearly white in colour with distinctive projection on thorax and abdomen. During the later stages of pupation, pigmentation starts to appear on eyes and underside of the wing buds (Lundi, 1940). Studies conducted in South Wales showed that the small hive beetle fed with pollen, torula yeast and honey, the pupal period ranges from 13-25 days (Haque and Levot, 2005). The pupal stages of small hive beetle which infest the genus *Apis* is mainly seen in soil, whereas the pupal stages of *Aethina* spp. which infest stingless bee (*T. iridipennis*) seen in the hive itself (Jayalekshmi, 2015).

As reported by Schmolke (1974), on the distal ventral portion bulbous projection is present for female pupae. Whereas, bulbous projection is absent for male pupae, which enabled the sex determination of pupae.

2.2.4. Adult

Ellis *et al.*, (2002) described the morphometric characters of adult small hive beetles (*Aethina tumida*) in USA. Adult small hive beetles were oval in shape, 5-7 mm in length and 3.2 mm width. Soon after emergence they appeared to be yellowish brown, but later on, changed to brown. The head, thorax and abdomen of the beetle are well separated, elytra is not covering abdomen completely and antennae ends in distinctive 3 segmented club.

2.2.4.1. Sex determination of adult

Male and female small hive beetle (F. Nitidulidae) could be distinguished by the method developed by Schmolke (1974). He reported that by holding the adult beetle in between thumb in such a way that ventral side of abdominal tip was visible. On gently squeezing the beetle abdomen, the female beetle might extend its ovipositor whereas; male beetle protrudes its 8th tergite. In other words, the beetle which extends her ovipositor without much effort is likely female beetle.

2.2.4.2. Oviposition behaviour

Studies conducted by Ellis (2004) in USA showed that, one week after emergence from soil, adult beetle became sexually mature. While, Mustafa and Williams (2002) reported that sexual behaviours of the beetle reach its peak at 2-3 weeks after emergence from soil.

The exact mating behaviour of the beetle is unknown hitherto. Neuman *et al.* (2001) described that beetle lay eggs in irregular masses of 20-30 in number, in crevice or cavity inside the hive. Mating and oviposition of adult beetle can be induced by slight shaking of the jar, which hold the beetle. The female adult beetle was capable of laying approximately 2000 eggs during her life time.

Ellis and Delaplane (2008) conducted a study to separate small hive beetle eggs from hive content. They proposed microscopic slide technique by utilizing the oviposition behaviour of adult female beetle.

2.2.4.3. Longevity

Longevity of small hive beetle adults was reported to vary depending upon the diet they take. Pettis and Shimanuki (2000) revealed that adult small hive beetle can survive for five days without food or water thus they can be easily transported into new areas. Ellis (2004) studied the longevity and reproductive success of newly emerged *A. tumida* and reported that longevity and reproductive success of the beetle was significantly higher in honey fed diet among the different treatments (control - unfed, pollen, empty brood comb, bee brood, fresh Kei apples and rotten Kei apples). According to Somerville (2003), the longevity of adult beetle was up to 1-16 days in field condition, whereas in laboratory condition the longevity extended up to 16 months.

2.2.4.4. Other characters

The olfactory cues used by the adult beetle help them to find appropriate host colony. Odours from different hive products such as honey and pollen stimulate the olfactory receptors of adult beetle. Studies conducted by Schmolke (1974) reported that *A. tumida* is active before or just after dusk whereas, Ellis *et al.* (2003) stated that beetle is more active during evening time, due to the existence of circadian rhythm. The young beetles readily after emergence were active fliers and show strong phototaxis. Later on, they became less active and hide to rear part of the colony.

Studies conducted in USA reported that at an adequate environmental condition was required for hive beetle to complete its life cycle with in a period of 32-44 days (Lundi, 1940). According to Somerville (2003), under moderate climatic

conditions of USA and South Africa the total life cycle period of small hive beetle ranges from 4-6 weeks.

2.2.5. Abiotic Factors

The effects of abiotic factors such as temperature, rainfall, relative humidity on the survival and longevity of small hive beetle is reviewed below:

2.2.5.1. Temperature

De Guzman and Frake (2007) reported that temperature had a pronounced effect on the development, body size and weight of small hive beetle. Studies conducted at Weslaco, USA by Meikle and Patt (2011) showed that the temperature has profound impact on the egg, larval and pupal period of small hive beetle *A. tumida* that infest *A. mellifera* colonies. The egg period was 61 hours at a temperature of 21 °C, but in 35 °C it became 22 hours. Likewise pupal period was also found to decrease at higher temperature. They also reported that adult life span decreased with increase in temperature.

Studies conducted by Somerville (2003) in USA asserted that the survival rates of small hive beetle larvae rapidly decreased with dropping temperatures. All the larvae survived at temperatures above 30°C, whereas at 20°C less than half of the larvae survived, while at 10°C no larvae survived. A similar study stated that during cool weather, at temperatures less than 21°C, where the adult beetle became totally inactive.

Studies conducted by De Guzman and Frake (2007) in USA reported that larval development of *A. tumida* was influenced by different thermal regimes. Larval development seems to be faster at a higher temperature.

2.2.5.2. Light

Duehl *et al.* (2012) stated that small hive beetle larvae and adults were attracted to wave length of 390 nm. They also observed that the early instar larvae were not significantly attracted to light, while wandering larvae and adult exhibited strong positive phototaxis.

2.2.5.3. Relative Humidity

Annand (2011) reported that an optimum relative humidity greater than 65 per cent was required for rapid hatching of small hive beetle eggs. He also stated that relative humidity less than 34 per cent is detrimental to the survival of eggs. Cuthbertson *et al.* (2013) confirmed that relative humidity has profound effect on the viability of *A. tumida* eggs. Decrease in relative humidity reduced the viability of hive beetle eggs.

2.2.5.4. Rainfall

Studies conducted in Kenya stated that trap captures of small hive beetle in front of infested colonies was low throughout the year, but the infestation was abundant during rainy season. Eighty per cent of beetles were trapped during rainy period only (Torto *et al.*, 2010).

According to Annand (2011), in Australia, the small hive beetle followed a cyclic pattern, which peaked in rainy season then declined through winter, at a minimum in late spring. Jaylekshmi (2015) reported that hive beetle incidence in stingless bee colonies was in peak during rainy season (June-July).

2.2.6. Laboratory Rearing Of Small Hive Beetle

For standardizing efficient laboratory rearing of small hive beetle, numerous studies were conducted worldwide, using different diets.

Somerville (2003) described a viable technique for laboratory rearing of small hive beetle. The larvae of small hive beetle reared on plastic bags with pollen, brood and honey, top of the bag was kept open and placed over a transparent container filled with 12 cm deep sand. Holes were provided on the lid of the container for ventilation. They moistened the container inside the bag to maintain relative humidity as 60 per cent. Murrle and Neumann (2004) standardised an economical technique for the mass production of small hive beetle. A diet comprised of pollen, torula yeast and honey was fed to the larvae in the laboratory conditions. After completion of four recognizable larval instars, it was transferred into plastic cylinders of 9 cm diameter holding 15 cm of sandy soil (moist and loose).

2.2.7. Rearing Procedures

2.2.7.1. Mass rearing

Studies conducted by Keller (2002) in USA, showed that mass rearing of small hive beetle can be done in plastic containers of size 2×2×3 inches. In order to maintain a high relative humidity within the containers, placed a moistened cotton wool.

2.2.7.2. Individual rearing

Neumann *et al.* (2013) proposed individual rearing of small hive beetle for biological studies in eppendorf tubes with necessary diet requirement. The tubes were placed in laboratory trays in dark conditions. Punctures were also made in the lids to provide aeration

2.2.7.3. Alternative food sources used for laboratory rearing

Small hive beetles are able to exploit a wide variety of alternative food sources for laboratory rearing. Complete life cycle of small hive beetle can be achieved in

laboratory in a number of fruits such as kei apples (Ellis *et al.*, 2003); mango, banana, cantaloupe, pineapple and green grapes (Keller, 2002).

2.3. ASSESSMENT OF DAMAGE CAUSED BY SMALL HIVE BEETLE

2.3.1. Damage Caused To Different Honey Bee Colonies

2.3.1.1. *European Bee (Apis mellifera scutellata) Colonies*

Delaplane (1998) reported that the small hive beetle larvae which infest *Apis mellifera scutellata* (L.) colonies of USA, tunnel through the comb, feeding and defecating there by, resulted in the fermentation of honey. The fermented honey leaks out of the bottom board and resulted in the absconding of adult bees. They also reported that foul smell and slimy residue was evident from the infected hive.

2.3.1.2. *African bee colonies (Apis mellifera capensis)*

Lundi (1940) stated that in its native range of Africa, small hive beetle considered as minor pest which causes very little damage to the *Apis mellifera capensis* (L.) colonies. Elzen *et al.* (2000) reported that the defense behaviour of African bee colonies was so prominent that they were able to maintain the hive beetle population below economic threshold.

2.3.1.3. *Stingless bee colonies*

Nitidulid beetle, *Epuraea (Haptoncus) luteolus* Erichson from multiple stingless bee species *Trigona thoracica* Smith, *Heterotrigona itama* Smith and *Tetragonula laeviceps* Smith in Malaysia reported to cause severe damage in the colonies by consuming brood, honey and pollen pots (Krishnan *et al.*, 2015).

Jayalekshmi (2015) reported that the grubs of *Aethina* spp. severely damage the colonies of stingless bee (*T. iridipennis*) colonies of Kerala. The larval stage of the beetle tunnels through the honeypot, brood cell and pollen pots, feeding and

defecating there by, resulted in fermentation and spoilage of honey. She also reported that in severe case of infestation the bees absconded from the colonies.

2.3.1.4. Bumble bee (*Bombus impatiens* Cresson)

Studies conducted by Stanghellini *et al.* (2000) in USA revealed a potential host shift of small hive beetle from *Apis mellifera* colonies to Bumble bee (*Bombus impatiens*) colonies. They also reported that the colony of bumble bees infested with hive beetle was collapsed completely. Oviposition of the adult female beetle in the colony was the damage traced out in the study conducted by Hoffman *et al.* (2008) in USA.

2.3.2. Yield Loss Caused By Small Hive Beetle

Yield loss caused by hive beetle in honey bee colonies are less studied, hence the literature available on this aspect was scanty. Elzen *et al.* (2001) reported that small hive beetle invasion resulted in a yield loss of US dollar 3 million in *Apis mellifera* colonies in USA. Studies conducted by Jayalekshmi (2015) reported an yield reduction of 93.93 per cent and 96.76 per cent from the districts of Kottayam and Idukki respectively due to the infestation of hive beetle in stingless bee colonies of Kerala.

2.3.3. Factors Contributing To Hive Beetle Incidence

2.3.3.1. Hive types

Studies conducted by Jayalekshmi (2015) in southern districts of Kerala revealed that pest incidence was relatively lesser in bamboo hives. Highest pest incidence was recorded from stingless colonies maintained in wooden boxes.

2.3.3.2. Height of the hive

Jayalekshmi (2015) studied the effect of height of the hive from the ground level with that of the pest incidence. The colonies placed at a height 9-11 ft from the ground level recorded highest incidence (5.10 per cent) of hive beetle followed by 6-8 ft (4.69 per cent). The study also revealed that the colonies which were placed at a height of 3-5 ft were free of pest incidence.

Materials and Methods

3. MATERIALS AND METHODS

The present study entitled “Bioecology of small hive beetles and assessment of their damage in stingless bee colonies of Kerala” was carried out in the AICRP on Honey Bees and Pollinators, Department of Agricultural Entomology, College of Agriculture, Vellayani (76 59’ 08” E longitude, 80 24’ 90” N latitude and a mean height of 29 m above MSL) during 2015-17. The study was conducted to find out the incidence, bioecology of small hive beetle and the extent of damage caused by the beetle in the southern part (Thiruvananthapuram, Kollam and Pathanamthitta districts) of Kerala.

3.1. ESTIMATION OF SMALL HIVE BEETLE INCIDENCE IN STINGLESS BEE COLONIES

The incidence of small hive beetle and other pests in the southern part of Kerala was recorded for a period of one year.

3.1.1. Selection of Apiaries and Collection of Hive Beetles From Stingless Bee Colonies

Ten apiaries were selected at random by purposive sampling from each district (Table 1.) (Plate 1.). The different coleopteran pests infesting stingless bee colonies were collected during the survey and was sorted and categorized into primary and secondary feeders. The beetles as well as different life stages of the beetle were preserved in small PCR tubes containing 70 per cent alcohol with labels intact. The beetles of family nitidulidae were sent to Dr. Alexander Kirejtshuk, Senior Scientist, Natural history Museum, Paris, France and tenebrionid beetles were sent to Dr. Maxim Nabozhenko, Scientist, Russian Academy of Sciences for identification.



1a. Stingless bee hives placed in the apiary



1b. Hives placed on the terrace

Plate 1. An apiary in Thiruvananthapuram district of Kerala

3.1.2. Incidence of Small Hive Beetle in Southern Kerala

Observations on the incidence of hive beetle were recorded at monthly intervals.

The stingless bee colonies infested with small hive beetle were brought to the laboratory for thorough examination. The following parameters were observed in the apiaries:

3.1.2.1. Estimation of percentage of infestation

The total number of colonies and the number of colonies infested with hive beetle were recorded in an apiary. The percentage of infestation was calculated as follows:

$$\text{Percentage of infestation} = \frac{\text{Number of colonies infested with small hive beetle}}{\text{Total number of colonies observed}} \times 100$$

3.1.2.2. Incidence of small hive beetle, *E. latissima* in different types of beehive

The number of hives under each category viz., wooden box, PVC pipes, mud pots and bamboo hives and small hive beetle incidence in the hives were recorded and was expressed in percentage. The quality and type of wood that were used for constructing the hives were also recorded. The hives which are made of soft woods considered as bad quality.

3.1.2.3. Influence of hive height on *E. latissima* incidence

The height of the hive from the ground level was categorized into 1-2 m, 2-3 m, 3-4 m and > 4 m. Percentage of hive beetle incidence in each category was worked out separately.

3.1.2.4. Influence of apiary conditions (shade and foraging plants) on *E. latissima* incidence

Shade conditions in the apiaries were categorized (based on visual canopy coverage) into low (< 30 per cent canopy coverage), moderate (30-80 per cent canopy coverage) and high (> 80 per cent canopy coverage) (Plate 2.) and the percentage incidence of hive beetle in each category was calculated.

The plants visited by stingless bee for pollen and nectar near the hive were also recorded from the apiaries in each district. The observations were recorded at hourly interval from 6 am to 6 pm.

