ORIGIN AND COMPOSITION OF STINGLESS BEE PROPOLIS

By

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THESIS

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2023

DECLARATION

I, hereby declare that this thesis entitled "**ORIGIN AND COMPOSITION OF STINGLESS BEE PROPOLIS**" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled "**ORIGIN AND COMPOSITION OF STINGLESS BEE PROPOLIS**" is a record of research work done independently by Mr. Abhijith R L (2020-11-053) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

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LIST OF ABBREVATIONS AND SYMBOLS USED

@ - At the rate of

°C - Degree Celsius

CD - Critical Difference

CRD - Completely Randomised Design

et al. - And others

g - Gram

g^{-1}- Per gram

h - Hour

L- Litre

m – Metre

m^2 – Square metres

cm^2- Square centimetres

m^{-2} - Per meter square

mg - Milligram

mL- Millilitre

mL^{-1}- Per milliliter

mm- Millimetre

kPa- Kilopascal

rpm - Rotations per minute

Fig.- Figure

sp. or spp.- Species (singular and plural)

min - Minutes

viz.- Namely

rpm - Revolutions per minute

LC-HRMS - Liquid chromatography coupled with high resolution mass spectrometry

GC-HRMS - Gas chromatography coupled with high resolution mass spectrometry

INTRODUCTION

1. INTRODUCTION

Meliponini (stingless bees) are a monophyletic group of eusocial insects that are part of a larger group known as the corbiculate bees (Hymenoptera: Apidae), which includes honey bees, bumble bees and orchid bees. The smallest of the honey producing bees, stingless bees are typically found in tropical and subtropical regions of the world. Over 500 different species of stingless bees have been identified worldwide (Michener, 2013). In the Indian subcontinent, stingless bees are usually referred to as "dammer bees" or "dammar bees" where, dammar is a resin found among dipterocarp trees and is with the regional name of “arakki" in Kerala (Nair, 2003). Shanas and Faseeh (2019) reported that *Tetragonula travancorica* Shanas and Faseeh are the most common species in peninsular India.

Stingless bees primarily forage for pollen and nectar, but they also collect substances like plant resin, water, etc. Resin is a substance that plants release from their wounds, buds, flowers and fruits for protection, pollination, etc. Stingless bees forage on natural resin secretion or wounds made by bees using their mandibles for days or even weeks.

Propolis or bee glue is a resinous mixture that honey bees produce by mixing beeswax with exudates gathered from various botanical sources like birch, poplar, pine, alder, willow, palm, mango, etc. The term propolis was derived from the Greek words ‘Pro’ which means ‘‘in front of’’ and ‘polis’ which means ‘community’ or ’city’ (Toreti *et al*., 2013) meaning hive defensive substance. Worker bee secretes wax which is mixed with resin collected from various trees especially redwood, jack and breadfruit forming a dark-colored sticky substance called cerumen (Marisa and Salni, 2012). Generally, propolis is composed of 50% resin and vegetable balsam, 30% wax, 10% essential oil and aromatics, 5% pollen and 5% other substances.

A single propolis sample can contain more than 300 components because the plant source and, consequently, the chemistry of the bee glue is dependent on the specificity of the local flora at the site of collection, which varies depending on geographic location (Bankova *et al.,* 2008). The bees utilize it for building the nest, brood, honey pot, pollen pot, pillars, connectives, entrance tube and internal canal and to cover gaps in their honeycombs and safeguard the hive entrance. Scientific studies attribute propolis with a broad range of beneficial health effects including antiviral, antibacterial, antioxidant, antifungal, anticancer and anti-inflammatory activities due to flavonoids, aromatic acid and phenolic compounds in it. Hence, it is widely used in complementary and alternative medicine, in food and beverages to improve health and to prevent diseases such as heart disease, inflammation, diabetes and cancer.

However, still, it is an underutilized substance in our country. More research is needed to find the hidden properties of stingless bees propolis and its applications. So, the investigations on it will provide ample scope for the future utility of this substance. Hence, the proposed investigation entitled resin foraging and characterization of stingless bee propolis has been undertaken with the following objectives:

- Assessment of resin foraging behaviour of stingless bee
- Origin of resin and characterization of bee propolis

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Propolis, also known as bee glue, is a viscous bee product created by combining insect secretions (saliva and wax) and plant resins. Resin is made up of lipid-soluble mixtures of volatile and non-volatile phenolic compounds, such as flavonoids, aromatic acids and benzopyrans, as well as terpenoids, such as mono-, di-, and sesquiterpenes, which have a range of anti-inflammatory, antifungal, antibacterial and antiviral properties (Langenheim, 2003). In stingless bees, resin use is even more extensive; many stingless bee species collect resin in copious amounts and use it to support multiple aspects of colony function (Araujo *et al*., 2016).

In this context, literature pertaining to resin foraging, the origin of stingless bee propolis, and the characterization of propolis are reviewed here.

2.1 PLANTS VISITED BY BEES FOR RESIN

The compounds found in propolis are determined by the substances secreted by various plants. Honeybees gather resources from virtually any prolific plant source in the vicinity of the hive, including populus, eucalyptus, pine, sugarcane, cashew nut, and orange trees (Bonvehi and Coll, 2000). Apart from resin, some of these substances are commonly lipophilic materials on the leaves, gums and lattices (Bankova, 2005).

According to Armbruster (1984), *Dalechampia* (Euphorbiaceae) and *Clusia* (Guttiferae) were the botanical resin sources of propolis. Wallace and Trueman (1995) discovered that the *Eucalyptus torelliana* F.Muell. fruit produced resin, which was harvested *by Trigona carbonaria* Smith stingless bee workers. *Agathis borneensis* kauri (Araucariaceae), of the nine tree species seen in Borneo, was identified by Leonhardt and Bluthgen (2009), as the most alluring resin source of ten stingless bee species. Marisa and Salni (2012) reported that the botanical source of propolis made by *Trigona* spp. identified were *Pterocarpus indicus* Willd. and *Artocarpus communis* Forst. from its bark. Botanical sources of propolis by *Tetragonula biroi* Friese were found to be mango, jackfruit and chico (Alvarez *et al.,* 2013). As per Massaro *et al.* (2014), the propolis of Australian stingless bees (*Tetragonula carbonaria* Smith) was sourced from *Corymbia torelliana* F.Muell. Due to the presence of xanthones, Sanpa *et al.* (2015), revealed that *Garcinia mangostana* L. was the most likely plant source of propolis from *Tetragonula laeviceps Smith* and *Tetrigona melanoleuca* Cockerell. Whereas, *Mimosa tenuiflora* (Willd.) Poir., was discovered to be the botanical source of geopropolis made by *Scaptotrigona postica* latreille in the state of Rio Grande do Norte (Ferreira *et al*., 2017). The yellow resin from the fruit surface of the *G.*

mangostana was compared to the propolis from Thailand's stingless bees and was determined to be the botanical source (Ishizu *et al*., 2018). A study conducted by Vazhacahrickal and Jose (2018), from Kerala found that *M. indica* L., *Artocarpus heterophyllus* Lam, *Manihot esculenta* Crantz, *Anacardium occidentale* L., *Moringa oleifera* Lam. and *Ficus benghalensis* L. were the resin sources of stingless bees. According to Georgieva *et al*. (2019), the stingless bee (*Lisotrigona cacciae Nurse)* propolis sample's botanical origins were determined to be *Dracaena cochinchinensis* (Lour.) S.C.Chen, *Cratoxylum cochinense* (Lour.) Blume, and *Mangifera indica* L. Pujirahayu *et al*. (2019) discovered from Southeast Sulawesi that the plant source of propolis of Tetragonula sapiens Cockerell was *M. indica.* For the first time, *Macaranga tanarius* (L.) Müll. Arg. and *M. indica* were identified as plant sources of Indonesian propolis produced by *T. laeviceps* in Banten and *Heterotrigona itama* Cockerell in South Kalimantan (Mulyati *et al*., 2020). According to Mizuno *et al*. (2022), propolis from Indonesia, was made by stingless bees using the plant *Calophyllum inophyllum* L.