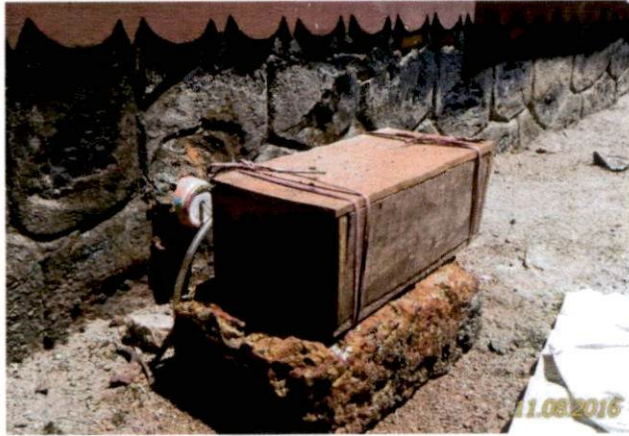
3.1.3. Symptoms of *E. latissima* Infestation

Hive beetle infested colonies were opened and observed to record the symptoms of damage, if any. The damage caused by the adult beetle and larvae to the internal hive structures *viz.*, brood cells, honey and pollen storage were also recorded. The symptoms were categorized into different phases based on the percentage of infestation.

Phase I	: <10 per cent infestation
Phase II	: 10-50 per cent infestation
Phase III	: 50-80 per cent infestation
Phase IV	: >80 percent infestation

3.1.4. Other Pests Infesting Stingless Bees

Apart from small hive beetle, the infestation by other pests, their nature of damage and symptoms of infestations were also recorded.



2a. Low (<30 % canopy coverage)



2b. Moderate (30-80 % canopy coverage)



2c. High (>80 % canopy coverage)

Plate 2. Different shade conditions in the apiary

3.2. BIOECOLOGY OF SMALL HIVE BEETLE

Stingless bee colonies free of hive beetle infestation were brought to the laboratory and were subjected to the following studies.

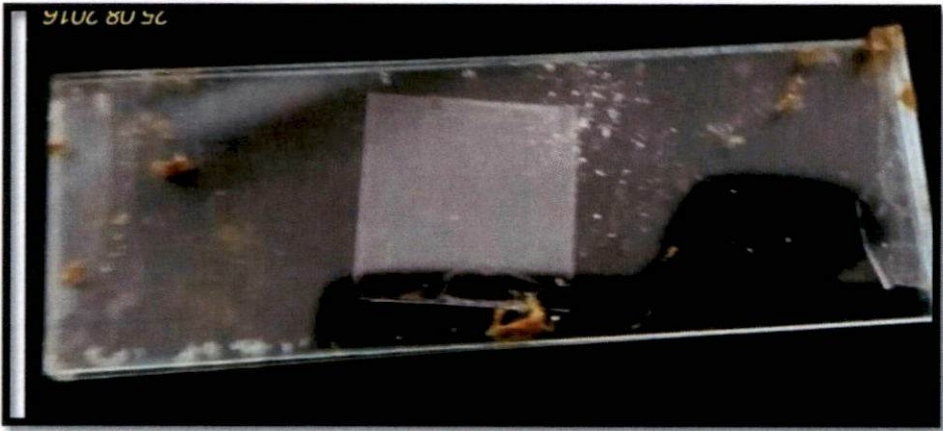
3.2.1. Biology of *E. latissima* beetle in Laboratory Condition

The duration of each stage of beetle was studied. For this, five colonies of stingless bees with pollen, brood and honey storage were maintained under the laboratory conditions. Ten numbers of adult beetles (male and female in the ratio 1:4) were introduced into these colonies. Since the hive beetles are small and fast moving, aspirator was used to transfer them to the hives. Male and female beetles were distinguished by means of the difference in their anal sclerite (Schmolke, 1974).

Microscopic slide technique developed by Ellis and Delaplane (2008) was utilized as a provision for egg laying by the beetle, so that the eggs can be viewed directly. Two microscopic slides were placed one above the other with a coverslip in the middle. The two adjacent ends of these microscopic slides were wrapped and fixed with cellotape so that a small space of 1 mm or less than 1 mm was created in between this slides which mimics the cracks and crevices of the hive. (Plate 3.) Such microscopic slides were placed into the hives maintained in the laboratory. Twenty four hours after introduction, slides were carefully observed with a hand lens (magnification 4 X) for the presence of eggs laid by the beetle. Three replications were maintained for this experiment.

3.2.1.1. Egg period

The egg period of small hive beetle was noted as number of days from oviposition to larval emergence. In order to find out egg period, the slide with beetle eggs (as described in 3.2.1) was transferred to a container and the date of egg laying was noted. The positions of 10 eggs were randomly marked using a permanent marker pen with the help of hand lens (magnification 4 X). Slides were inspected at



3a. Microscopic slide developed for egg laying by *E. latissima*



3b. Eggs laid by the *E. latissima* between glass slides

Plate 3. Microscopic slide technique

frequent intervals of 8 hours to find out whether the eggs are hatched or not. Once the egg hatched, egg period was recorded. Pollen blocks and honey were provided as nourishment food in the container for the emerging larvae. The whole container was covered with black cloth, since the first instar larva prefer a dark condition. The duration of egg period was recorded as the mean number of days.

3.2.1.2. Larval period

Each individual larva soon after the emergence was carefully transferred into a small Eppendorf tube using a camel hair brush with the aid of a hand lens of magnification 4 X. The sides of the tubes were carefully examined for the presence larval moult skin to quantify the number and period of each larval instar. The larval period is recorded as the mean number of days.

3.2.1.3. Pupal period

The pupal period of small hive beetle was noted as the mean number of days from pupation to adult emergence. The last instar larva along with some hive contents were transferred into a small eppendorf tube. Small pin holes were made on the top of the tubes for providing aeration.

3.2.2. Morphological and Morphometric Characters of Different Life Stages of *E. latissima*

The images and measurements (length and width) of different life stages were taken by stereo microscope provided with high speed digital fire wire live camera, LAS measurement module and data transfer.

3.2.2.1. Egg

Microscopic slides with beetle eggs (as described in 3.2.1) were observed under leica image analyzer system. Morphological characters as well as length and width of egg stage was noted as mean (10 randomly marked eggs) in mm.

3.2.2.2. Larvae

The morphological features of different larval instars were observed under the stereo microscope. The mean length and width of beetle larvae (10 Nos.) were also recorded in mm.

3.2.2.3. Pupa

Pupae were taken out by cutting open the pupation chamber. Morphological characters as well as mean length and width of pupal stages (10 Nos.) were noted in mm.

3.2.2.4. Adult

The morphological features of adult beetle were recorded by observing under stereo microscope. The mean length and width of adult beetles (10 Nos.) were noted in mm.

3.3. ASSESSMENT OF EXTENT OF DAMAGE CAUSED BY BEETLE

The small hive beetle infested hives were brought to the laboratory and the following studies were conducted.

3.3.1. Nature of Damage by Different Stages of *E. latissima*

The nature of damage caused by the adult beetle and larvae were recorded. The hives infested with small hive beetle were carefully examined to find out the exact damage caused by larvae and adult stage of the beetle. The replication of the experiment was made as three.



4a. Wooden box of dimension $15 \times 5 \times 10$ cm



4b. Wooden box with pollen block 4 cm^3

Plate 4. Experimental set up for the damage assessment
Of *Epuraea latissima*

3.3.2. Time Taken for the Complete Destruction of Hive Materials by *E. latissima*

The time taken by the larvae for the complete destruction of hive materials is quantified by the procedure as follows. Pollen, brood and honey blocks of 4 cm³ were cut out from healthy honey bee colonies using a sharp blade and transferred to the wooden box of dimension 15×5×10 cm (Plate 4.). Moistened cotton pads were also placed inside the box to maintain sufficient relative humidity. Second instar larvae (10 Nos.) were released into the box. The time taken by the larvae for the complete deterioration of pollen, brood and honey block was recorded as the mean number of days. The replication maintained was five.

3.3.3. Population Dynamics of *E. latissima*

Ten hive beetle infested colonies of stingless bees maintained at College of Agriculture, Vellayani were utilized to study the population dynamics of the pest. Hives which possess equal volume of pollen, brood and honey were selected for the study. The grub population was taken at fortnightly intervals for a period of thirteen months (from May 2016 to May 2017). For this, 1 cm³ volume of pollen block was cut out from the infested colonies at fortnightly intervals and using destructive sampling method, number of grubs with in each block was counted and worked out the mean.

3.3.4. Correlation Studies

The meteorological data during this period was collected from Meteorological Department, College of Agriculture, Vellayani, Thiruvananthapuram. Weather data such as mean daily temperature, monthly mean temperature, average rainfall and relative humidity were collected for the period from May 2016 to May 2017. The weather data at fortnightly intervals was calculated from monthly data and it was

correlated with the grub population of small hive beetle. Thus the correlation coefficients are worked out.

Table 1. Geographic details of the apiaries from Southern Kerala

Districts	Sl. No.	Locations	Longitude	Latitude
Thiruvananthapuram	1	Nedumangad	77.0046° E	8.6080° N
	2	Tholikkode	77.0551° E	8.6452° N,
	3	Perukavu	77.0118° E	8.4941° N,
	4	Koonanvenga	77.0032° E	8.6024° N
	5	Poovar	77.0708 ° E	8.3177° N
	6	Madathara	77.0133° E	8.8157° N
	7	Vilappilshala	77.0430° E	8.5302 ° N
	8	Cheruvarakkonam	77.1514° E	8.3310°N
	9	Kattakkada	77.0852° E	8.5033° N
	10	Malayinkeezhu	77.0361° E	8.4902° N
Kollam	1	Kadakkal	76.9222° E	8.8293° N
	2	Mayyanad	76.6498 ° E	8.8509 ° N
	3	Ayoor	76.6172° E	8.8895° N
	4	Veliyam	76.7668° E	8.9162° N
	5	Anchal	76.9065° E	8.9300° N
	6	Kottarakkara	76.8002° E	9.0017° N
	7	Pattazhi	76.8002° E	9.0017° N
	8	Sooranad	76.6932° E	9.0714°N
	9	Vettikkavala	76.8274° E	8.9966° N
	10	Valakom	76.8418° E	8.9566° N
Pathanamthitta	1	Adoor	76.7308° E	9.1512° N
	2	Pandalam	76.6785° E	9.2251° N
	3	Elanthoor	76.7279° E	9.2892° N
	4	Thiruvalla	76.5741° E	9.3835° N
	5	Kadammanitta	76.7686° E	9.3199° N
	6	Ranni	76.7856 ° E	9.3866 ° N
	7	Vadasherikkara	76.8209 ° E	9.3307 ° N
	8	Vaipur	76.7019° E	9.4483° N
	9	Chunkappara	76.7456° E	9.4519° N
	10	Konni	76.8497° E	9.2267° N

Results

4. RESULT

The investigation on "Bioecology of small hive beetles and assessment of their damage in stingless bee colonies of Kerala" was carried out in All India Co-ordinated Research Project (AICRP) on Honey bees and Pollinators, Department of Agricultural Entomology, College of Agriculture, Vellayani during 2015-17. The results of the study are presented in this chapter.

4.1. ESTIMATION OF SMALL HIVE BEETLE INCIDENCE IN STINGLESS BEE COLONIES

4.1.1. Selection of Apiaries and Collection of Hive Beetles From Stingless Bee Colonies

Seven different beetles were collected from stingless bee colonies in the selected apiaries of southern Kerala (Plate 5, 6 and 7). The collected beetles were classified into primary and secondary feeders of contents in the stingless bee hive (Table 2).

The hive beetles collected from stingless bee hives were identified as *Epuraea latissima* Kirejtshuk, *Carpophilus marginellus* Motschulsky, *Carpophilus* spp -1, *Carpophilus* spp – 2 and *Phenolia picta* Macley belonging to the family nitidulidae. Others were identified as *Palorinus humeralis* Gebien belonging to tenebrionidae and cryptophagid beetles belonging to cryptophagidae. All the beetles collected from the hive (except *E. latissima*) were found as secondary feeders from the stingless bee colonies of southern Kerala. The incidence of *E. latissima*, *P. humeralis* and cryptophagid beetles were recorded from all the districts, while the incidence of *Carpophilus* spp -1 and *P. picta* was recorded only from the Kollam district. Incidence of *C. marginellus* and *Carpophilus* spp - 2 was recorded from both Thiruvananthapuram and Pathanamthitta districts.

Table 2. Beetles associated with the stingless bee hives in Southern Kerala

Sl. No.	Hive beetles	Family	Districts			Infestation	
			TVM* ¹	KLM* ²	PTA* ³	P* ⁴	S* ⁵
1.	<i>Carpophilus marginellus</i>	Nitidulidae	+	-	+	-	+
2.	<i>Carpophilus</i> spp - 1	Nitidulidae	-	+	-	-	+
3.	<i>Carpophilus</i> spp - 2	Nitidulidae	+	-	+	-	+
4.	<i>Phenolia picta</i>	Nitidulidae	-	+	-	-	+
5.	<i>Eपुरaea latissima</i>	Nitidulidae	+	+	+	+	-
6.	<i>Palorinus humeralis</i>	Tenebrionidae	+	+	+	-	+
7.	Cryptophagid beetle	Cryptophagidae	+	+	+	-	+

*¹ Thiruvananthapuram *² Kollam *³ Pathanamthitta*⁴ Primary *⁵ - Secondary

‘+’ Indication of presence

‘-’ Indication of absence



5a. *Carpophilus marginellus* Motschulsky



5b. Nitidulid beetle *Carpophilus* spp-1

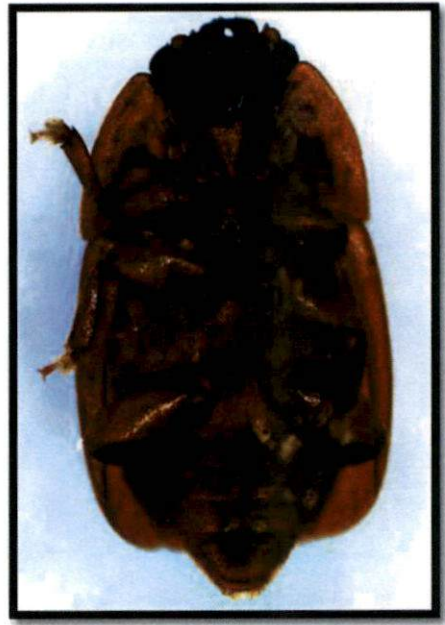


5c. Nitidulid beetle *Carpophilus* spp-2

Plate 5. Nitidulid beetles collected from stingless bee hives
(Dorsal and ventral view)



6a. Nitidulid beetle *Phenolia picta* Macl.

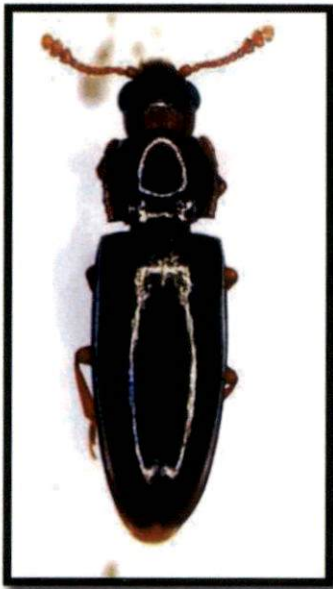


6b. Nitidulid beetle *Epuraea latissima* K.