When poplar species are not available, trees including pine, birch, elm, alder, beech and horse-chestnut species are good sources of resin for temperate honey bees (Alfonsus, 1933; Ghisalberti, 1979; Crane, 1990). While in another study from northern hemisphere, some of the more significant resin sources were *Populus* spp., *Betula* spp*., Ulmus* spp., *Pinus* spp., *Quercus* spp., *Aesculus hippocastanum* L. and *Eucalyptus* spp. (Popravko and Sokolov, 1980; Dimov *et al*. 1992; Bonvehi *et al*. 1994). Martos *et al.* (1997) discovered that the leaf exudate of *Cistus* spp. as a plant source of propolis in Tunisia. Accordingly, the botanical origins of propolis identified in Säo Paulo state were *B. dracunculifolia, A. angustifolia,* and *Eucalyptus citriodora Hook*. Also, *B. dracunculifolia* was the most abundant component and was collected by *Apis mellifera* L. (Bankova *et al*., 1999). Bankova *et al.* (2000) discovered that the main source of *A. mellifera* propolis from temperate zones was *Populus nigra* (Du Roi) Moench. Nyeko *et al.* (2002) revealed that *Alnus* sp. appeared to be the resin source of propolis of Ugandan honey bees. Cuesta-Rubio *et al*. (2002) depicted floral resin from the *Clusia rosea* Jacq. as the botanical source of *A. mellifera* propolis sourced from Cuba. According to Teixeira *et al.* (2005), shoot apices of *B. dracunculifolia* was the source of green propolis produced by *A. melllifera*. While, Daugsch *et al.* (2008), delineated that *Dalbergia ecastophyllum* L. was the resin source of propolis from Northeastern Brazil. Silva *et al.* (2008) revealed that the botanical origin of *A. mellifera* propolis was *A. occidentale*. When the profiles of Argentinian Andean propolis were compared to those of *Larrea nitida* Cav. exudates, it was discovered to contain lignan and volatile organic compounds, providing strong evidence for its botanical origin (Agüero *et al.,* 2011). The primary plant source of the

Mediterranean propolis was *Cupressus sempervirens* L. (Popova *et al.,* 2012). According to Tran *et al.* (2012), *A. mellifera* was observed to collect resin from the sticky exudate on the stem and seed pods of *Acacia paradoxa* DC. and reported it as botanical resin source. Barth *et al.* (2013) discovered *Baccharis* plants and *Eupatorium* plants as sources of propolis collected by *A. mellifera*. The botanical origin of *A. mellifera* propolis sourced from the Hawaiian and Okinawan regions was found to be *M. tanarius* fruit (Inui *et al.,* 2014). As per Jain *et al.* (2014), the origin of Brazilian red propolis from *D. ecastaphyllum* and was collected by *A. mellifera*. Dimkić *et al.* (2016) found that *Populus* spp. as the major botanical source of *A. mellifera* propolis. According to Herrera-López *et al. (2019), Bursera simaruba* L. was the resin source of *A. mellifera* propolis sourced from Yucatan Peninsula region. As per González *et al*. (2019), Amaicha propolis was discovered to be sourced from *Zuccagnia punctata Cav.* which was collected by *A. mellifera*. Sonoran propolis was discovered to be comparable to *Populus fremontii* S. Watson resins and *Ambrosia ambrosioides* (Cav.) Payne as a secondary plant source (Alday *et al.,* 2019). The Giovanini de *et al.* (2021) revealed that *A. mellifera* propolis botanical origins were *Araucaria angustifolia* var. nigra*, Pinus elliottii* var. densa and *Pinus taeda* Linn.

Based on analysis of propolis of *A. mellifera* and five indigenous species of stingless bees, from tropical Venezuela revealed that *Clusia* species, such as *C. major* and *C. minor* were the botanical origin of propolis (Tomas-Barberan *et al.,* 1993).

2.2 RESIN FORAGING BEHAVIOUR

Inoue *et al.* (1985) investigated *H. itama* and found that it collected resin continuously throughout the day. While, do Nascimento and Nascimento (2012), delineated that in the dry season, resin collection peaked at 0700h, whereas in the rainy season, it peaked between 0800h and 1000h, also 6,213 and 118 bees were seen bringing back resin during the dry and rainy seasons, respectively. The best period for foragers to leave and return to the hive was between 0800h and 1000h. in the morning, and it rapidly fell after 1200h, according to Ghazi *et al*. (2014). In another study Ghazi *et al*. (2014), reported that the best period for foragers to leave and return to the hive was between 0800h and 1000h in the morning, and it rapidly fell after 1200h. Harano *et al*. (2020) found that under undisturbed conditions in a stingless bee colony, 11.3 Per cent of these emigrating bees contained resin, and in cases of experimental disturbance, they carried resin burdens (90.5%). As per Wicaksono *et al*. (2020), from *Lepidotrigona terminata Smith* found that at 0700h to 0800h and 1600h to 1700h, there were fewer bees departing and returning to the nest. However, the peak hours were from 1000h until 1200h, when 8 and 6 individuals per minute respectively left and returned to the nest.

Devanesan *et al*. (2002) investigated *Tetragonula iridipennis* Smith foraging behaviour in Kerala and found that foraging activity peaked in July and was at its lowest in December and January. After 0900h and evenly dispersed throughout the day, resin foraging increased, but it decreased after 1600h. Moreover, just 3–10% of their total fight was spent collecting resin (Saravanan and Alagar, 2007). Vijayan *et al.* (2018) revealed that resin foragers started foraging from 0700h to 0800h, and resin foraging activity reached its peak between 0800h and 1100h, reduces to a minimum from 1700h to 1800h.

The number of foragers at naturally occurring resin wounds remained constant over the course of the observation period, while bees discovered artificially induced wounds within 1-2 days. Forager numbers at artificial wounds increased over the course of the next 5 days until resin secretion stopped or the resin hardened (Leonhardt and Bluthgen, 2009).

2.3 CORRELATION BETWEEN THE NUMBER OF RETURNING FORAGERS WITH RESIN LOAD AND WEATHER PARAMETERS.

Abiotic elements like temperature, humidity, solar radiation, wind and foraging behaviour have an additional impact on bees' daily flying activity and foraging behaviour. (Heard and Hendrikz, 1993; Hilario *et al*. 2012; Oliveira *et al.* 2012; Polatto *et al.* 2014).

Based on the comparison, temperature and the output of propolis not correlated, while a correlation was shown with rainfall (Pereira *et al.*, 2009). Bastos *et al.* (2011) reported that on *B. dracunculifolia, the number of resin foragers was high during the rainy season.* Foraging activity do reduce by over 90% during the rainy seasons (do Nascimento and Nascimento, 2012). Temperature and light levels were positively connected with bees' flight behaviours, which brought nectar and resin as well as leaving the nest without garbage (Wicaksono *et al*., 2020).

2.4 COLOUR, TEXTURE, AND ODOUR OF PROPOLIS

Propolis has a wide range of scents, textures and colours. Some varieties of propolis have a resinous scent, while others have no fragrance. Propolis is sticky and moldable at normal temperature and are stiff and friable at low temperature. Light cream, green, red, brown, or black propolis are all possible colours (Ghisalberti, 1979; Kuropatnicki *et al*. 2013). At temperatures ranging from 25 to 45 °C, propolis is a soft, pliable and sticky substance. It becomes hard and brittle, especially when frozen. Even at higher temperatures, it will remain brittle. When the temperature rises above 45 °C, it becomes increasingly sticky and gummy. Propolis will become liquid at 60 to 70 °C, but the melting point of some samples may be as high as 100 °C (Wagh, 2013).

2.5 COMPOSITION OF PROPOLIS

As per Marisa and Salni (2012), a propolis sample examined in Indonesia, contained compounds such as terpenoid (tannin) for redwood sap and tannin and alkaloid for breadfruit sap. Alvarez *et al.* (2013) reported that flavonoids and phenolic compounds identified from the propolis were pinobanksin- 5,7-dimethyl ether and artepillin C, pinobanskin-3-(butyrate or isobutyrate), luteolin-5-methyl ether and kaempferide. According to Sanpa *et al.* (2015), *T. laeviceps* propolis contained six xanthones, one triterpene, and one lignane, while *T. melanoleuca* propolis primarily contained triterpenes. Whereas, flavanols such as methoxy chalcones and quercetin methyl ethers were discovered by Ferreira *et al.* (2017). Whereas, Ishizu *et al*. (2018) characterized the Thailand stingless bees propolis, and components identified were α-mangostin, garcinone C, γ-mangostin, garcinone D, β-mangostin, gartanin, 8-deoxygartanin, 9-hydroxycalabaxanthone and mangostanol. Another study by Pujirahayu *et al*. (2019) found that propolis collected from Southeast Sulawesi, Indonesia, contained compositions of mangiferolic acid, cycloartenol, ambonic acid, mangiferonic acid, and ambolic acid, which are cycloartane-type triterpenes. Georgieva *et al*. (2019) observed eighteen components in stingless bee *L. cacciae* propolis, including phenols, triterpenes, homoisoflavanes, flavanes, and xanthones. While, Mizuno *et al.* (2022), discovered Calophylloidic acid A, a novel chromanone derivative from stingless bee propolis.

The presence of compounds belonging to classes flavonoids, flavanones, flavones and flavonols; benzoic acid and derivatives; benzaldehyde derivatives; cinnamyl alcohol, cinnamic acid and its derivatives; aliphatic hydrocorbons; sugar; nicotinic acid, pantothenic acid, chalcones and dihydrochalcones; amino acids: esters: other acids and derivatives; alcohol, ketones, phenols and heteroaromatic compounds; terpene, sesquiterpene, alcohol and derivatives; sesquiterpene and triterpene hydrocorbons; sterols and steroid hydrocarbons; fatty acids (C7-C18 acids); ketones; enzymes; waxy acids; aliphatic acids and aliphatic esters; alcohol and aliphatic acids in the bee propolis were reported by various researchers (Walker and Crane, 1987; Kuropatnicki *et al.* 2013; Anjum *et al.,* 2019).

According to Greenaway *et al*. (1990), flavonoids, alcohols, aldehydes, aliphatic and aromatic acids, chalcones, terpenoids, steroids, sugars, and amino acids were also found in propolis. Garcia *et al*. (1993) revealed that flavones, flavanones, and flavanols were the primary physiologically active components found in European and North American propolis. Islapinin, ermanin, pectolinarigenin, sakuranetin, isosakuranetin, quercetin-3,30-dimethyl ether, 3-acetyl pinobanksin, betuletol, isorhamnetin, kaempferide, rhamnazin, rhamnetin, alnusin, alpinetin, alnusitol, pinostrobin, pinocembrin, chrysin, tectochrysin, acacetin, rhamnocitrin, quercetin, galangin, apigenin, pinobanksin, kaempferol, rutin, catechin, luteolin

and naringenin were the major compounds under flavonoids, flavanones, flavones and flavonols present in bee propolis (Walker and Crane, 1987).

Marcucci (1996) found that compounds belonging to classes nicotinic acid, pantothenic acid, chalcones and dihydrochalcones such as alpinetin chalcone, naringinen chalcone, pinobanksin chalcones, pinobanksin-3-acetate chalcone, pinostrobin chalcone, pinocembrin chalcones, sakuranetin chalcone, 20,60, a-trihydroxy-40-methoxy chalcone, 20,6, dihydroxy-40-methoxy dihydro chalcone, 20,40,6-trihydroxy dihydro chalcone. Marcucci (1996) delineated waxy acids such as archid acid, behenic acid, cerotic acid, lauric acid, linoleic acid, lignoceric acid, and montanic acid. While, aliphatic acids and aliphatic esters included were acetic acid, angelic acid, butyric acid, crotonic acid, fumaric acid, isobutyric acid, methyl butyric acid, isobutyl acetate, isopentyl acetate, isopentinyl acetate. Benzene methanol, cinnamyl alcohol, glycerol, a-glycerophosphate, phenethyl alcohol, isobutenol, hydroquinone, and prenyl alcohol were among the alcohols (Marcucci, 1996) and characterized amino acids such as alanine, b-alanine, a-amino butyric acid, d-amino butyric acid, arginine, asparagine, aspartic acid, cystine, cysteine, glutamic acid, glycine, histidine, hydroxyproline, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, proline, pyroglutamic acid, sarcosine, serine, threonine, tryptophane, tyrosine, valine.

Bankova *et al*. (1999) identified components such as p-coumaric acid, dihydrocinnamic acid, cinnamic acid, prenyl-p-coumaric acid, diprenyl-p-coumaric acid, gallic acid, E/Z communic acid, kaempferide, coumaric acid, aromadendrine-4'-methyl ether, cycloartenol, and ß-amyrine from propolis sourced in Säo Paulo state. Cuesta-Rubio *et al.* (2002) characterised nemorosone as the most prevalent polyisoprenylated benzophenone, while a combination of xanthochymol and guttiferone E was shown to be less prevalent. (Abd and Hegazi, 2002) reported the esters such as methyl palmitate, cinnamyl-trans-4- coumarate, ethyl palmitate, stearic acid, methyl ester, phthalate ester, benzyl benzoate, benzyl-trans-4-coumarate, 3-Methyl-3-butenyl isoferulate, 3-Methyl-2-butenyl isoferulate, 3-Methyl-3-butenyl caffeate, 2-Methyl-2-butenyl caffeate, 3-Methyl-2-butenyl caffeate, benzyl caffeate, phenylethyl caffeate, cinnamyl caffeate, tetradecyl caffeate, tetradecenyl caffeate, tetradecenyl caffeate (isomer), tetradecanyl caffeate, hexadecyl caffeate. Also, he recognised alpha-amino acids such as acetic acid, malic acid, and lactic acid 5,3-dihydroxy valeric acid, 2,3-dihydroxypropanoic acid, 2,3,4,5-tetrahydroxypentanoic acid-1,4-lactone, 2,3,4,5 tetrahydroxy pentanoic acid-1,4-lactone, pentonic acid, 2-deoxy-3,5-dihydroxy-c-lactone, tetrahydroxy pentanoic acid-1,4-lactone, nonanoic acid, palmitic acid, oleic acid, decanoic acid, tetradecanoic acid, hepta- decanoic acid, succinic acid, octadecenoic acid, tetracosanoic acid, eicosanoic acid, hexacosanoic acid and 2-hydroxyhexacosanoic acid. Teixeira *et al.*

(2005) identified classes such as prenylated and nonprenylated phenylpropanoids and terpenoids. Silva *et al.* (2008) described that the triterpenoids like α-amyrin, β-amyrin, lupeol, cycloartenol, and 24-methylenecycloartanol and the phenolic derivatives such as cardanol, cardol, and anacardic acid from propolis produced by *A. mellifera.* Propolis from Argentina, included flavonoids included the flavones chrysin, tectochrysin, pinocembrin, pinobanksin and galangin (Lima *et al*., 2009).

According to Tran *et al.* (2012), 2'-hydroxy-3',4'-dimethoxychalcone, 2',3',4'-trimethoxychalcone, 7-dihydroxy-2,3-dihydroflavonol 3-acetate (pinobanksin 3-acetate), 2',4'-dihydroxy-3'-methoxychalcone, and 5,7-dihydroxy-6-methoxy-2,3-dihydroflavonol 3-acetate were components identified in Australian propolis. Shashikala *et al*., (2016) found thirteen chemicals, discovered as major constituents by GC-MS analysis of propolis from the *A. mellifera*, includes ethyl hexanol, 3 ethyl 3 methyl heptane, dodecane, 1,1 dimethyl ethyl, tetradecane, 4,6 dimethyl, tetracosane. diethyl phthalate, dibutyl phthalate, hexadecanoic acid, octadecenoic acid, 1.2 benezene-dicarboxylic acid and hexatricontane. Dimkić *et al.* (2016) characterized components like, p-hydroxybenzoic acid, p-coumaric acid, caffeine, chrysin, apigenin, quercetin, pinocembrin, pinobanksin, and galanin as major components.

Benzaldehyde derivatives identified were vanillin, caproic aldehydes, isovanillin p-hydroxybenzaldehyde, protocatechualdehyde (Marcucci, 1996); (Walker and Crane, 1987); (Abdulkhani *et al.*, 2017); (Akbay *et al.,* 2017). Sonoran propolis was found to contain kaempferol-3-methyl-ether as well as polyphenols (Alday *et al.,* 2019). Trans-linalool oxide (furanoid), linalool, 6-camphenone, trans-pinocarveol, p-cymen-8-ol, and 2,3,6-trimethylbenzaldehyde were the major volatile compounds of propolis by *A. mellifera* from North-west Argentina (González *et al*., 2019). Mangiferolic acid, iso-mangiferolic acid, and dammarenediol II were listed as propolis constituents from the Yucatan peninsula (Herrera-López *et al.,* 2019). By using static headspace gas chromatography coupled to mass spectrometry, 99 volatiles were discovered; monoterpenes and sesquiterpenes were the most prevalent classes (Giovanini de *et al.,* 2021). Luteolin, p-coumaric acid, naringenin, caffeic acid, catechin, gallic acid, ferulic acid, syringic acid, vanillic acid, kaempferol, apigenin, quercetin, and rutin were some of the phenolic components found in the Egyptian propolis extract (Yong and Liu, 2021). Gallic acid, vanillin acid, ferulic acid, ellagic acid, kaempferol, quercetin, caffeic acid, chlorogenic acid, gentisic acid, cinnamic acid, and syringic acid were the 11 phenolic compounds found (Tylkowski *et al*., 2010; Ibrahim *et al*., 2022).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The materials used and methodology followed for the current investigation are detailed in this chapter. This investigation focused on studying the resin foraging behaviour of stingless bee *T. travancorica* and the origin and composition of stingless bee propolis.