Plate 6. Nitidulid beetles collected from stingless bee hives
(Dorsal and ventral view)



7a. Tenebrionid beetle *Palorinus humeralis* Gebien



7b. Cryptophagid beetle

Plate 7. Different types of beetles collected from stingless bee hives

4.1.1.1. Nitidulid beetles

E. latissima, *C. marginellus*, *Carpophilus* spp (2 Nos.) and *P. picta* were the nitidulid beetles which were collected from stingless bee hives in southern Kerala.

The adult beetle of *E. latissima* was dark brown in colour and about 3mm and 1mm in length and width respectively.

Carpophilus spp-1 was dark brown coloured beetle with small yellow patch on the base of elytra. The length of the beetle measured up to 3.50 mm. The size *Carpophilus* spp-2 was almost same as that of *Carpophilus* spp-1, however they are easily distinguishable by a yellow patch on the elytra. *C. marginellus* was dark brown beetle with 2.923 mm length and 0.987 mm width.

P. picta was a dark black beetle with striations on the elytra. It was relatively bigger in size and ranges from 6-7 mm.

Among these, the primary infestation was recorded only in the case of *E. latissima*. The other nitidulid beetles occasionally visited stingless bee colonies as a secondary feeder.

4.1.1.2. Tenebrionid beetle

The reddish brown coloured adult beetle, *P. humeralis* and its larvae were recorded as secondary pests in the stingless bee colonies. Length of the beetle was measured up to 3 mm. The beetle larvae tunneled through the pollen storage in abandoned hives.

4.1.1.3. Cryptophagid beetle

Adult beetle is shiny black in colour and about 5 mm in length. The beetle larvae and adults were observed as secondary feeders of hive contents in the stingless bee hive (Plate 8). All the life stages of the beetle were observed within the bee hive.



8a. Cryptophagid beetle



8b. Larvae feed on hive content



8c. Cryptophagid beetle (Pupa)



8d. Adult beetle feeding on hive content



8e. Fully matured larva

Plate 8. Cryptophagid beetle infesting on stingless bee hive contents

4.1.2. Incidence of *E. latissima* in Southern Kerala

Results of the studies on the monthly incidence of *E. latissima* (being the primary pest of stingless bee hive) conducted in three southern districts of Kerala viz., Thiruvananthapuram, Kollam and Pathanamthitta for a period of 13 months (from May 2016 to May 2017) are detailed below.

The monthly incidence of small hive beetle, *E. latissima* varied among the apiaries of three districts. In Thiruvananthapuram district, the hive beetle incidence was recorded during June, July and August. Maximum infestation was recorded during the month of June from the apiaries viz., Malayam, Kattakkada, Malayinkeezhu and Nedumangad. The incidence was also observed from the apiary at Tholicode during the month of July and August. The incidence of beetle was recorded during the month of July also from the location, Malayam (Table 3).

In Kollam district, the hive beetle incidence was observed during the month of June, July, September and October from the apiary at Sooranad. Apart from this, the hive beetle incidence was also recorded from Kottarakkara during the month of June and from Pattazhi during the month of July (Table 4).

The hive beetle incidence was recorded only from two apiaries, Kadammanitta and Ranni of Pathanamthitta district during the month of May (Table 5).

4.1.2.1. Estimation of percentage of infestation

Of the three districts surveyed in the Southern Kerala, maximum percentage of hive beetle incidence was recorded from Kollam (19 %) followed by Thiruvananthapuram (16 %) and Pathanamthitta (3 %) (Table 6).

Table 3. Monthly incidence of the small hive beetle, *E. latissima* in Thiruvananthapuram district

Sl. No.	Locations	Months												
		Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	
1.	Cheruvarakkonam	-	-	-	-	-	-	-	-	-	-	-	-	-
2.	Poovar	-	-	-	-	-	-	-	-	-	-	-	-	-
3.	Malayam	-	-	-	-	-	+	+	-	-	-	-	-	-
4.	Kattakkada	-	-	-	-	-	+	+	-	-	-	-	-	-
5.	Malayinkeezhu	-	-	-	-	-	+	+	-	-	-	-	-	-
6.	Perukavu	-	-	-	-	-	-	-	-	-	-	-	-	-
7.	Tholicode	-	-	-	-	-	-	+	+	-	-	-	-	-
8.	Nedumangad	-	-	-	-	-	-	+	-	-	-	-	-	-
9.	Koonanvenga	-	-	-	-	-	-	-	-	-	-	-	-	-
10.	Madathara	-	-	-	-	-	-	-	-	-	-	-	-	-

'+' Indication of hive beetle incidence '-' Indication of absence of hive beetle incidence

Table 4. Monthly incidence of the small hive beetle, *E. latissima* in Kollam district

Sl. No.	Locations	Months													
		Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.		
1.	Kadakkal	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2.	Mayyanad	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3.	Ayoor	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4.	Veliyam	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5.	Anchal	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6.	Kottarakkara	-	-	-	-	-	+	-	-	-	-	-	-	-	-
7.	Pattazhi	-	-	-	-	-	-	+	-	-	-	-	-	-	-
8.	Sooranad	-	-	-	-	-	+	+	+	+	+	-	-	-	-
9.	Vettikkavala	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10.	Valakam	-	-	-	-	-	-	-	-	-	-	-	-	-	-

‘+’ Indication of hive beetle incidence

‘-’ Indication of absence of hive beetle incidence

Table 5. Monthly incidence of the small hive beetle, *E. latissima* in Pathanamthitta district

S.No.	Locations	Months														
		Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.			
1.	Adoor	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2.	Pandalam	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3.	Elanthoor	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4.	Thiruvalla	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5.	Kadammanitta	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
6.	Ranni	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
7.	Vadasserikkara	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8.	Vaipur	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9.	Chunkappara	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10.	Konni	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+ Indication of hive beetle incidence *- Indication of absence of hive beetle incidence

Table 6. Infestation *E.latissima* in Southern Kerala

Sl. No.	Districts	Percentage of infestation
1.	Thiruvananthapuram	16
2.	Kollam	19
3.	Pathanamthitta	3

4.1.2.2. Incidence of small hive beetle, *E. latissima* in different types of beehive

Mud pots, wooden boxes, PVC pipes and bamboo hives were generally used by the beekeepers for rearing the stingless bees in Thiruvananthapuram, Kollam and Pathanamthitta districts of Kerala (Plate 9).

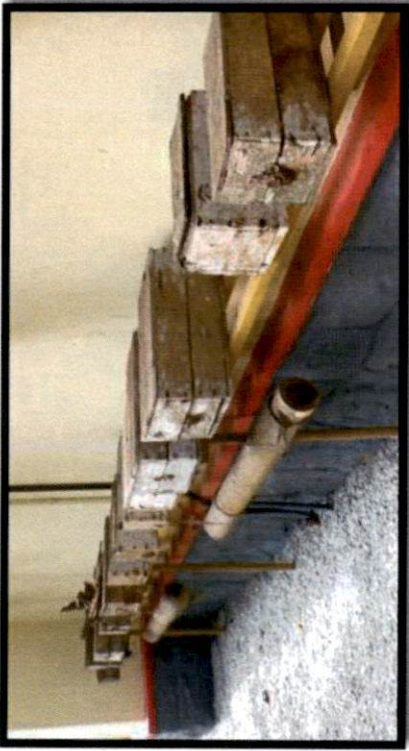
Majority of beekeepers in Thiruvananthapuram district maintained stingless bee colonies in the wooden box (61 Nos.) followed by mud pot (20 Nos.), PVC pipes (11 Nos.) and bamboo hives (8 Nos.). The hive beetle incidence was recorded only from the stingless bee colonies maintained in wooden box (24.61 per cent) and the maximum incidence was noticed from Tholicode (Table 7).

In Kollam district also, the most preferred type of hive was wooden box (63 Nos.) followed by mud pot (21 Nos.), PVC pipes (12 Nos.) and bamboo hive (4 Nos.). Hive beetle incidence was recorded from the stingless bee colonies maintained in wooden box (25.39 per cent) as well as mud pots (14.28 per cent). Severe infestation was noticed from Sooranad where stingless bee hives were maintained in wooden boxes (Table 8).

Beekeepers in Pathanamthitta district, maintained stingless bee colonies mostly in wooden box (54 Nos.), followed by PVC pipes (26 Nos.), mud pots (15 Nos.) and bamboo hives (5 Nos.). The percentage incidence of hive beetles was



9a. Mud pots



9b. Wooden box



9c. PVC pipes



9d. Bamboo hives

Plate 9. Different types of hives used by beekeepers of Southern Kerala

Table 7. Incidence of the small hive beetle, *E. latissima* in Thiruvananthapuram district on different types of hives

Sl. No.	Locations (10 hives/ location)	Types of hives									
		Mud pot		Wooden box		PVC pipes		Bamboo hive			
		Total	Infested	Total	Infested	Total	Infested	Total	Infested		
1.	Nedumangad	2*	0	6	1	1	0	1	0	1	0
2.	Tholicode	0	0	8	6	0	0	2	0	0	0
3.	Perukavu	2	0	8	0	0	0	0	0	0	0
4.	Koonanvenga	0	0	10	0	0	0	0	0	0	0
5.	Poovar	5	0	4	0	1	0	0	0	0	0
6.	Madathara	3	0	0	0	2	0	1	0	0	0
7.	Cheruvarakkonam	3	0	5	0	2	0	2	0	1	0
8.	Malayinkeezhu	2	0	6	2	1	0	0	0	0	0
9.	Malayam	0	0	10	4	0	0	0	0	0	0
10.	Kattakkada	1	0	4	3	3	0	2	0	0	0
Total		18	0	61	16	10	0	7	0	0	0
Hive beetle incidence (%)		0		24.61		0		0		0	

Table 8. Incidence of the small hive beetle, *E. latissima* in Kollam district based on hive types

Sl. No.	Locations (10 hives/ location)	Mud pot		Wooden box		PVC pipes		Bamboo hive	
		Total	Infested	Total	Infested	Total	Infested	Total	Infested
1.	Kadakkal	0	0	10	0	0	0	0	0
2.	Mayyanad	2	0	7	0	1	0	0	0
3.	Ayoor	2	0	6	0	1	0	1	0
4.	Veliyam	1	0	5	0	3	0	1	0
5.	Anchal	1	0	2	0	6	0	1	0
6.	Kottarakkara	1	0	9	7	0	0	0	0
7.	Pattazhi	5	3	5	0	0	0	0	0
8.	Sooranad	0	0	10	9	0	0	0	0
9.	Vettikkavala	3	0	7	0	0	0	0	0
10.	Valakom	6	0	2	0	1	0	1	0
Total		21	3	63	16	12	0	4	0
Hive beetle incidence (%)		14.28		25.39		0		0	

5.55 per cent in wooden boxes with relatively higher infestation from Kadammanitta location. The other hive types were free of the hive beetle infestation (Table 9).

No hive beetle incidence was observed from PVC pipes and bamboo hives in three districts. The hive beetle incidence in mud pot was recorded only from the Kollam district.

The incidence of small hive beetle, *E.latissima* was more prominent in the stingless bee hives made of wooden box in three districts. Quality of the wood is an important parameter which determines the hive beetle incidence in the apiaries. Influence of quality of wood on the incidence of hive beetle is represented in Table 10. The hives made of teak was considered as good quality hives, whereas hives made of locally available wood viz., mango, *Clerodendron* spp. and pine wood are considered as bad quality ones (Plate 10).

Considering the influence of the quality of wooden boxes on the incidence of small hive beetle, *E.latissima*, out of 25 hives made from bad quality wood, 15 were infested with hive beetle in Thiruvananthapuram district (Table 10.). Similarly, in Kollam district, out of 32 bad quality hives, 16 were infested with small hive beetle. Twenty bad quality hives were recorded from Pathanamthitta district, in which three hives were infested with hive beetle. Out of 36 good quality hives were from Thiruvananthapuram only one hive was infested with hive beetle, whereas none of the good quality wooden box in Kollam and Pathanamthitta was infested with hive beetle.

4.1.2.3. Influence of hive height on *E. latissima* incidence

The number of stingless bee colonies maintained at different heights from the ground level (1-2 m, 2-3 m, 3-4 m and > 4 m) and percentage incidence of hive beetle in Southern Kerala are presented in Table 11.



10a. Cracks in the bad quality hives



10b. wooden box made of locally available soft wood

10. Bad quality wooden boxes

Table 9. Incidence of the small hive beetle, *E. latissima* in Pathanamthitta district based on hive types

Sl. No.	Locations (10 hives/ location)	Types of hives									
		Mud pot		Wooden box		PVC pipes		Bamboo hive			
		Total	Infested	Total	Infested	Total	Infested	Total	Infested		
1.	Adoor	1	0	7	0	2	0	0	0		
2.	Pandalam	0	0	10	0	0	0	0	0		
3.	Elanthoor	5	0	5	0	0	0	0	0		
4.	Thiruvalla	2	0	3	0	5	0	0	0		
5.	Kadammanitta	0	0	7	2	3	0	0	0		
6.	Ranni	0	0	5	1	4	0	1	0		
7.	Vadasserikkara	1	0	4	0	5	0	0	0		
8.	Vaipur	2	0	4	0	2	0	2	0		
9.	Chunkappara	0	0	6	0	3	0	1	0		
10.	Konni	4	0	3	0	2	0	1	0		
Total		15	0	54	3	26	0	5	0		
Hive beetle incidence (%)		0		5.55		0		0			

Table 10. Incidence of the small hive beetle, *E. latissima* in wooden boxes based on the wood quality

Sl. No.	Thiruvananthapuram				Kollam				Pathanamthitta			
	Wooden box(GQ ¹)		Wooden box(BQ ²)		Wooden box(GQ)		Wooden box(BQ)		Wooden box(GQ)		Wooden box(BQ)	
	Total No. of hives	Infested hives	Total No. of hives	Infested hives	Total No. of hives	Infested hives	Total No. of hives	Infested hives	Total No. of hives	Infested hives	Total No. of hives	Infested hives
1.	6	1*	0	0	1	0	9	9	4	0	3	0
2.	1	0	7	6	7	0	0	0	5	0	5	0
3.	8	0	0	0	4	0	2	0	5	0	0	0
4.	8	0	2	0	2	0	3	0	2	0	1	0
5.	4	0	0	0	2	0	0	0	0	0	7	2
6.	0	0	0	0	1	0	8	7	4	0	1	1
7.	4	0	1	0	5	0	0	0	4	0	0	0
8.	1	0	5	2	0	0	10	9	4	0	0	0
9.	4	0	6	4	7	0	0	0	6	0	0	0
10.	0	0	4	3	2	0	0	0	0	0	3	0
Total	36	1	25	15	31	0	32	16	34	0	20	3

*1- Good quality *2- Bad quality

Table 11. Incidence of the small hive beetle, *E. latissima* in Southern Kerala based on the height from ground level

Districts	Hives at different heights							
	1-2 m		2-3 m		3-4 m		>4 m	
	Total	Infested	Total	Infested	Total	Infested	Total	Infested
Thiruvananthapuram	12	0	21	4	58	12	9	0
Kollam	15	1	32	2	48	16	5	0
Pathanamthitta	16	0	34	1	15	2	35	0
Total	43	1	87	7	121	30	49	0
Incidence of hive beetle (%)	2.33		8.04		24.79		0	

Out of 300 stingless bee colonies surveyed in Southern Kerala, 121 hives were maintained at a height of 3-4 m, followed by 2-3 m (87 Nos.), > 4m (49 Nos.) and 1-2 m (43 Nos.). The hives maintained at 3-4 m height recorded the highest incidence of hive beetle (24.79 %) followed by 2-3 m (8.04 %). None of the colonies maintained at a height > 4 m showed hive beetle incidence.