3.1 STUDY LOCATION

The study was conducted at the stingless bee hives of dimensions (30 cm x 14 cm x 15 cm) in four different locations *viz.,* Vellayani, Nedumangad, Mariapuram and Navaikulam of Thiruvananthapuram district at monthly intervals from September, 2021 to August, 2022 and the details of which are presented in Table 1. Each location was visited and the details of the botanical sources, resin foraging behavior, and physical and chemical properties of propolis samples were recorded.

Table 1. Locations selected for resin foraging study

Location ID	Location	Longitude (°N)	Latitude (°E)	Elevation (m)
L1	Vellayani	8.43	76.98	23
L2	Nedumangad	8.59	76.99	58
L3	Mariapuram	8.36	77.11	38
L4	Navaikulam	8.80	76.80	89

3.2 RESIN FORAGING BEHAVIOUR

3.2.1 Identification of botanical sources

Trees and plants upto a 100 m radius around beehives were observed at each location. Resin sources were identified by breaking the branches, making wounds on the stem, breaking petioles, or making a deep cut on stems in the case of trees, followed by monitoring them for the next three days for resin production. Trees with wounds that didn't start producing resin until the third day was discarded. Plants and trees with resin-flowing wounds were observed daily at an hourly interval from morning 0700h to evening 1800h to record whether foraging bees are visiting. If foraging bee visits were recorded, the tree or plant can be confirmed as a botanical source.

3.2.2 Grading of resin wounds

Wounds, both artificial and natural, were monitored for three days for resin production on the major botanical sources identified. Then the resin-flowing wounds were graded to get an awareness of the resin content. The wound area equivalent to the amount of resin secretion was visually evaluated and scored from 1 to 4, with 1 denoting invisible resin flow, 2 denoting

an area of less than 2 cm^2 covered in resin, 3 denoting 2 to 5 cm^2, and 4 denoting more than 5 cm^2 (Leonhardt and Bluthgen, 2009).

3.2.3 Bee visitation at resin wounds

The identified major botanical sources with naturally occurring or artificially produced resin-flowing wounds were observed for three days following injury or wound detection. Hourly observations for 10 minutes daily were taken till the cessation of the resin foraging period.

3.2.4 Resin foraging activity of *T. travancorica*

Returning foragers with resin load in their hind legs were observed at beehives for a period of 10 minutes each hour from 0700h to 1800h at monthly intervals from September, 2021 to August, 2022. For confirmation, stingless bees carrying resins were trapped using a small insect net and suspected resin load from hind legs was felt using hands. Further, weather parameters at hive locations were collected for correlation with the number of returning foragers with resin load.

3.2.5 Volume of resin inside the hive

The volume of resin was measured by multiplying the length, breadth, and height of the resin-covered region. These observations were taken three times during the observation period *viz*., October 2021, March 2022, and August 2022 from the same bee hive in each of the four locations.

3.3 CORRELATION BETWEEN THE NUMBER OF RETURNING FORAGERS WITH RESIN LOAD AND WEATHER PARAMETERS

The relationship between number of returning foragers with resin load with weather parameters such as wind velocity, temperature, relative humidity, rainfall, sunshine hours were assessed.

3.4 SAMPLE COLLECTION AND STORAGE OF PROPOLIS

Propolis samples were collected from a bee hive at each of the four locations. Propolis was spotted inside the bee hive and was removed by scraping it with a sharp knife. The collected propolis sample was washed with clean water to remove the dirt in it. The cleaned samples were stored in plastic bottles in the refrigerator at 1-4 °C.

3.4. PHYSICAL PROPERTIES OF PROPOLIS

3.4.1 Colour, texture and odour of propolis

The colour, texture, and odour of the four propolis samples were recorded. Colour of the propolis was determined using the Royal Horticultural Society colour chart. The texture of the propolis samples was determined by the hand-feeling method. Also, the propolis was checked for odour and classified as aromatic and non-aromatic.

3.4.2 Solubility of propolis

The propolis sample each weighing 0.1 g was dissolved in different solvents including hexane, diethyl ether, ethyl acetate, ethanol, methanol, acetonitrile, and distilled water based on the polarity of each solvent. Then the sample was shaken in the vortex for 5 minutes and the solubility of propolis was recorded.

3.5 COMPOSITION OF PROPOLIS

3.5.1 Sample Extraction

Propolis samples were grounded into fine particles using a pestle and mortar. Then, 1 g of each of the grounded propolis samples was taken in a labeled 50 mL centrifuge tube. To this 15 mL of 70% ethanol was added and this mixture was vortexed at 2490 rpm for 14 minutes. After thorough mixing in the vortex, samples were placed in the shaker at 200 rpm for 24 h at 26 °C. After which the centrifuge tubes were stored in the refrigerator at -4 °C until further processing.

3.5.2 GC-HRMS analysis of propolis

Gas chromatography coupled with high resolution mass spectrometry (GC-HRMS) was used to analyse samples of propolis to determine its chemical composition. The model used was AccuTOF GCv Jeol make, mode of ionization was electron impact (EI) with a mass range of 10-2000 amu and mass resolution 6000. A capillary column with measurements of 30 m x 0.25 mm x 0.25 mm was used for GC. The temperature at the injector was 250 °C. Pre-column pressure was 80 kPa, and the split ratio was 40:1. The initial column oven temperature was at 60 °C and heated at rate was 6 °C/min up to 280 °C. Helium gas served as the carrier gas with a flow rate at 1.0 mL/min.

3.5.3 LC-HRMS analysis of propolis

LC-HRMS is used to distinguish exact mass so precisely and to characterize each component of propolis. Propolis samples were analyzed on the Thermo Scientific make Q-

exactive mass spectrometer combined with the LC Ultimate 3000 model UHPLC, the analytical separation was carried out by using C-18column with dimension of (100mmX 2.1mm, 5 µm). It is equilibrated with 0.1% solvent A (HCOOH in H_2O), %5 B (Methanol). Flow rate 0.200 [ml/min] was constant, solvent B concentration ranges from 5% to 95% in 75 minutes, from 70 to 75 minutes 90% to 95% and then, it reduced to 5% at 75 minutes. Here, mass to charge ratio measurements are carried out with a range of 100 to 1500 m/z. The oven was set to a temperature of 110 °C, while the auto sampler temperature set at 10 °C. The Q-exactive mass spectrometer was operated by electro spray ionization (ESI) of positive and negative polarity at 70,000 resolution, Maximum IT of 200 ms AGC target of 1000000, for Full MS – SIM analysis. dd-MS^2 (TopN), was operated at a resolving power of 70,000, IT 100 ms, ACG of target 3000000. While, an inclusion list-based data-dependent scan (dd-MS^2 / dd-SIM), was set up at 70,000 resolving power, Maximum IT of 50 ms, AGC target of 100000, isolation windows at 2.0 m/z, and collision energy at 30.

3.6 STATISTICAL ANALYSIS

Data obtained from different experiments were statistically analyzed using KAU GRAPES (Gopinath *et al.,* 2021).

RESULTS

4. RESULTS

The results of the current investigation on the resin foraging behaviour of stingless bees and the origin and composition of propolis are presented under different heads in this chapter.

4.1 RESIN FORAGING BEHAVIOUR

4.1.1 Plants visited by bees for resin

The different botanical resin sources of *T. travancorica* identified across all the locations were *Mangifera indica* L., *Artocarpus heterophyllus* Lam., *Artocarpus altilis* Parkinson, *Anacardium occidentale* L., *Garcinia xanthochymus* Hook., *Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg., *Garcinia cambogia* Syn., *Araucaria araucana* Molina, *Artocarpus hirsutus* Lam. and *Macaranga peltate* (Roxb.) Müll.Arg. is given in Table 2 and Plate 1.