4.1.2.4. Influence of apiary conditions (shade and foraging plants) on *E. latissima* incidence

The number of stingless bee colonies maintained in different shade categories such as low (< 30 % canopy coverage), moderate (30-80 % canopy coverage) and high (> 80 % canopy coverage) and the percentage incidence of hive beetle in each category are represented in Table 12.

Of the three districts, 222 number of the colonies were placed in moderate shade conditions followed by high (71 Nos.) and low (7 Nos.) shade conditions. Highest *E. latissima* incidence (50.70 %) were recorded from the apiaries with high shade conditions (> 80 % canopy coverage) followed by moderate shade conditions (0.90 %). *E. latissima* were absent in apiaries with low shade conditions among the three districts.

The important foraging plants visited by the stingless bees in and around the apiaries of the districts for pollen and nectar sources are depicted in the Table 13.

A total of 31 foraging plants were visited by the stingless bees which are potential food source in the three Southern districts of Kerala. Out of the 31 foraging plants, 12 were nectar providers, 9 were pollen providers, whereas 10 plants were both nectar and pollen providers. Foraging plants of the family Euphorbiaceae was prominent in all the apiaries that were selected for the study.

Table 12. Incidence of *E. latissima* in apiaries under different shade conditions

Districts	Canopy coverage					
	Low (< 30 %)		Moderate (30-80 %)		High (> 80 %)	
	Total	Infested	Total	Infested	Total	Infested
Thiruvananthapuram	1	0	72	1	27	15
Kollam	2	0	68	1	30	18
Pathanamthitta	4	0	82	0	14	3
Total	7	0	222	2	71	36
Hive beetle incidence (%)	0.00		0.90		50.70	

Table 13. Plants visited by stingless bees for their food source

Sl. No.	Foraging plant	Scientific name	Family	Foraging source		
				N	P	N+P
1	Coconut	<i>Cocos nucifera</i> L.	Areaceae		+	
2	Rubber	<i>Heavea brasiliensis</i> L.	Euphorbiaceae	+		
3	Cashew	<i>Anacardium occidentale</i> L.	Anacardiaceae			+
4	Papaya	<i>Carica papaya</i> L.	Caricaceae		+	
5	Citrus	<i>Citrus</i> spp.	Rutaceae	+		
6	Mango	<i>Mangifera indica</i> L.	Anacardiaceae		+	
7	Banana	<i>Musa</i> spp.	Musaceae	+		
8	Rose apple	<i>Eugenia jambosa</i> L.	Myrtaceae			+
9	Passion fruit	<i>Passiflora grandiflorum</i> S.	Passifloraceae		+	
10	Lovi lovi	<i>Flaciourtia inermis</i> (Burm.f.)	Salicaceae			+
11	Rambutan	<i>Nephelium lappaceium</i> L.	Sapindaceae	+		
12	Bilimbi	<i>Averrhoabilimbi</i> L.	Oxalidaceae		+	
13	Anjili	<i>Artocarpus hirsute</i> Lam.	Moraceae			+
14	Asoka tree	<i>Saracaindica</i> (Roxb.)	Fabaceae			+
15	Anthurium	<i>Anthurium andreanum</i> L.	Araceae		+	
16	Tulsi	<i>Ocimum sanctum</i> L.	Lamiaceae	+		
17	Neem	<i>Azadiracta indica</i> L.	Meliaceae			+
18	Henna plant	<i>Lawsonia inermis</i> L...	Lythraceae		+	
19	Touch me not	<i>Mimosa pudica</i> L.	Fabaceae			+
20	Ixora	<i>Ixoracoccinea</i> L.	Rubiaceae	+		
21	Balsam	<i>Impatiens balsaminae</i> L.	Balsaminaceae	+		
22	Rosa	<i>Rosa</i> sp.	Rosaceae			+
23	Rangoon creeper	<i>Antigonon leptopus</i> L.	Polygonaceae			+

24	China rose	<i>Hibiscus rosasinensis</i> L.	Malvaceae		+	
25	Canna	<i>Canna indica</i> L.	Cannaceae	+		
26	Ten O'clock plant	<i>Portulaca grandiflora</i> H.	Portulacaceae		+	
27	Trumpet bush	<i>Tecoma stans</i> L.	Begoniaceae	+		
28	Sponge gourd	<i>Luffa cylindrica</i> M. Roem.	cucurbitaceae	+		
29	Wild tapioca	<i>Manihot glaziovii</i> M.	Euphorbiaceae	+		
30	Teak	<i>Tectona grandis</i> L.	Lamiaceae			+
31	Tapioca	<i>Manihot esculenta</i> C.	Euphorbiaceae	+		
	Total			12	9	10

4.1.3. Symptoms of *E. latissima* Infestation

The damage symptoms due to the infestation by small hive beetle were almost similar in three districts in Southern Kerala. The symptoms caused by the beetle are categorized into distinct phases. A visual scoring method was developed based on percentage infestation of *E. latissima* in stingless bee colonies.

Phase I (< 10 % infestation)

There was no visible sign of infestation at this phase. The beetles mainly occupied the rear portion of hive thus unable to distinguish the infestation of the beetle at this phase (Plate 11).

Phase II (10-50 % infestation)

Honey breaks and flow out of the honey pots. Pollen pots and brood cells were intact externally with feeding larvae inside (Plate 11).

Phase III (50-80 % infestation)

Fermentation and discoloration of honey was prominent during this stage due to feeding and defecation of beetle larvae. Moreover, pollen pot, honey pot and brood cells became undistinguishable. Fermenting smell of honey in the apiary was an indication of small hive beetle at this phase. Honey obtained from such infested hives became unfit for human consumption due to fermentation and frothiness. All the life stages of beetle *viz.*, egg, larvae, pupae and adults were also apparent in the hive at this phase (Plate 12).

Phase IV (> 80 % infestation)

At this phase beetle and grubs leave the colony after the complete destruction of pollen pots, honey pots and brood cells. Light brown coloured fermented entangled mass of substance only remained in such hives (Plate 12).



11a. Phase I (< 10% infestation)



11b. Phase II (10-50 % infestation)



Plate 11. Symptoms of *Epuraea latissima* infestation



12a. Phase III (50- 80 % infestation)



12b. Phase IV(>80 % infestation)



Plate 12. Symptoms of *Epuraea latissima* infestation

4.1.4. Other Pests Infesting Stingless Bees

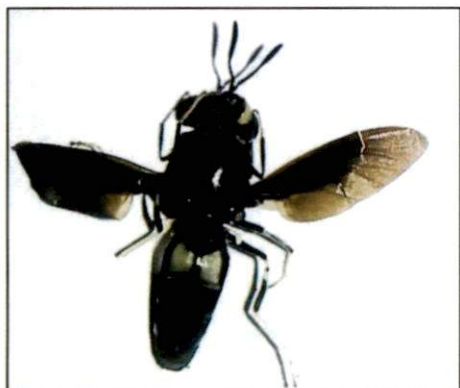
Apart from small hive beetle, other pest species which infested the stingless bee colonies in Southern Kerala and their nature of damage and symptoms were also documented (Table 14).

4.1.4.1. Black soldier fly *Hermitia illucens* F. Stratiomyidae

The black soldier fly, *Hermitia illucens* belonging to the family Stratiomyidae is a pest of stingless bee colonies. The adult fly was black coloured and its length measures up to 16 mm. It possesses two small transparent spots on the first abdominal segments and that make them easily recognizable from other flies. The maggots were dull white in colour, dorso-ventrally flattened, 10-12 mm length, 4-5 mm width and leathery with a tiny black head (Plate 13). The adult fly lived up to seven days.

4.1.4.1.1. Nature of damage and symptoms

The flies were a general scavenger in organic waste materials and break down organic food materials and release nutrients in to the soil. Occasionally the adult fly enters into the weak colonies of stingless bees and lay eggs inside the hives. Even though the adult fly never feed on the hive contents, the emerging maggot severely damaged the colony by tunneling through the brood, honey and pollen pots. The affected colonies were completely damaged and bees abandoned the hives. The infested colonies exhibit a foul smell due to the excreta of feeding maggots. The hive contents get transformed into black coloured entangled mass 2 weeks after infestation (Plate 13). The infestation of black soldier flies is generally seen in cool, moist and damp places.



13a. Adult fly



13b. Maggot of *Hermetia illucens* L.



Plate 13. Different life stages and damage symptoms of Black soldier fly
Hermetia illucens L.



Table 14. Other pests recorded from the stingless bee hives in Southern Kerala

Sl. No.	pests	Incidence			Infestation	
		TVM* ¹	KLM* ²	PTA* ³	Primary	Secondary
1	Black soldier fly <i>Hermetia illucens</i> L.	+	+	+	+	-
2	Pollen mites	+	-	+	+	
3	Ants	-	+	-	-	+

*¹ - Thiruvananthapuram *² - Kollam *³ - Pathanamthitta ' + ' Presence - Absence

4.1.4.2. *Ants*

Certain species of ants (unidentified) were found to infest stingless bee colonies as secondary feeders (Plate 14).

4.1.4.3. *Pollen mite*

The mites mainly infested pollen pots and successively they spread over the brood cell. The infested pollen pots were completely powdered (Plate 14).

4.2. BIOECOLOGY OF SMALL HIVE BEETLE

4.2.1. *Biology of E. latissima in Laboratory Condition*

Among the beetles collected from stingless bee hive, primary damage was observed only in the case of *E. latissima*. Hence biology of *E. latissima* was studied under laboratory conditions (Plate 15). The data pertaining to duration of different life stages of the beetle are illustrated in Table 15.

4.2.1.1. *Egg period*

The mean egg period of *E. latissima* was recorded as 2.2 ± 0.537 days. During the biology studies, some of the hive beetle eggs were infested with a predatory mite, which directly feeds on hive beetle egg content. Within two days after infestation the eggs were found damaged completely (Plate 16).

4.2.1.2. *Larval period*

The mean larval period of *E. latissima* was recorded as 14.2 ± 1.686 days including all the four recognizable larval instars.

4.2.1.3. *Pupal period*

The mean number of days from pupation to adult emergence was recorded as 17.77 ± 0.963 days.



14 a. Secondary infestation ants



14b. Egg laid by the ants inside the hive

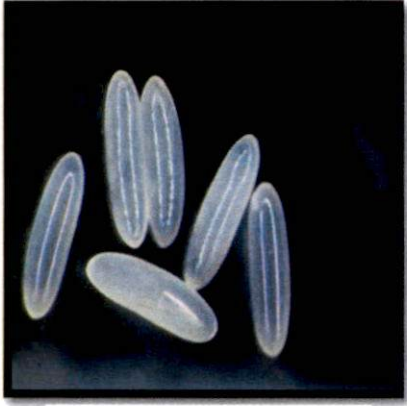


14c. Initial stages of pollen mite infestation



14d. Final stage of pollen mite infestation

Plate 14. Infestation of nest other than *E. latissima* in stingless bee colonies



15a. Egg



15b. Larva

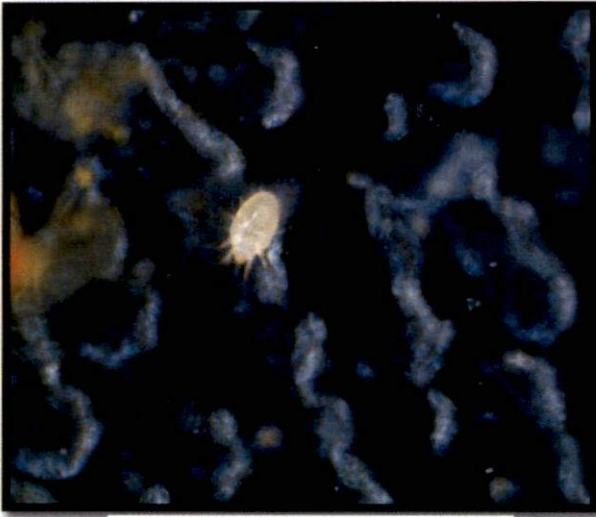


15c. Adult



15d. Pupa

Plate 15. Life cycle of *Epuraea latissima*



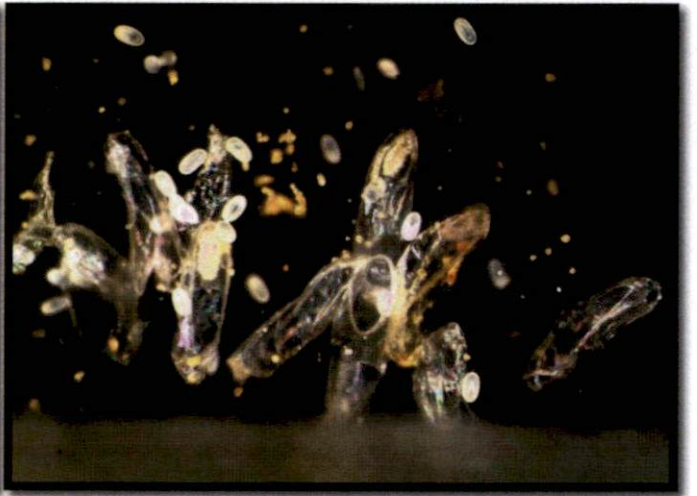
16a. Predatory mite



16 b. Direct feeding of *E. latissima* eggs by mites



16c. Initial stage of predation



16d. Final stage predation

Plate 16. Predation of *Epuraea latissima* eggs by mites

4.2.2. Morphological and Morphometric Characters of Different life stages of *E. latissima*

Data pertaining to morphometric characters of *E. latissima* are presented in Table 16 (Plate 17).