Stingless bees visiting the major botanical resin sources are given in Plate 1. *T. travancorica* was found collecting white coloured resin from the cut stem of *M. indica, A. altilis, A. araucana.* In *A. heterophyllus,* stingless bee collected white coloured resin from both cut stalk and fruit surface. Stingless bee was found collecting yellowish resin from cut stem of *G. cambogia* and golden yellowish colored resin from the cut stem of *A. occidentale*. Yellowish coloured resin was collected by stingless bee from both the natural and artificial cut on the fruit surface of *G. xanthochymus.*

4.1.2 Bee visitation at resin wounds

An artificial cut was made on the major botanical sources identified and was graded from 1 to 4, based on resin flow from the sources. *M. indica,* with resin covered area of 5.4 cm^2 of grade 4 was the highest grade. However, *A. araucana* and *G. xanthochymus* with resin covered area of 0.48cm^2 and 0.6 cm^2 with grade of 2 was the lowest. Other resin sources *viz*., *A. heterophyllus*, *A. altilis*, *A. occidentale, G. cambogia* with resin areas of 3.4 cm^2, 3.2 cm^2, 2.8 cm^2, and 4 cm^2 respectively belonged to grade 3 (Table 3).

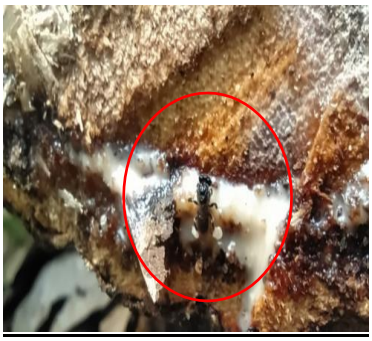
Table 2. Botanical resin sources from different locations

	Locations			
Resin sources	Vellayani	Nedumangad	Mariapuram	Navaikulam
Mangifera indica	✓	✓	✓	✓
Artocarpus heterophyllus	✓	✓	✓	✓
Artocarpus altilis	✓	✓	✓	✓
Artocarpus hirsutus	✓	✓	✓	✓
Anacardium occidentale	✓	-	-	-
Garcinia xanthochymus	✓	-	-	-
Garcinia cambogia	✓	✓	✓	✓
Araucaria araucana	✓	-	-	-
Macaranga peltate	✓	✓	✓	✓
Hevea brasiliensis	-	✓	✓	✓

Table 3. Visitation of *Tetragonula travancorica* at resin wounds

Resin sources	Area of resin covered (cm^2)	Grade*	Average number of bees visited per day
Mangifera indica	5.4	4	11.53
Artocarpus heterophyllus	3.4	3	6.5
Artocarpus altilis	3.2	3	9.5
Anacardium occidentale	2.8	3	8.2
Garcinia cambogia	4	3	16.71
Garcinia xanthochymus	0.6	2	6.55
Araucaria araucana	0.48	2	2.17

*The wounded area was visually assessed and graded as 1(invisible resin flow),2(<2cm^2), 3(2-5 cm^2) and 4(>5cm^2).