4.2.2.1. Egg

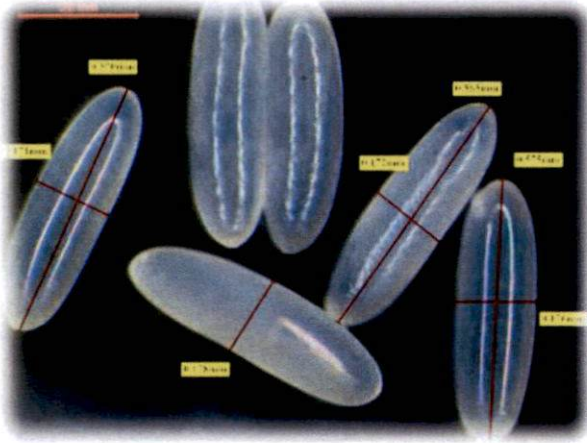
The mean length and width of hive beetle egg was 0.551 ± 0.037 mm and 0.173 ± 0.003 mm respectively.

The eggs were hyaline pearly white, deposited in irregular masses in the cracks and crevices of the hive. Sometimes they deposit eggs in the brood cells and pollen pots also. The eggs were glued to the substratum very loosely (Plate 18).

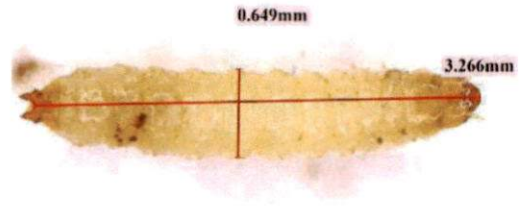
4.2.2.2. Larvae

The small hive beetle larvae showed four recognizable larval instars. The mean size of 10 randomly marked larvae of 1st, 2nd, 3rd and 4th instar of small hive beetle were recorded as $1.336 \pm 0.297 \times 0.199 \pm 0.059$ mm, $2.615 \pm 0.231 \times 0.581 \pm 0.159$ mm, $3.677 \pm 0.165 \times 0.742 \pm 0.136$ mm and $4.471 \pm 0.159 \times 1.062 \pm 0.025$ mm respectively.

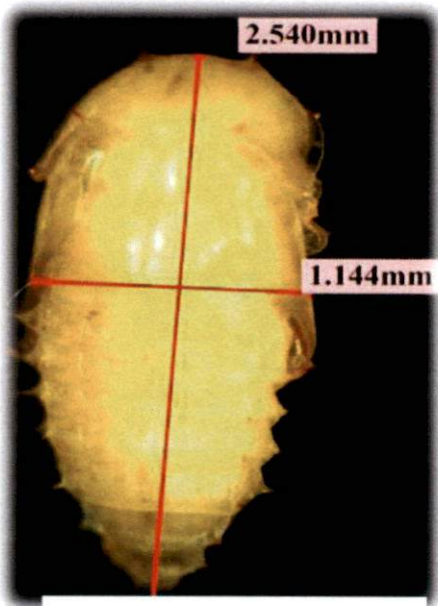
The larvae were pale white in colour, later pale yellow with sclerotized brownish head capsule. The larval body was dorso-ventrally flattened, sub cylindrical in appearance and possess three pairs of thoracic legs. The dorsal surface was provided with rows of hard spines along with dorsal spike at the end of the abdomen. It was considered as the distinguishable feature of beetles of this family (Plate 19 & 20).



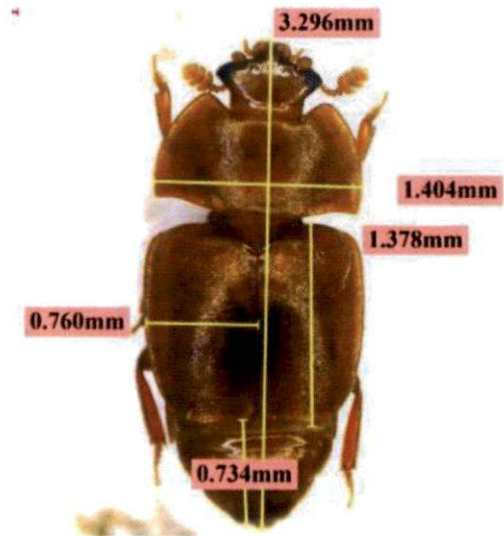
17a. Egg



17b. Larva



17c. Pupa



17d. Adult

Plate 17. Morphometrical observations on different stages of *E. latissima*

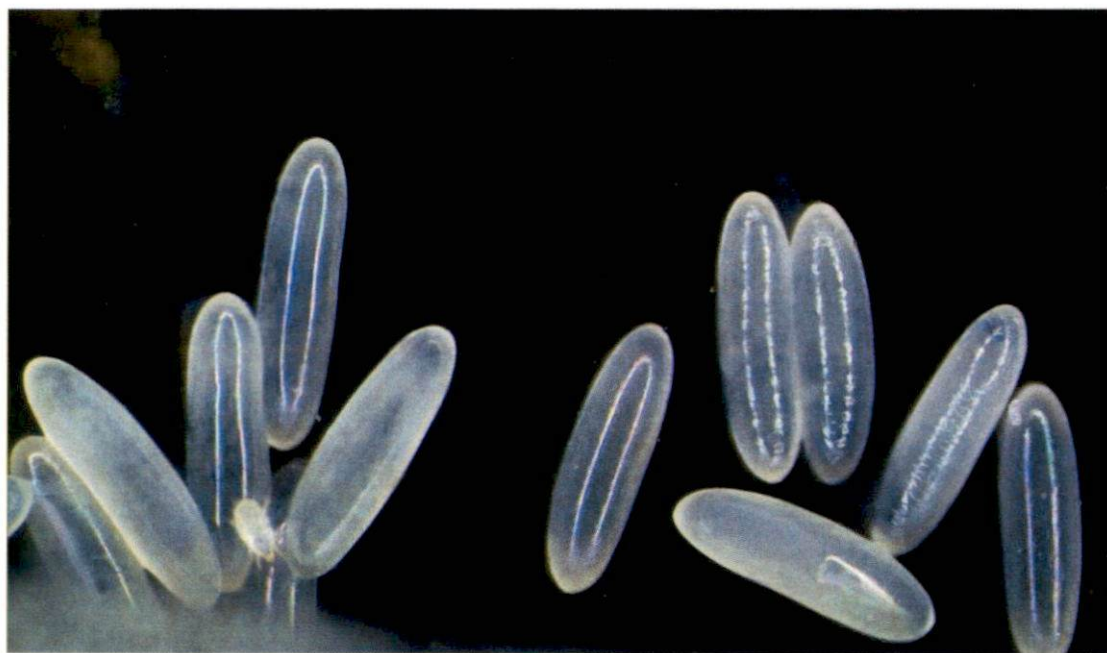


Plate 18. Eggs of *Epuraea latissima*



19a. Fully matured larva



19b. Rows of spines

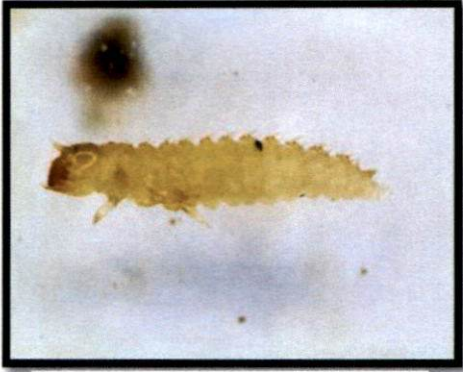


19c. Dorsal spine

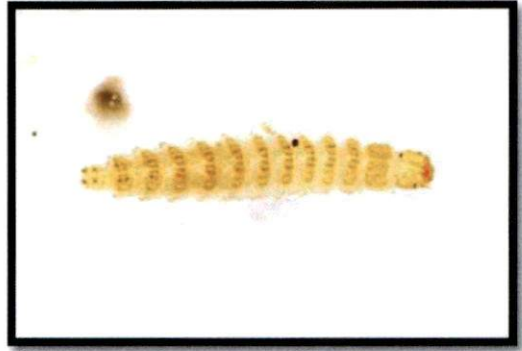


19d. Three pairs of forelegs

Plate 19. Morphological characters of *Epuraea latissima* larva



20a. 1st instar larva



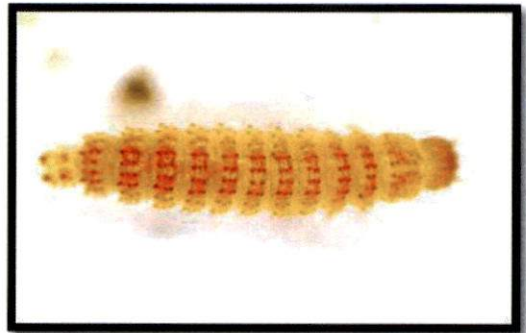
20b. 2nd instar larva



20c. 4 larval instars



20d. 3rd instar larva



20e. 4th instar larva

Plate 20. Different larval instars of *Epuraea latissima*

Table 15. Duration of life stages of *E. latissima*

Sl. No.	Life stages	Duration (days)*
1.	Egg	2.2 ± 0.537
2.	Larva	14.2 ± 1.686
3.	Pupa	17.77 ± 0.963
4.	Total (Egg to adult emergence)	33.2 ± 0.576

*- Mean±SD

Table 16. Morphometric parameters of different stages of *E. latissima*

Sl. No.	Life stages	Parameters *	
		Length (mm)	Width (mm)
1.	Egg	0.551±0.037	0.173±.003
2.	Larvae 1 st instar	1.336±0.297	0.199±0.059
	2 nd instar	2.615±0.231	0.581±.159
	3 rd instar	3.677±0.165	0.742±0.136
	4 th instar	4.471±0.159	1.062±0.025
3.	Pupa	2.515±0.123	1.1124±.044
4.	Adult	3.0897±0.454	1.378±0.0758

*- Mean±SD

4.2.2.3. Pupa

The pupae were exarate with the size ranging from $2.515 \pm 0.123 \times 1.1124 \pm 0.044$ mm. The head of the pupa bear two short erect spines immediately above the eyes. Apart from this, a series of spines are arranged in prothorax, femoro-tibial joints of all legs and either side of the abdomen. The pupation of the beetle takes place within the stingless bee hive. The pupation chamber was made up of pollen and cerumen (Plate 21).

4.2.2.4. Adult

The adult beetle (*E. latissima*) was dark brown in colour with a moderately convex body. The size of the beetle ranged from $3.0897 \pm 0.454 \times 1.378 \pm 0.0758$ mm. Antennae of the beetle was 11 segmented and was seen concealed under prothorax when not in use. The last three segments of the antennae form a distinct club, which makes them easily recognizable from other groups. Antennal grooves were also evident in adult beetles. The hard brownish elytra of the beetle were short with the last three abdominal segments exposed. Hind coxae were widely separated compared to fore and mid coxae (Plate 22).

4.3. ASSESSMENT OF EXTENT OF DAMAGE CAUSED BY *E. latissima*

4.3.1. Nature of Damage by Different Stages of *E. latissima*

All stages of small hive beetle were seen in decaying hive materials. Larvae and adults caused damage to the bee colony (Plate 23).

4.3.1.1. Damage caused by larvae

The damage caused by the larvae was more pronounced when compared with the adult beetles. The grubs tunnelled through the honey pots, pollen pots and brood cells. Honey breaks out of the honey pots due to larval feeding. The excreta and feeding activity of larvae caused discoloration and fermentation of honey. The grubs



21a. Dorsal view



21b. Ventral view



21c. Mature larvae start to pupate



21d. Pupation chamber

Plate 21. Pupal characters of *Epuraea latissima*



22a. Adult beetle



22b. Moderately convex body



22c. Antenna



22d. Concealed antennae under prothorax



23e. Widely spaced hind coxae



22f. Transparent hind wings

feed inside the pollen pots and periodically remove the pollen frass to outside. Sometimes the pollen pots and brood cells remained intact externally with feeding larvae inside.

4.3.1.2. Damage caused by adults

The adults caused relatively less damage to the bee colony. They mainly occupied the bottom portion of the hive and nibbled the hive contents.

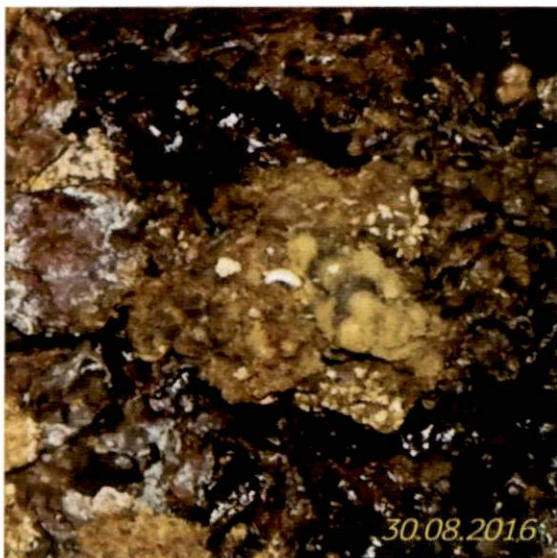
4.3.2. Time Taken by *E. latissima* for the Complete Destruction of Hive Materials

It was observed that the most preferred food by the beetle larvae was pollen. The time taken by *E. latissima* larvae to completely damage 4 cm³ volume of pollen pots, honey pots and brood cells was recorded (Table 17). The results showed that the mean number of days taken by the grubs for the complete destruction of pollen block was 16.00 days, followed by brood cells (13.50 days) and honey pots (10.83 days) (Plate 24).

4.3.3. Population Dynamics of *E. latissima*

The results of the studies on the population dynamics of the small hive beetle, *E. latissima* conducted from 1st fortnight of May 2016 to 2nd fortnight of May 2017 is presented in Table 18.

The population of small hive beetle (larvae) showed fluctuation throughout the study period. Population of the grubs of *E. latissima* were observed for a period ranging from 1st fortnight of May 2016 to 2nd fortnight of January 2017. The peak population of larvae was observed during 2nd fortnight of May, 2016 with 20.67 grubs cm⁻³ volume of pollen block. During that peak period, maximum number of larvae was in second instars, which caused more damage to the colonies. A pronounced grub population was also observed during 2nd fortnights of June and July 2016 and 1st



23a. Larvae tunneling through pollen pots



23b. Adult beetle nibbling the hive content

Plate 23. Damage caused by *E. latissima*



24a. Five days after the release of *E. latissima* larvae



25b. Eight days after the release of *E. latissima* larvae

Plate 24. Time taken for complete destruction of the pollen block

Table 17. Time taken for complete destruction of 4 cm³ volume of hive content

Sl. No.	Parameters	Time for destruction (days)
1.	Brood cells	13.50
2.	Pollen pots	16.00
3.	Honey pots	10.83

Table 18. Grub population of *E. latissima* at fortnight intervals under laboratory conditions.

Sl. No.	Month	Grub population (No of grubs cm ⁻³ volume of pollen block)
1.	I FN* May 2016	8.67
2.	II FN May 2016	20.67
3.	I FN June 2016	11.67
4.	II FN June 2016	19.67
5.	I FN July 2016	13.33
6.	II FN July 2016	15.00
7.	I FN Aug 2016	10.33
8.	II FN Aug 2016	7.33
9.	I FN Sep 2016	8.67
10.	II FN Sep 2016	8.00
11.	I FN Oct 2016	5.67
12.	II FN Oct 2016	5.67
13.	I FN Nov 2016	12.00
14.	II FN Nov 2016	6.00
15.	I FN Dec 2016	10
16.	II FN Dec 2016	9.33
17.	I FN Jan 2017	2.33
18.	II FN Jan 2017	0
19.	I FN Feb 2017	0
20.	II FN Feb 2017	0
21.	I FN March 2017	0

Table 18. continued

Sl. No	Fortnight	Grub population (No of grubs cm ³ volume of pollen block)
22	II FN March 2017	0
23.	I FN April 2017	0
24.	II FN April 2017	0
25.	I FN May 2017	0
26.	II FN May 2017	0

*- Fortnight

Table 19. Correlation coefficient of weather parameters with grub population of *E. latissima*

Parameters	Grub population
Grub population	1
Max temperature	-0.40385**
Rainfall	0.766371*
RH	0.351187**

*Significant at 5% level

** Significant at 1% level

fortnight of July 2016 where the mean number recorded was 19.67, 15.00 and 13.33 grubs cm^{-3} volume of pollen block respectively.

4.3.4. Correlation Studies

The fortnightly data of weather parameters *viz.*, maximum temperature, rainfall, relative humidity were correlated with the population of small hive beetle 2016-17

The results indicated that correlation coefficients between the population of *E. latissima* and weather parameters (Table 19.) showed a significant positive correlation with rainfall and relative humidity whereas the maximum temperature had a strong negative correlation with the population of *E. latissima*.

Discussion

5. DISCUSSION

The present investigation was conducted at AICRP on Honey Bees and Pollinators, Department of Agricultural Entomology, College of Agriculture, Vellayani to study the bioecology of small hive beetles and assessment of their damage in stingless bee colonies. The results obtained in the present study are discussed below:

5.1. ESTIMATION OF HIVE BEETLE INCIDENCE IN STINGLESS BEE COLONIES

A survey (100 colonies from each district) was conducted in three Southern districts (Thiruvananthapuram, Kollam and Pathanamthitta) of Kerala during 2015-17 to estimate the incidence of small hive beetle.

In the present study, seven different beetles were collected from the stingless bee hives of Southern Kerala. Out of which five beetles belong to the family Nitidulidae (*Epuraea latissima*, *Carpophilus marginellus*, *Carpophilus* spp (2 Nos.) and *Phenolia picta*, one to the family Tenebrionidae (*Palorinus humeralis*) and other belongs to Cryptophagidae. Among these beetles collected, primary infestation to the stingless bee colonies was noticed only in the case of *E. latissima*. Nitidulid beetle of the same genus, *Epuraea (Haptoncus) luteolus* was reported by Krishnan *et al.* (2015) which caused severe damage in the colonies by consuming brood, honey and pollen pots of multiple stingless bee species *Trigona thoracica*, *Heterotrigona itama* and *Tetragonula laeviceps* in Malaysia. Jayalekshmi (2015) also reported the incidence of a nitidulid beetle, *Aethina* spp which infests stingless bee colonies of Kerala. The small hive beetle, *E. latissima* was not reported as a pest of stingless bee in India hitherto.

The other nitidulids recorded during the study was found to infest stingless bee colonies as secondary feeders. This observation being strengthened by the results

of Torto *et al.* (2007) who asserted that fermenting hive products in hive beetle infested colonies attract other nitidulid beetles as secondary infestation. Secondary infestation of these nitidulids in stingless bee colonies was also a new record. The tenebrionid beetle *P. humeralis* was recorded as a pollen feeder (secondary infestation) in stingless bee colonies. The beetle has already been reported as a stored grain pest in Indonesia in milled rice and maize grains (Semple, 1986). But their association with stingless bee colonies has not been reported so far.

Studies conducted on monthly incidence of small hive beetle in Southern Kerala revealed that peak month of *E. latissima* infestation was June in the case of Thiruvananthapuram district. While in Kollam district, it was recorded in the month of June and July and in Pathanamthitta district in the month of May. From the study, it was asserted that the incidence of *E. latissima* was more prominent in apiaries of Southern Kerala during rainy season (Fig 1.). According to Arbogast *et al.* (2010), the peak period of infestation of small hive beetle (*A. tumida*) was May and June (spring and summer months). Similarly, the studies conducted by Torto *et al.* (2010) from Kenya are in agreement with the results obtained in the current study. They also revealed that small hive beetle (*A. tumida*) was abundantly caught from apiaries during the rainy season.

In the present study, maximum percentage of hive beetle (*E. latissima*) incidence was recorded from Kollam (19 %) followed by Thiruvananthapuram (16 %) and Pathanamthitta (3 %) (Fig 2.). Jayalekshmi (2015) reported the incidence of hive beetle, *Aethina* spp from the Kottayam (4 %) and Idukki (5 %) districts, while Thiruvananthapuram, Kollam and Pathanamthitta districts were free from hive beetle infestation. This infers that over the years, hive beetle incidence has been spreading to new areas.

Studies on the influence of type of hives on incidence of hive beetles are scanty. In Kerala, where meliponiculture was flourishing more rapidly, beekeepers

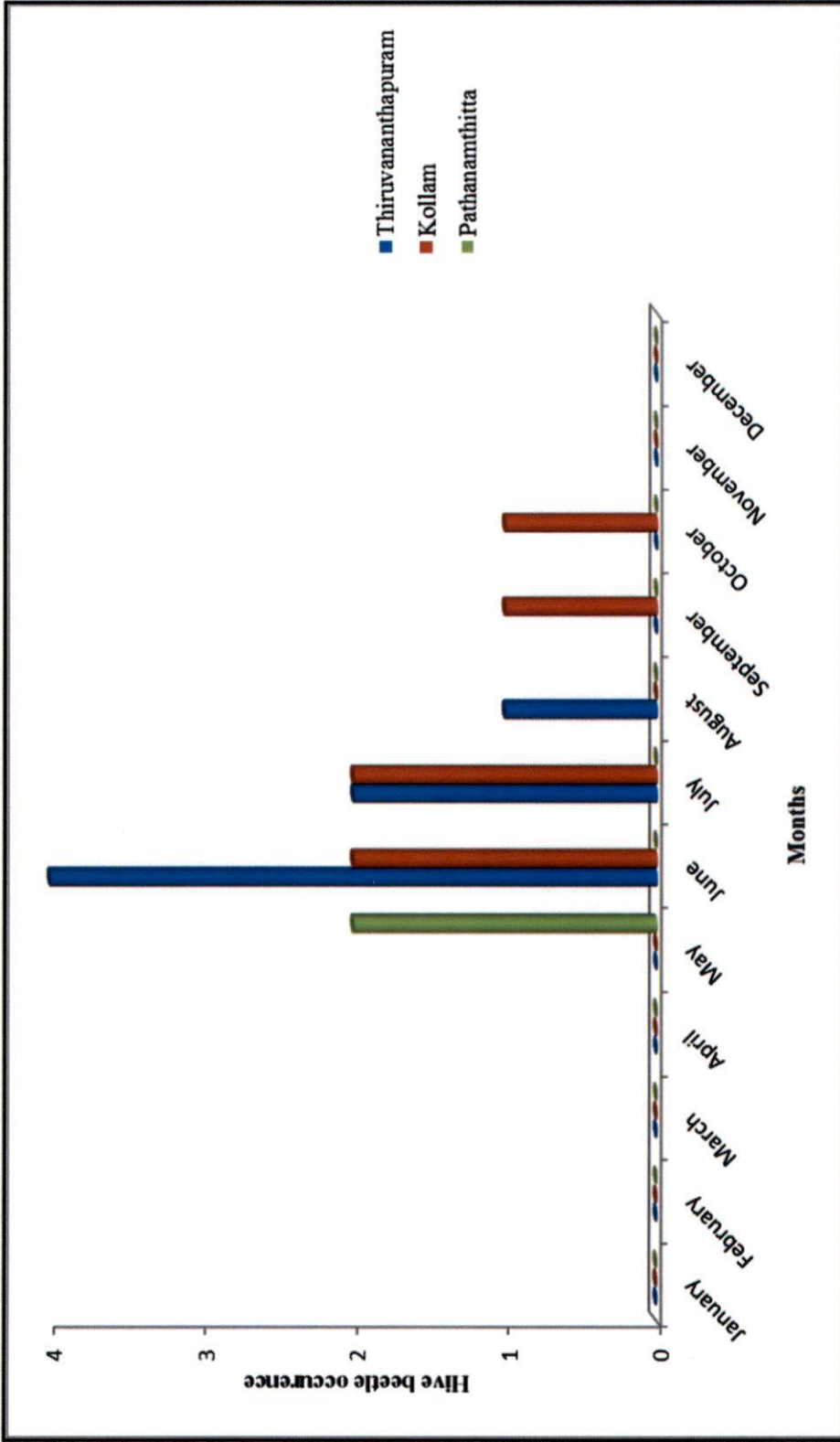


Fig 1. Monthly incidence of *E. latissima* in Southern Kerala

themselves are experimenting hives of different types for a pronounced yield. Though different types of hives are used by the beekeepers, wooden boxes are mostly preferred by them throughout the three districts (Fig. 3). When we take the percentage incidence of small hive beetles in relation to hive types, highest incidence was recorded from the wooden boxes in all the three districts (Table 7, 8 and 9).

The small hive beetle incidence was more prominent in bad quality wooden boxes which were made from the locally available woods *viz.*, *Clerodendron* spp, mango, pine wood while that made of good quality wood of teak was free of the hive beetle infestation. Such type of poor quality wood is more liable to absorb moisture. During rainy season, bad quality wood absorbs moisture which enables a favourable condition for the hive beetle multiplication. During summer months these types of woods undergo contraction and expansion which leads to the formation of cracks and crevices through which the hive beetle may enter into the hives, facilitating oviposition and further multiplication.

Generally colonies with good strength are not attacked by enemies. For good colony strength, the material used for hive construction is an important factor. In the present study, small hive beetle incidence was meagre in bamboo hives. Nisha (2002) conferred that bamboo hives were highly suited for stingless beekeeping, since the brood development, honey and pollen storage were higher in bamboo hives. The brood development in this type of hive is maximum resulting in a strong colony which has more number of worker bees. Such a colony can actively defend the intrusion of small hive beetle into the stingless bee colonies. This reason might have contributed to less incidence of hive beetle in bamboo hives.

Another aspect of the study was on the influence of height of the hive from the ground level on the incidence of small hive beetle. Based on the survey conducted during the study period, majority of beekeepers in Southern Kerala preferred to maintain stingless bee hives in 3-4 m height (40.33 %) (Fig 4.) followed