Mangifera indica

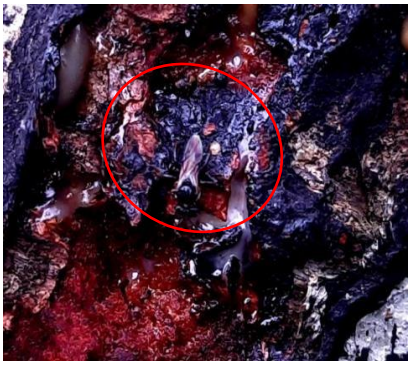
Artocarpus heterophyllus

Artocarpus altilis

Anacardium occidentale

Garcinia xanthochymus

Garcinia cambogia



Araucaria araucana

Plate 1. Botanical resin sources of *T. travancorica*

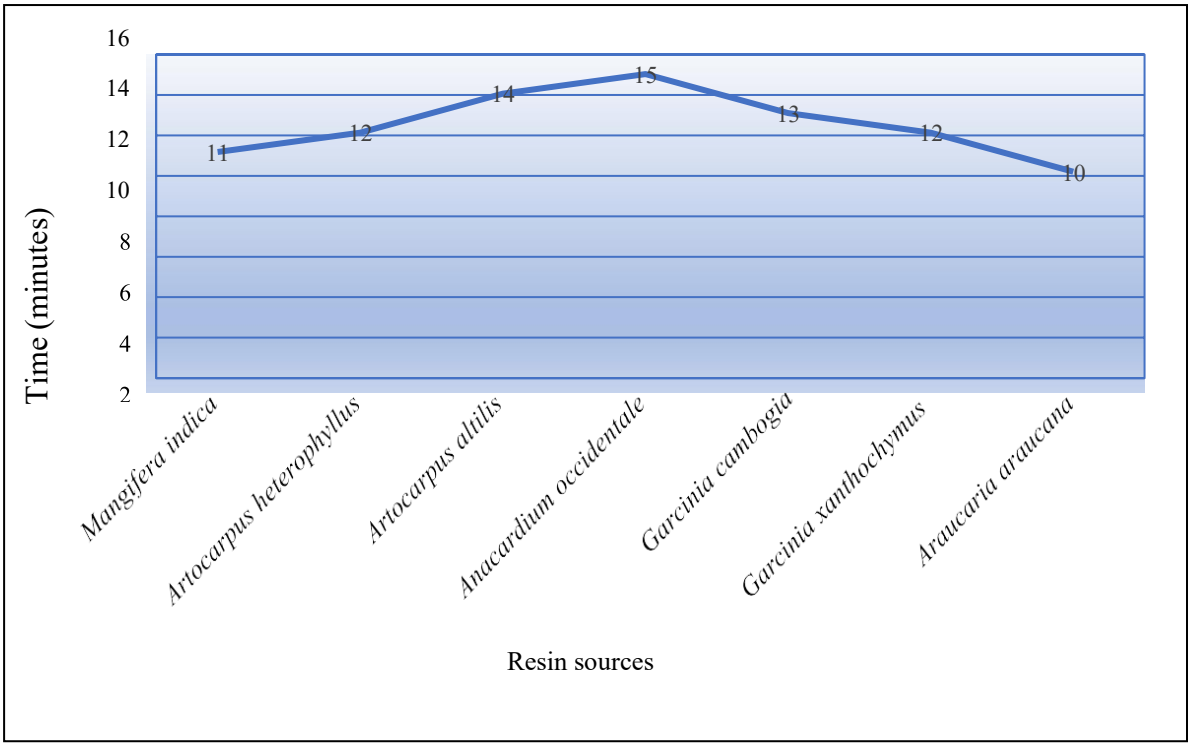


Fig. 1. Time spent by *T. travancorica* at resin wounds

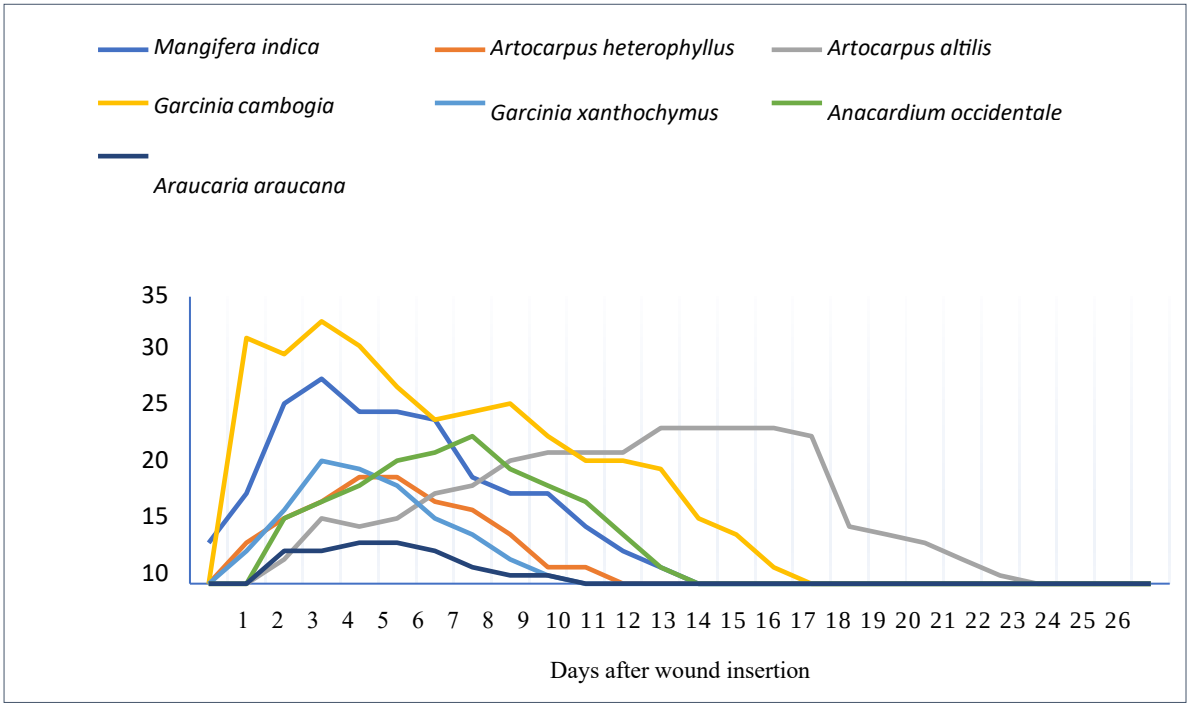


Fig. 2. Visitation of *T. travancorica* at various resin sources

The total number of bees visited per wound on a daily basis is given in Table 3. The artificial cut made on *G. cambogia* was with 16.71 foragers per day whereas, *A. araucana* was with 2.17 foragers per day. *M. indica, A. heterophyllus*, *A. altilis, A. occidentale,* and *G. xanthochymus* were with 11.53, 6.5, 9.5, 8.2 and 6.55 foragers, respectively.

The time taken by a stingless bee to complete resin foraging ranged from 10 to 15 minutes on the identified resin sources as shown in Fig. 1. Here, based on the observations, the stingless bee spent the maximum time on *A. occidentale* at 15 minutes, followed by *A. altilis* at 14 minutes and G. *cambogia* at 13 minutes. The time spent by a stingless bee collecting resin was only 12 minutes in *A. heterophyllus* and *G. xanthochymus*. Whereas, the lowest time spent by stingless bees was on *M. indica* and *A. araucana* with 11 and 10 minutes, respectively.

Visitation of *T. travancorica* in the wound of different sources is given in Fig. 2. It was found that stingless bees started foraging on *M. indica* resin wound on the 1st day, attained peak on the 4th day, and reduced to a minimum on the 14th day. Whereas in *A. heterophyllus,* foraging commenced on the 1st day, the 5th and 6th days were observed as peak foraging periods and foraging declined to a minimum on the 14th day. Although, in *A. altilis,* bee foraging started only on the 2nd-day resin, during the 13 to 16th foragers attained their peak, and foraging diminished on the 23rd day. From this source also, foraging initiated on the 2nd day, attained peak on the 8th day, and reduced to a minimum on the 14th day on *A. occidentale*. Visitation of *T. travancorica* in *G. cambogia* wound started on 1st day after wound insertion, attained maximum on the 4th day, and reduced to a minimum on the 17th day. Whereas, on *G. xanthochymus* foraging activity commenced on 1st day itself, peak foraging was observed on the 4th day, and on the 11th day foraging came to an end. In *A. araucana,* foraging commenced on the 2nd day after wound insertion, the maximum population was observed on the 5th and 6th day and ceased on the 11th day.

4.1.3 Resin foraging activity of *T. travancorica*

Observations on the number of returning foragers with resin load in September, 2021 across all the locations varied from 0.33 to 21.66 (Table 4). Regarding the observations at Vellayani, peak foraging activity was observed during 0900-1000h (10.33), which was on par with 1000h-1100h (9.66) and 1100h-1200h (9.66). The least foraging activity was observed during 0700h-0800h, 1600h-1700h, and 1700h-1800h with 1.33 foragers only. Observations at Nedumangad showed that increased foraging activity was found during 1100h-1200h (3.33), 1300h-1400h (2.66), which was on par with 1000h-1100h (2.33) and 1200h-1300h (2.33). While minimum foraging activity was observed during 0700h-0800h, 1400h-1500h, 1500h-1600h, 1600h-1700h, and 1700h-1800h with 0.33, 1.00, 1.00, 0.66, and 0.33 foragers respectively. Peak foraging activity at Mariapuram was found to be 1100h-1200 h (21.66) and minimum during

0700h-0800h (3.33) and 1700h-1800h (3.66). At Navaikulam, the highest foraging activity was found during 1200h-1300h (8.33), 1400h-1500h (8.33), which was on par with 1000h-1100h (7.66) and 1300h-1400h (7.33). Whereas the lowest foraging activity observed was during 0700h-0800h and 1700h-1800h with a population of 2.33 and 2.66 foragers, respectively.

The resin foraging rate during October month is given in Table 5 which ranged from 0.67 to 21.33 across different locations. At Vellayani, the highest foraging was observed during 1100h-1200h (14.67) and 1400h-1500h (14.33) which was on par with 1300h-1400h (13.33). Minimum foraging was observed during 0700h-0800h (2.67). Peak foraging activity at Nedumangad was during 1100h-1200h (14.67), while the least foraging activity was during 0700h-0800h (1.67). At Mariapuram, increased foraging activity during 1100h-1200h (21.33) was on par with 1200h-1300h (20.00). Whereas, minimum foraging activity was observed during 0700h-0800h (3.67). Observations from Navaikulam revealed that 1100h-1200h and 1300h-1400h were the peak foraging period whereas, the least foraging was during 0700h-0800h (0.67).

Observations in November as given in Table 6 ranged from 0.67 to 18.67. At Vellayani, 1500h-1600h (18.67) was the peak foraging time, and 0700h-0800h (1.33), and 0900-1000h (1.33) were the least foraging time. The highest foraging activity at Nedumangad was 0800h-0900h (16.00), 0900-1000h (17.00), and 1000h-1100h (16.33). Whereas, minimum foraging activity was during 1500h-1600h (2.33), 1600h-1700h (2.33), 1700h-1800h (2.67), and 0700h-0800h (3.00). At Mariapuram, peak foraging activity was during 1500h-1600h (18.00) and minimum during 0700h-0800h (0.67). Peak foraging activity at Navaikulam was 0900-1000h (7.67) and 1400h-1500h (7.67) which was on par with 1300h-1400h (6.67) and 1500h-1600h (6.67). Minimum foraging activity was observed during 0700h-0800h (2.67) and 1100h-1200h (3.33).

Table 4. Number of returning foragers during September, 2021

Time interval	Number/10 minutes			
	Vellayani	Nedumangad	Mariapuram	Navaikulam
0700-0800h	1.33^{f}	0.33^{c}	3.33^{f}	2.33^{e}
0800-0900h	4.33^{c}	1.33b^{c}	6.00^{e}	6.66^{bc}
0900-1000h	10.33^{a}	1.33b^{c}	15.00^{c}	5.33^{cd}
1000-1100h	9.66^{a}	2.33^{ab}	16.66^{b}	7.66^{ab}
1100-1200h	9.66^{a}	3.33^{a}	21.66^{a}	5.66^{cd}
1200-1300h	6.33^{b}	2.33^{ab}	17.00^{b}	8.33^{a}
1300-1400h	2.66^{e}	2.66^{a}	14.33^{c}	7.33^{ab}
1400-1500h	3.33d^{e}	1.00^{c}	16.66^{b}	8.33^{a}
1500-1600h	3.66^{cd}	1.00^{c}	9.00^{d}	6.66^{bc}
1600-1700h	1.33^{f}	0.66^{c}	5.33^{e}	4.66^{d}
1700-1800h	1.33^{f}	0.33^{c}	3.66^{f}	2.66^{e}
CD (0.05)	0.978	1.021	1.474	1.503