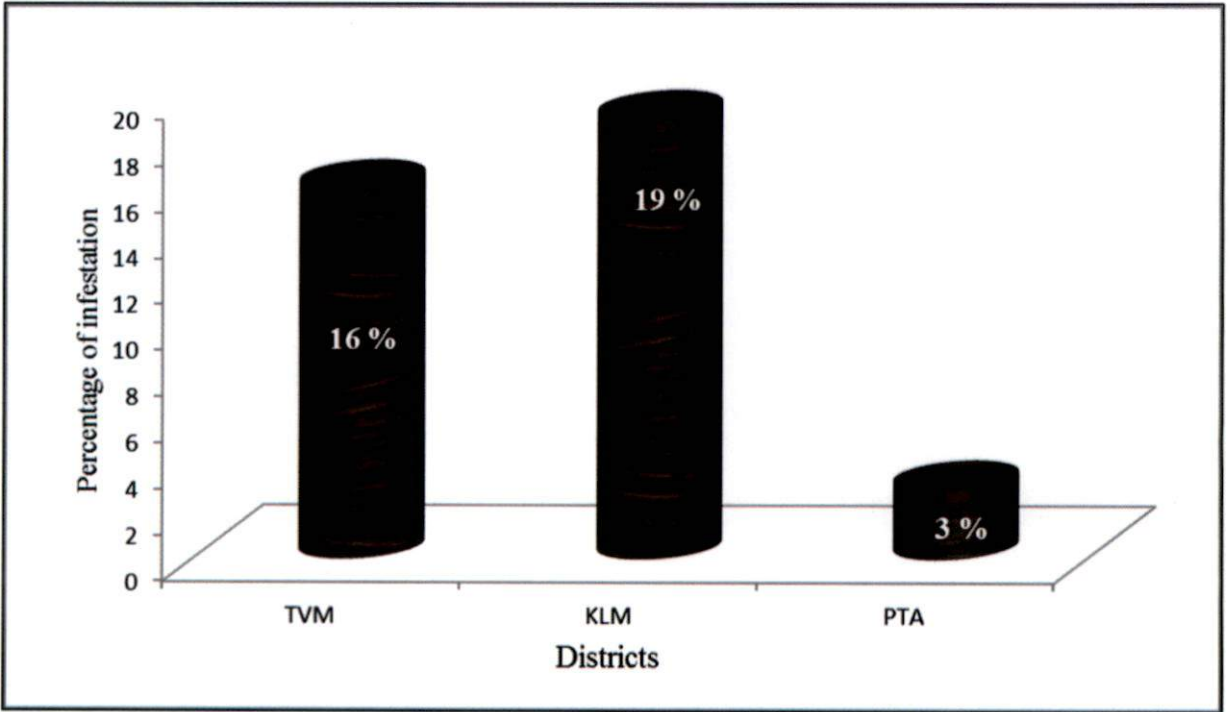


Fig. 2. Incidence of small hive beetle (*E. latissima*) in Southern Kerala

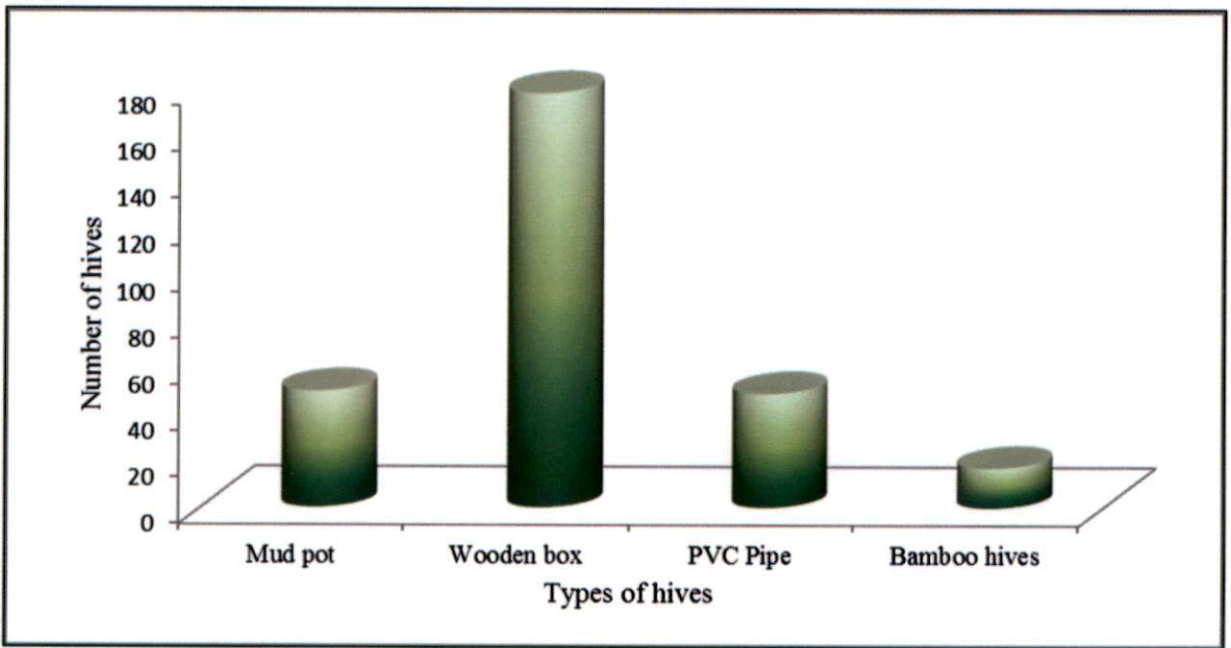


Fig 3. Types of hives used by beekeepers of Southern Kerala

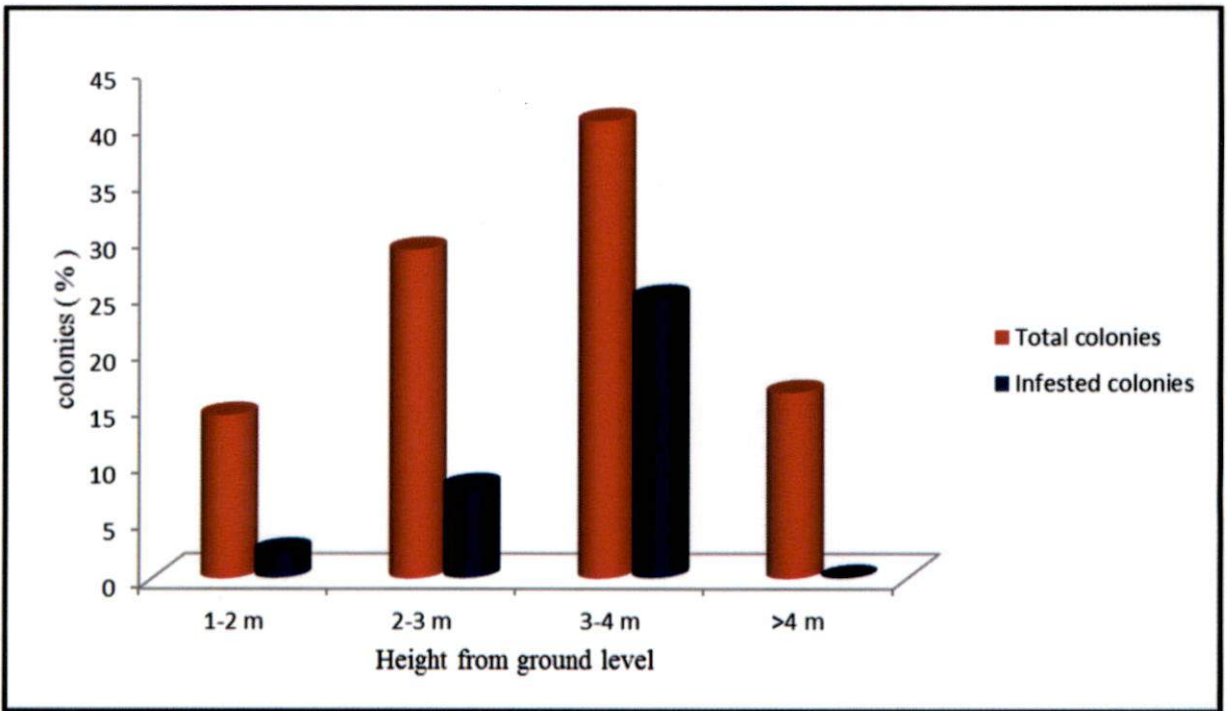


Fig. 4. *E. latissima* incidence in apiaries with different height from ground level

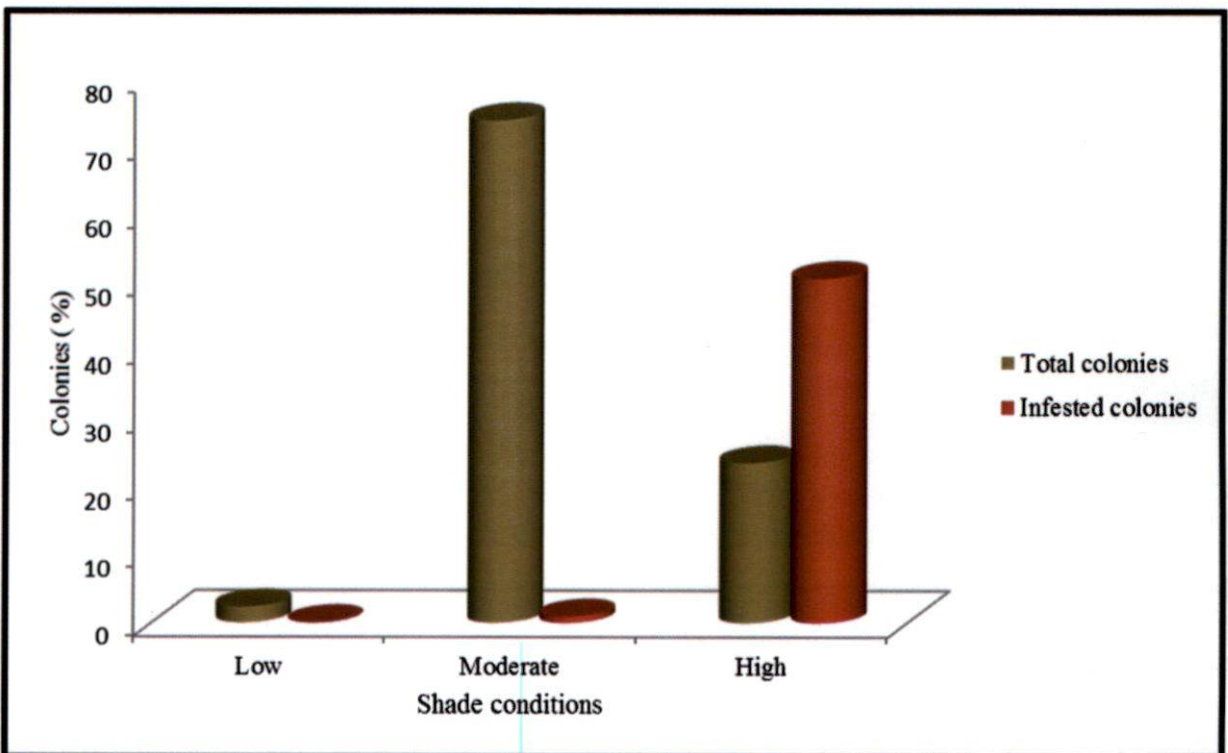


Fig. 5. *E. latissima* incidence in apiaries with different shade conditions

by 2-3 m (29 %), > 4 m (16.33 %) and 1-2 m (14.33 %). Management of stingless bees is not much strenuous as that of the Indian honey bee, where we need to open the hive for two times in a year *i.e.*, the honey harvesting (April) and colony division (October-November) time. So the beekeepers place stingless bee hive on sunshades while some hang the hives in the support from the sunshade (3 - 4 m) and also on the terraces (> 4 m) so that they can also avoid other disturbance at the ground level. In the present study, highest hive beetle incidence was recorded from the stingless bee colonies maintained at a height of 3-4 m (24.79 %) from the ground level, followed by 2-3 m (8.04 %). No hive beetle incidence was recorded in the colonies maintained at a height > 4 m. The results of the study are in line with the findings of Jayalekshmi (2015) who reported that pest and disease problems were more prominent in apiaries with the hives placed at height of 2.7 m to 3.3 m. The more pronounced incidence of small hive beetle may be due to the flight behaviour of small hive beetle. Further future line of studies can only reveal the exact relation between flight behaviour and hive beetle incidence at this specified height.

Shade by the canopy is an important factor which determines the pest incidence. Of the shades categorized based on the visual scoring of canopy (Table 12.), 74 per cent of the colonies were placed in moderate shade condition followed by high (23.66 %) and low (2.34 %) shade conditions. Majority of hive beetle incidence (50.70 %) were recorded from the apiaries with high shade conditions (> 80 % canopy coverage) (Fig 5.). Small hive beetles were not recorded in apiaries with low shade conditions in all the three districts. This was a first attempt to find out the effect of shade condition on hive beetle incidence hence no other work has been found so far in this regard. Annand (2011) reported that an optimum relative humidity greater than 65 per cent is required for rapid hatching of small hive beetle eggs. The high shade condition in the apiary results in increased relative humidity which might have contributed for high incidence.

The important symptoms of damage recorded from *E. latissima* infested hives were tunnels through honey pots, pollen pots and brood cells, fermentation and discolouration of honey due to the feeding and defecation by the beetle larvae and finally a light brown coloured undistinguished mass of hive contents. These findings are endorsed by earlier reports of Krishnan *et al.* (2015) where he reported that *Eपुरaea (Haptoncus) luteolus* from multiple stingless bee species *Trigona thoracica*, *Heterotrigona itama* and *Tetragonula laeviceps* in Malaysia caused severe damage symptoms in the colonies by consuming brood, honey and pollen pots. The *Aethina* spp which infested stingless bee colonies of Kerala (Jayalekshmi, 2015) also produced similar damage symptoms. Many of the beekeepers are not aware of the symptoms of beetle infestation as they are not much prominent during the initial stages of infestation. Once the honey pots are infested the beekeepers came to know about the incidence where the honey breaks out of the pots which start fermenting and finally come out of the hive. Unless the fermentation odour of honey occurs, the colony will be retained as such. In some cases the number of foraging bees also gets reduced.

Apart from this, *Hermitia illucens*, pollen mite and ants were also observed as pests of stingless bee colonies. In *H. illucens* infested colonies, maggot severely damaged the colony by tunnelling through the brood, honey and pollen pots. The infested colonies exhibit a foul smell due to the excreta of feeding maggots. The hive content gets transformed into black coloured mass within two weeks after infestation. *H. illucens* has already been reported as a pest from stingless bee colonies of Kerala by Nisha (2002) and the recorded damage symptoms also corroborate with studies conducted by Jayalekshmi (2015).

The pollen mites found to infest the pollen pots of stingless bee colonies and infested pots were completely converted into a powdered mass. The infestation of pollen mites (*Amblyseius* spp) has already been reported from stingless bee colonies

of Kerala by Nisha (2002). The damage symptoms of mite recorded from the present study was in agreement with the previous findings.

During the study, a species of ant (unidentified) was observed as secondary pest in stingless bee colonies of Southern Kerala. Studies conducted by AICRP (2004) recorded ants (*Solenopsis geminata*) from the stingless bee colonies, which were found to enter into the hives just after colony division. The ants observed in the present study was not the one already described, since they are secondary feeders.

With regard to study on the food sources (nectar and pollen) of stingless bees in and around the apiaries, it was revealed that out of the 31 foraging plants visited by the stingless bees, 12 were nectar providers, 9 were pollen providers, whereas 10 plants were both nectar and pollen providers. Foraging plants of the family Euphorbiaceae was prominent in all the apiaries that were selected for the study. Stingless bees also depend on these plants for the collection of propolis. The alkaloid present in this propolis is an important factor which determines the quality of the honey as well as the defense behaviour of the bees against the hive intruders.

5. 2. BIOECOLOGY OF SMALL HIVE BEETLE

Study on the biology of *E. latissima* infesting stingless bee colonies was not attempted earlier. In the present study it was observed that the egg period, larval and pupal period of the beetle is 2.2 ± 0.537 , 14.2 ± 1.686 and 17.77 ± 0.963 days respectively. Regarding the morphometric studies, the length and width of egg, larva, pupa and adult was recorded as $0.551 \pm 0.037 \times 0.173 \pm 0.003$ mm, $4.471 \pm 0.159 \times 1.062 \pm 0.025$ mm, $2.515 \pm 0.123 \times 1.1124 \pm 0.044$ mm and $3.0897 \pm 0.454 \times 1.378 \pm 0.0758$ mm respectively.

Morphological characters of the eggs, larvae, pupa and adult of *E. latissima* corroborate with the previous findings of Lundi (1940) on eggs and Hood (2000) on larvae of *Aethina tumida*, nitidulid beetle infesting the *Apis* spp. While in the case of pupa, though the characters were almost same, pupation of *A. tumida* was reported in

the soil outside the hive. But in the present study, the pupation was observed within the hive itself (Haque and Levot, 2005). Ellis *et al.*, (2002) described the morphometric characters of adult small hive beetles (*A. tumida*) in USA. He reported that the adult beetles of *A. tumida* possess 5-7 mm in length and 3.2 mm width where the beetle was found to be larger than *E. latissima*.

5. 3. ASSESSMENT OF EXTENT OF DAMAGE CAUSED BY SMALL HIVE BEETLE

Studies on the nature of damage caused by different stages of the beetle revealed that the larvae caused more pronounced damage by tunneling through the honey pots, pollen pots and brood cells. Honey breaks out of the honey pots due to larval feeding. The excreta and feeding activity of larvae cause discoloration and fermentation of honey. The adult cause relatively lesser damage to stingless bee colonies, which nibble the hive contents. These were in accordance with findings of Jayalekshmi (2015) who reported the nature of damage caused by the nitidulid beetle which infested stingless bee colonies.

In the present study on the assessment of population and damage by small hive beetle revealed that for complete destruction of pollen pot, brood cells and honey pot of uniform block size (4 cm^3) the small hive beetle grubs took 16.00 , 13.50 and 10.83 days respectively. The compact nature of pollen block might have resulted in time consumption for the complete destruction of the hive, since the beetle larvae took less number of days (10.83 days) to damage the 4 cm^3 honey block completely.

Studies on the correlation between grub population of *E. latissima* and weather parameters showed a significant positive correlation with rainfall and relative humidity (Fig 6.). The positive correlation of grub population of *E. latissima* with rainfall is supported by the findings of Anand (2011) who reported that the small hive beetle *A. tumida* followed a cyclic pattern, which peaked in rainy season then declined through winter and recorded a minimum in late spring. He also reported that

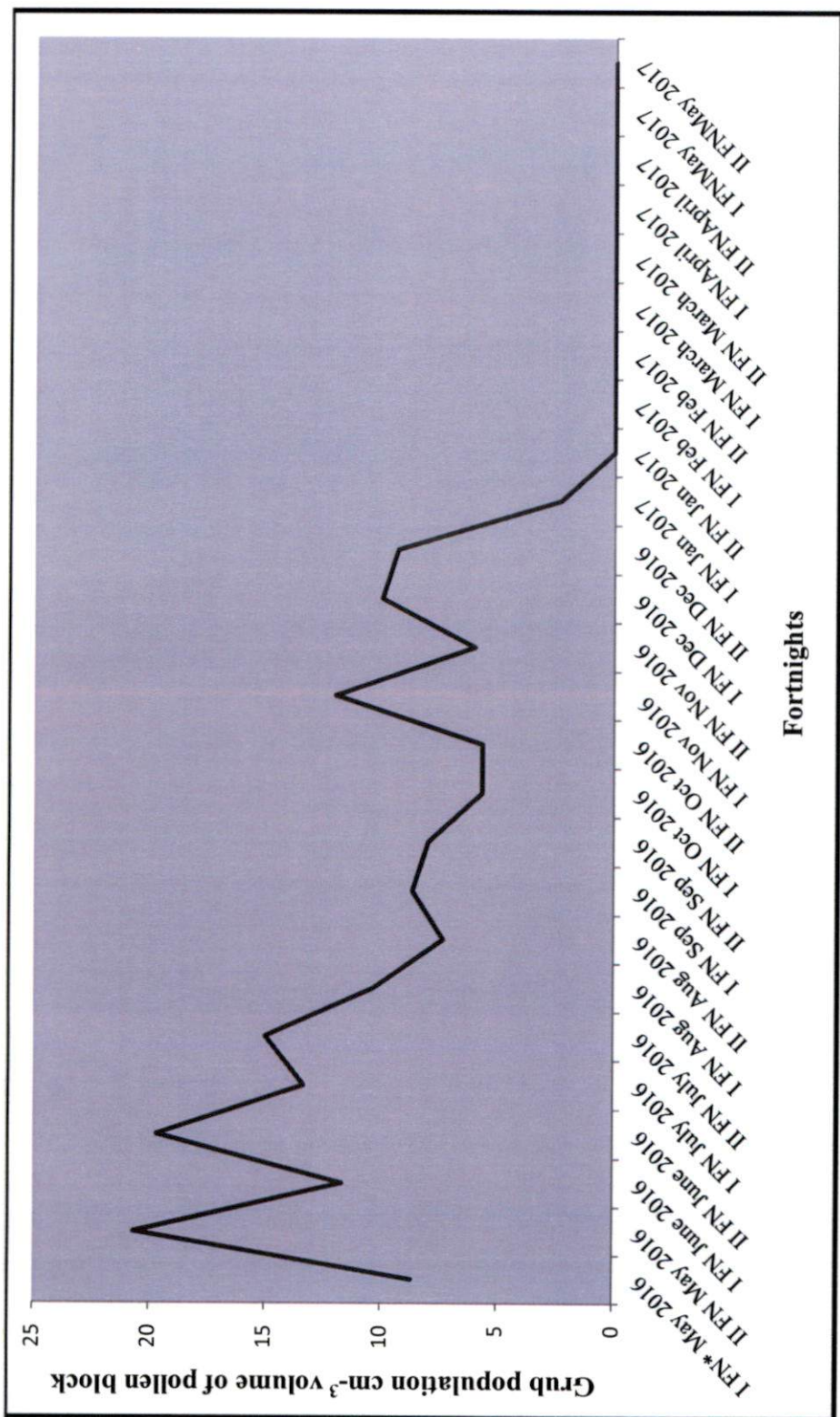


Fig.6. Grub population of *E. latissima* at fortnight intervals under laboratory conditions
 *FN- Fortnight

an optimum relative humidity greater than 65 per cent is required for rapid hatching of small hive beetle eggs while relative humidity less than 34 per cent is detrimental to the survival of eggs. Cuthbertson *et al.* (2013) also confirmed that relative humidity has a profound effect on the viability of *A. tumida* eggs. Decrease in relative humidity reduced the viability of hive beetle eggs. These conditions are different in USA where Somerville (2003) who asserted that survival rates of small hive beetle larvae rapidly decreased with dropping temperature.

Summary

6. SUMMARY

The present investigation on “Bioecology of small hive beetles and assessment of their damage in stingless bee colonies” was carried out as three separate experiments, which includes, estimation of small hive beetle incidence in Southern Kerala (Thiruvananthapuram, Kollam and Pathanamthitta), bioecology of small hive beetle and assessment of population and damage by small hive beetle at AICRP on Honey Bees and Pollinators, Department of Agricultural Entomology, College of Agriculture, Vellayani. The study was conducted with an objective to study the incidence, bioecology and the extent of damage by small hive beetles in stingless bee colonies. Ten locations each with ten apiaries from each districts were selected for the study.

Seven different beetles belonging to the family Nitidulidae, Tenebrionidae and Cryptophagidae were collected from the selected apiaries in Southern Kerala. Among this, *E. latissima* was identified as the primary pest infesting the stingless bee colonies and its incidence was recorded from all the three districts. Studies on the monthly incidence of the small hive beetle revealed that the peak incidence of hive beetle was during the months of May, June and July. In Thiruvananthapuram, peak period of infestation was recorded in the month of June whereas in Kollam district, peak period of infestation was noted as June and July. However, from Pathanamthitta district, incidence was recorded only in the month of May. Highest incidence of small hive beetle was recorded from Kollam (19 %), followed by Thiruvananthapuram (16 %) and Pathanamthitta (3 %) districts.

Majority of bee keepers maintained stingless bee colonies in wooden boxes (59.33 %) followed by mud pots (18.66 %), PVC pipes (16.33 %) and bamboo hives (5.66 %). Regarding the incidence of hive beetle in hive types, Highest infestation was recorded from stingless colonies maintained in wooden boxes particularly, bad quality wooden boxes made up of locally available woods in Thiruvananthapuram

(60 %), Kollam (50 %) and Pathanamthitta (15 %) districts. Colonies maintained in PVC pipes and bamboo hives were free of *E. latissima* infestation.

With regard to the influence of height of the hive on beetle incidence, it was found that the hives maintained at 3-4 m recorded the highest incidence of hive beetle (24.79 %) followed by 2-3 m (8.04 %). None of the colonies maintained at a height > 4 m had hive beetle incidence in all the three districts

Shade conditions in the apiaries also had a profound influence on small hive beetle incidence where apiaries with high shade (> 80 % canopy coverage) conditions recorded highest hive beetle incidence (50.70 %) followed by the moderate shade (30-80 % canopy coverage) conditions (0.90 %). Hive beetle incidence was absent in apiaries with low shade conditions in all the three districts.

A total of 31 plants were recorded as potential foraging plants of stingless bees in three Southern districts of Kerala. Out of which, 12 were nectar providers, 9 were pollen providers, whereas 10 plants were both nectar and pollen providers.

As far as the damage symptoms are concerned, fermentation and discolouration of honey due to the feeding and defecation by the beetle larvae and finally a light brown coloured undistinguished mass of hive contents is the prominent one noticed in the hives infested with *E. latissima*

Other pest species recorded from the stingless bee colonies are Black soldier fly *Hermitia illucens*, ants and pollen mites.

Regarding the biology studies, egg, larval and pupal period of the beetle was recorded as 2.2 ± 0.537 , 14.2 ± 1.686 and 17.77 ± 0.963 days respectively. The length and width of egg, 1st instar larva, 2nd instar larva, 3rd instar larva, 4th instar larva, pupa and adult was recorded as $0.551 \pm 0.037 \times 0.173 \pm 0.003$ mm, $1.336 \pm 0.297 \times 0.199 \pm 0.059$ mm, $2.615 \pm 0.231 \times 0.581 \pm 0.159$ mm, $3.677 \pm 0.165 \times 0.742 \pm 0.136$ mm,

4.471 ± 0.159 × 1.062 ± 0.025mm, 2.515 ± 0.123 × 1.1124 ± 0.044mm and 3.0897 ± 0.454 × 1.378 ± 0.0758 mm respectively. Apart from these, the morphological characters of the different stages were also observed where the eggs were hyaline pearly white, larvae pale white with sclerotized brownish head capsule, exarate pupa in pupation chamber and dark brown adult with 11 segmented antennae.

Studies on the assessment of damage and population by small hive beetle revealed that for complete destruction of pollen pot, brood cells and honey pot of uniform block size (4cm³) the hive beetle grubs took 16.00, 13.50 and 10.83 days respectively. The hive beetle population was recorded for a period ranging from 1st fortnight of May to 2nd fortnight of January whereas the peak population was observed during 2nd fortnight of May, 2016 with 20.67 grubs/cm³ volume of pollen block.

Regarding the correlation studies, the beetle population had a significant positive correlation with the rainfall and relative humidity, whereas the temperature (maximum) showed a strong negative correlation.

Thus the present study clearly revealed that the small hive beetle which caused severe damage to stingless bee colony is the nitidulid beetle, *E. latissima*. The incidence was more prominent in stingless bee colonies maintained in bad quality wooden boxes, placed at high shade conditions (> 80 per cent canopy) and also those kept at a height of 2-3 m. Among the weather parameters, high rainfall and relative humidity predisposes hive beetle (*E. latissima*) infestation in the apiaries.

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**BIOECOLOGY OF SMALL HIVE BEETLES AND
ASSESSMENT OF THEIR DAMAGE IN STINGLESS BEE
COLONIES**

by

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Abstract of the thesis

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ABSTRACT

The study entitled “Bioecology of small hive beetles and assessment of their damage in stingless bee colonies” was conducted with the objectives to study the incidence, bioecology and the extent of damage by small hive beetles in stingless bee colonies. The study was conducted during the period 2015-2017 at Department of Agricultural Entomology, College of Agriculture, Vellayani.

A survey was conducted in Southern districts of Kerala viz., Thiruvananthapuram, Kollam and Pathanamthitta. Seven beetles (five nitidulids, one cryptophagid and one tenebrionid) were collected from the hives during the survey. Among these, the nitidulid beetle which caused the primary infestation is identified as *Epuraea latissima* Kirejtshuk and it is the first report from stingless bee hive. Monthly incidence of the hive beetle revealed that peak period of infestation was recorded from Thiruvananthapuram in the month of June, whereas in Kollam district, it was during June and July. However, the incidence was recorded only during the month of May from Pathanamthitta district. Among the three districts, highest incidence of small hive *E. latissima* beetle was recorded from Kollam (19.00 %), followed by Thiruvananthapuram (16.00 %) and Pathanamthitta (3.00 %).

The type of hive used for maintaining the stingless bee colony is an important factor which determines the hive beetle incidence. Majority of bee keepers maintained stingless bees in wooden box (59.33 %) followed by mud pots (18.66 %). Among the wooden boxes, the incidence was more prominent in bad quality wooden boxes (made of locally available soft wood) in Thiruvananthapuram (60 %), Kollam (50 %) and Pathanamthitta districts (15 %). Studies on the influence of height of the stingless bee hive from the ground level with the hive beetle incidence revealed that the hives kept at 3-4 m recorded the highest incidence (24.79 %) followed by 2-3 m (8.04 %). The colonies maintained at a height of > 4 m were free from hive beetle incidence. Regarding the incidence of small hive beetle in different shade conditions, highest hive beetle incidence (50.70 %) was recorded from apiaries under high shade

condition (>80 % canopy coverage) followed by moderate (30-80 % canopy coverage) shade condition (0.90 %).

The damage symptoms recorded from infested colonies were fermentation and discolouration of honey due to the feeding and defecation by the beetle larvae and a light brown coloured undistinguished mass of hive contents. Apart from the small hive beetles, black soldier fly, *Hermetia illucens* L., pollen mites and ants were also documented as pests of stingless bees from the apiaries of Southern Kerala.

Regarding the studies on biology of the hive beetle, egg period, larval and pupal period of the beetle was recorded as 2.2 ± 0.537 , 14.2 ± 1.686 and 17.77 ± 0.963 days respectively. The length and width of egg, larva, pupa and adult was recorded as $0.551 \pm 0.037 \times 0.173 \pm 0.003$ mm, $4.471 \pm 0.159 \times 1.062 \pm 0.025$ mm, $2.515 \pm 0.123 \times 1.1124 \pm 0.044$ mm and $3.0897 \pm 0.454 \times 1.378 \pm 0.0758$ mm respectively.

The larvae caused severe damage by tunneling and feeding the pollen pots while the adults nibbled the hive contents. Studies on the correlation between larval population and weather parameters showed a significant positive correlation with rainfall and relative humidity. Highest population of grubs was recorded on 2nd fortnight of May. The time taken by the grubs for complete destruction of pollen block (4 cm^3) was recorded as 16 days, whereas for the destruction of brood cells and honey block of 4 cm^3 , it took 13.50 days and 10.83 days respectively.

The small hive beetle which primarily infested the stingless bee colonies is identified as the nitidulid beetle, *E. latissima*. The hive beetle incidence can be reduced by using good quality wooden boxes, where the bad quality wooden boxes along with high rainfall predisposes hive beetle incidence in the apiaries.

Appendices

APPENDIX 1
SURVEY SHEET

Estimation of small hive beetle in stingless bee colonies

Sl. No	Parameters	Observations
1	Date of collection	
2	District name	
3	Location name	
3a.	Altitude	
3b.	Latitude	
4	No of colonies	
5	No of infested colonies(with hive beetle)	
6	Infested colonies due to other reasons	
7	Symptoms of damage -Damage to brood -Damage to pollen source -Damage to honey and adult bees	
8	Other conditions of apiary	
9	Shade conditions	
10	Hive material/ type of wood	
11	Hive beetle incidence in different hives	
12	Height of the hive from ground level	
13	Other pests	
14	Foraging plants in the area	
15	Previous hive beetle incidence(Month)	

APPENDIX II

Fort night weather data from May 2016- May 2017

Fortnight	Maximum temperature(°C)	Rainfall (mm)	Relative humidity(%)
I FN May 2016	35.41	21.3	89.29
II FN May 2016	33.67	369	94
I FN June 2016	32.92	73	89
II FN June 2016	29	359.2	95.5
I FN July 2016	31.4	82.8	93.71
II FN July 2016	31.82	87.6	92.78
I FN Aug 2016	31.42	38.9	93.21
II FN Aug 2016	31.74	7	90.28
I FN Sep 2016	31.85	30.4	91.57
II FN Sep 2016	31.59	2.2	91.21
I FN Oct 2016	31.97	2.6	91.57
II FN Oct 2016	31.73	0	91
I FN Nov 2016	31.81	200	89.14
II FN Nov 2016	31.73	30	91.93
I FN Dec 2016	31.84	2	91.93
II FN Dec 2016	32.09	34.2	93.5
I FN Jan 2017	32.74	9.8	95.56
II FN Jan 2017	32.52	18.8	93.8
I FN Feb 2017	31.9	0	94.07
II FN Feb 2017	32.99	6	92.29

I FN March 2017	33.06	10	94.29
II FN March 2017	33.3	0	87.64
I April 2017	34.1	26.6	86.93
II April 2017	34.01	0.8	89.29
I May 2017	33	0	88
II May 2017	32	34	88

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