Table 5. Number of returning foragers during October, 2021

Time interval	Number/10 minutes			
	Vellayani	Nedumangad	Mariapuram	Navaikulam
0700-0800h	2.67^{g}	1.67^{h}	3.67^{g}	0.67^{e}
0800-0900h	7.00^{f}	4.67^{g}	8.00^{f}	4.33^{d}
0900-1000h	7.33^{f}	10.33^{d}	10.67^{de}	5.67^{c}
1000-1100h	10.33^{de}	11.67^{bc}	15.00^{c}	7.67^{b}
1100-1200h	14.67^{a}	14.67^{a}	21.33^{a}	8.67^{a}
1200-1300h	12.33^{bc}	12.67^{b}	20.00^{ab}	7.67^{b}
1300-1400h	13.33^{ab}	11.33^{cd}	19.00^{b}	8.67^{a}
1400-1500h	14.33^{a}	12.00^{bc}	10.67^{de}	7.33^{b}
1500-1600h	11.00^{cd}	11.67^{bc}	10.00^{e}	6.33^{c}
1600-1700h	9.00^{e}	9.00^{e}	12.00^{d}	7.67^{b}
1700-1800h	6.00^{f}	6.00^{f}	7.00^{f}	4.33^{d}
CD (0.05)	1.383	1.215	1.560	0.978

The number of returning foragers per hive per 10 minutes with resin load during December month ranged from 1.33 to 18.00 (Table 7). Increased foraging activity at Vellayani was during 0900-1000h (17.00), 1100h-1200h (18.00), and minimum foraging activities were observed during 0700h-0800h (1.33) and 0800h-0900h (1.66). At the Nedumangad location, peak foraging activities were observed during 1100h-1200h (13.33) and at least during 1700h-1800h (2.66). Peak foraging activity at Mariapuram was during 1200h-1300h (16.00) and the least foraging activity was during 0700h-0800h (2.33). At Navaikulam, the highest foraging activity was observed during 1000h-1100h (8.66), it was on par with 0900-1000h (8.33), 1100h-1200h (8.33), and 1300h-1400h (8.33) whereas, minimum during 0700h-0800h (2.66) and 1700h-1800h (3.33).

The resin foraging rate during January, 2022 ranged from 0.33 to 17.66 as given in Table 8. At Vellayani, the highest foraging activity was during 0900-1000h (7.33) and minimum during 1700h-1800h (1.33), 0700h-0800h (1.66) and 1400h-1500h (1.66). Peak foraging activity at Nedumangad was during 1100h-1200h (7.33), 1200h-1300h (7.66), and the least foraging activity was during 0700h-0800h (1.66). At Mariapuram increased foraging activity was observed during 0900-1000h (17.66) and minimum during 1600h-1700h (1.00) and 0500-0600h (0.33). The highest foraging activity at Navaikulam was observed during 1000h-1100h (4.33) it was on par with 1600h-1700h (3.33). Whereas, minimum foraging activity was observed during 1300h-1400h (0.66).

Observations during February, 2022 varied from 0.33 to 11.66 at various locations (Table 9). The highest foraging activity at Vellayani was 1000h-1100h with 8.00 foragers. While minimum foraging activity was observed during 0700h-0800h, 0800-0900h, 1500h-1600h, 1600h-1700h, and 1700h-1800h with 0.66, 1.33, 0.33, 1.33, and 1.00 foragers respectively. At Nedumangad, peak foraging activity was observed during 0900-1000h, 1000-1100h, and 1200h-1300h with the number of foragers carrying loads were 8.33, 7.33, and 7.66 respectively. Whereas, increased foraging activity was observed during 1700h-1800h with 0.33 foragers. Increased foraging activity at Mariapuram during 0900-1000h (11.66) and 1500h-1600h (11.00), which was on par with 1200h-1300h (10.66) and minimum during 0700h-0800h with 1.66.

Table 6. Number of returning foragers during November, 2021

Time interval	Number/10 minutes			
	Vellayani	Nedumangad	Mariapuram	Navaikulam
0700-0800h	1.33^{g}	3.00^{e}	0.67^{h}	2.67^{e}
0800-0900h	1.00^{g}	16.00^{a}	1.33^{gh}	5.33^{c}
0900-1000h	1.33^{g}	17.00^{a}	2.33^{g}	7.67^{a}
1000-1100h	4.00^{f}	16.33^{a}	3.67^{f}	5.00^{c}
1100-1200h	8.33^{d}	13.33^{b}	8.00^{d}	3.33^{e}
1200-1300h	6.67^{e}	8.33^{c}	5.67^{e}	3.67^{de}
1300-1400h	9.67^{cd}	4.67^{d}	9.67^{c}	6.67^{ab}
1400-1500h	10.00^{c}	2.67^{e}	9.33^{c}	7.67^{a}
1500-1600h	18.67^{a}	2.33^{e}	18.00^{a}	6.67^{ab}
1600-1700h	12.00^{b}	2.33^{e}	14.00^{b}	5.67^{bc}
1700-1800h	10.00^{c}	2.67^{e}	10.00^{c}	4.67^{cd}
CD (0.05)	1.444	1.414	1.285	1.063

Table 7. Number of returning foragers during December, 2021

Time interval	Number/10 minutes			
	Vellayani	Nedumangad	Mariapuram	Navaikulam
0700-0800h	1.33^{e}	4.33^{fg}	2.33^{g}	2.66^{f}
0800-0900h	1.66^{e}	5.33^{ef}	4.33^{f}	5.66^{de}
0900-1000h	17.00^{a}	8.00^{c}	7.33^{e}	8.33^{ab}
1000-1100h	14.66^{b}	11.33^{b}	9.33^{d}	8.66^{a}
1100-1200h	18.00^{a}	13.33^{a}	10.33^{cd}	8.33^{ab}
1200-1300h	15.00^{b}	10.33^{b}	16.00^{a}	7.33^{c}
1300-1400h	12.33^{c}	8.66^{c}	11.33^{c}	8.33^{ab}
1400-1500h	8.33^{d}	5.66^{de}	9.33^{d}	6.33^{d}
1500-1600h	8.33^{d}	6.66^{d}	11.33^{c}	5.33^{e}
1600-1700h	8.66^{d}	4.00^{g}	13.00^{b}	7.66^{bc}
1700-1800h	9.33^{d}	2.66^{h}	8.00^{e}	3.33^{f}
CD (0.05)	1.318	1.021	1.215	0.978

Table 8. Number of returning foragers during January, 2022

Time interval	Number/10 minutes			
	Vellayani	Nedumangad	Mariapuram	Navaikulam
0700-0800h	1.66^{fg}	1.66^{f}	6.66^{d}	1.33^{de}
0800-0900h	4.66^{bc}	3.66^{cde}	12.00^{b}	2.66^{bc}
0900-1000h	7.33^{a}	5.66^{b}	17.66^{a}	2.33^{bcd}
1000-1100h	5.33^{b}	4.66^{bc}	13.66^{b}	4.33^{a}
1100-1200h	5.66^{b}	7.33^{a}	12.00^{b}	2.66^{bc}
1200-1300h	3.66^{cde}	7.66^{a}	8.66^{c}	1.66^{cde}
1300-1400h	4.00^{cd}	4.33^{cd}	5.00^{de}	0.66^{e}
1400-1500h	1.66^{fg}	3.66^{cde}	5.66^{de}	1.33^{de}
1500-1600h	3.00^{de}	3.33^{de}	4.00^{e}	2.33^{bcd}
1600-1700h	2.66^{ef}	2.66^{ef}	1.00^{f}	3.33^{ab}
1700-1800h	1.33^{fg}	2.66^{ef}	0.33^{f}	3.00^{b}
CD (0.05)	1.251	1.103	1.91	1.063

Table 9. Number of returning foragers during February, 2022

Time interval	Number/10 minutes			
	Vellayani	Nedumangad	Mariapuram	Navaikulam
0700-0800h	0.66^{e}	2.00^{c}	1.66^{e}	1.00^{f}
0800-0900h	1.33^{e}	4.00^{b}	4.00^{d}	1.33^{f}
0900-1000h	6.33^{b}	8.33^{a}	11.66^{a}	2.66^{e}
1000-1100h	8.00^{a}	7.33^{a}	8.00^{c}	4.66^{cd}
1100-1200h	2.66^{d}	5.33^{b}	9.00^{bc}	5.33^{c}
1200-1300h	4.33^{c}	7.66^{a}	10.66^{ab}	5.33^{c}
1300-1400h	4.33^{c}	4.00^{b}	9.00^{c}	7.00^{b}
1400-1500h	3.33^{cd}	4.66^{b}	8.66^{c}	3.66^{de}
1500-1600h	0.33^{e}	1.66^{cd}	11.00^{a}	6.66^{b}
1600-1700h	1.33^{e}	1.00^{cd}	8.33^{c}	4.33^{cd}
1700-1800h	1.00^{e}	0.33^{d}	5.00^{d}	8.66^{a}
CD (0.05)	1.142	1.56	1.817	1.251

foragers respectively. At Navaikulam, the highest foraging activity was observed during 1700h-1800h with 8.66 foragers. Whereas, minimum foraging activity was observed during 0700h-0800h and 0800h-0900h with 1.00 and 1.33 numbers, respectively.

Observations on the number of returning foragers with resin load in March 2022 are given in Table 10 across all the locations varying from 1.00 to 12.33. At Vellayani, the highest foraging activity was during 1000h-1100h (10.00) and the minimum during 0700h-0800h (1.33). Increased foraging activity at Nedumangad was during 0900-1000h (6.33), 1000h-1100h (6.00), and 1500h-1600h (5.33). Peak foraging activity at Mariapuram was at 1100h-1200h (12.33) and at least during 0700h-0800h (2.00). At Navaikulam, peak foraging activity was observed during 1000h-1100h (6.66), 1100h-1200h (7.33), 1200h-1300h (6.33),1500h-1600h (6.33) 1600h-1700h (7.33) and 1700h-1800h (6.33). While least foraging activity was during 0700h-0800h with 1.66 foragers.

The number of returning foragers per hive per 10 minutes with resin load during April 2022 as given in Table 11 ranged from 0.66 to 18.66. At Vellayani, peak foraging activity was during 1100h-1200h (18.00) and least during 0700h-0800h (1.00), 1600h-1700h (1.33), and 1700h-1800h (0.66). At Nedumangad, increased foraging activity was observed during 1100h-1200h (18.66) and minimum during 0700h-0800h (2.33). The highest foraging activity at Mariapuram was during 1100h-1200h (12.66) and the minimum foraging activity was during 0700h-0800h (2.66). At Navaikulam, peak foraging activity was observed during 1000h-1100h (10.33), 1100h-1200h (10.66), and 1200h-1300h (10.33), and minimum foraging activity was during 0700h-0800h (3.66). At Navaikulam, increased foraging activity was observed during 0900-1000h, 1000-1100h, and 1100h-1200h with 11.33 foragers only. While the least foraging activity was observed during 1700h-1800h with a population of 4.66 foragers.

Table 10. Number of returning foragers during March, 2022

Time interval	Number/10 minutes			
	Vellayani	Nedumangad	Mariapuram	Navaikulam
0700-0800h	1.33^{h}	1.00^{c}	2.00^{h}	1.66^{d}
0800-0900h	1.66^{gh}	3.33^{b}	5.66^{g}	3.33^{c}
0900-1000h	3.66^{ef}	6.33^{a}	7.00^{ef}	4.66^{b}
1000-1100h	10.00^{a}	6.00^{a}	7.66^{de}	6.66^{a}
1100-1200h	6.66^{cd}	3.00^{b}	12.33^{a}	7.33^{a}
1200-1300h	8.66^{b}	2.33^{bc}	10.33^{b}	6.33^{a}
1300-1400h	2.66^{fg}	2.66^{b}	9.33^{bc}	4.33^{bc}
1400-1500h	4.33^{e}	2.66^{b}	7.33^{def}	4.33^{bc}
1500-1600h	2.66^{fg}	5.33^{a}	8.33^{cd}	6.33^{a}
1600-1700h	5.66^{d}	2.00^{bc}	8.33^{cd}	7.33^{a}
1700-1800h	7.00^{c}	2.00^{bc}	6.33^{fg}	6.33^{a}
CD (0.05)	1.142	1.615	1.142	1.215

Table 11. Number of returning foragers during April, 2022

Time interval	Number/10 minutes			
	Vellayani	Nedumangad	Mariapuram	Navaikulam
0700-0800h	1.00^{f}	2.33^{g}	2.66^{i}	3.66^{f}
0800-0900h	5.33^{de}	7.33^{d}	5.66^{h}	6.66^{d}
0900-1000h	10.00^{c}	11.00^{c}	8.33^{fg}	8.66^{b}
1000-1100h	14.00^{b}	15.66^{b}	10.33^{c}	10.33^{a}
1100-1200h	18.00^{a}	18.66^{a}	12.66^{a}	10.66^{a}
1200-1300h	10.33^{c}	12.00^{c}	11.33^{b}	10.33^{a}
1300-1400h	6.67^{d}	8.33^{d}	9.66^{cd}	8.33^{bc}
1400-1500h	4.66^{e}	5.66^{e}	9.33^{de}	8.33^{bc}
1500-1600h	2.00^{f}	5.33^{e}	8.66^{ef}	7.33^{cd}
1600-1700h	1.33^{f}	3.66^{f}	7.66^{g}	6.66^{d}
1700-1800h	0.66^{f}	4.00^{f}	5.66^{h}	5.33^{e}
CD (0.05)	1.351	1.215	0.978	1.215

Observation on number of returning foragers with resin load in the month of May 2022 across all the locations varied from 0.33 to 13.33 is given in Table 12. Highest foraging activity at Vellayani was during 0200-0300h and minimum foraging activity was at 0700-0800h with 8.66 and 0.33 foragers respectively. At Nedumangad, peak foraging activity was observed during 1100-1200h and least during 0700-0800h with 13.33 and 2.33 foragers respectively. Peak foraging activity at Mariapuram was during 1100-1200h (12.66) and this was on par with 1000-1100h (11.66). Whereas, least foraging activity was observed during 0700-0800h at the rate of 5.33 foragers. At Navaikulam, increased foraging activity was observed during 0900-1000h, 1000-1100h and 1100-1200h with 11.33 foragers only. While, least foraging activity was observed during 0500-0600h with the population of 4.66 foragers.

The observations from June 2022 varied from 0.66 to 16.33 as given in Table 13. At Vellayani, peak foraging activity was during 1100h-1200h (14.33) and the least foraging activity was observed during 0700h-0800h (2.33). The highest foraging activity at Nedumangad was during 1200h-1300h (4.66) and 1000h-1100h (0.33). Peak foraging activity during Mariapuram was observed during 1100h-1200h (15.66) and 1200h-1300h (15.00). While the least foraging activity was at 0700h-0800h (3.33). At Navaikulam, increased foraging activity was during 1000h-1100h (16.33) and minimum during 1600h-1700h (2.66) and 1700h-1800h (1.66).

The resin foraging rate during July, 2022 ranged from 0.33 to 22.66 (Table 14). Peak foraging activity at Vellayani was observed during 1100h-1200h and 1200h-1300h with only 10.66 and 10.33 foragers respectively. While minimum foraging activity was observed during 0700h-0800h with only 3.66 foragers. At Nedumangad, peak foraging activity was observed during 1000h-1100h and least during 1700h-1800h with 4.33 and 0.33 foragers respectively. Increased foraging activity at Mariapuram was observed during 1200h-1300h (21.66), 1300h-1400h (22.66), and minimum foraging activity at 0700h-0800h (3.66). At Navaikulam, the highest foraging activity was during 1000h-1100h with 3.66 foragers. While minimum foraging activity was observed during 0700h-0800h (1.00), 0800h-0900h (1.33), 0900-1000h (1.33), 1100h-1200h (1.33), 1200h-1300h (1.00), 1300h-1400h (1.33), 1400h-1500h (1.00), 1500h-1600h (1.00), 1600h-1700h (1.00) and 1700h-1800h (0.66).

Table 12. Number of returning foragers during May, 2022

Time interval	Number/10 minutes			
	Vellayani	Nedumangad	Mariapuram	Navaikulam
0700-0800h	0.33^{i}	2.33^{f}	5.33^{f}	6.66^{e}
0800-0900h	1.33^{gh}	5.66^{e}	7.33^{e}	9.66^{bc}
0900-1000h	2.33^{ef}	7.66^{d}	9.66^{d}	11.33^{a}
1000-1100h	6.33^{cd}	10.33^{b}	11.66^{ab}	11.33^{a}
1100-1200h	6.66^{c}	13.33^{a}	12.66^{a}	11.33^{a}
1200-1300h	2.66^{e}	10.00^{bc}	11.33^{bc}	10.33^{b}
1300-1400h	5.66^{d}	8.66^{cd}	10.66^{bcd}	9.66^{bc}
1400-1500h	8.66^{a}	8.66^{cd}	10.33^{cd}	9.33^{c}
1500-1600h	7.66^{b}	8.33^{d}	9.66^{d}	8.33^{d}
1600-1700h	1.66^{fg}	8.66^{cd}	8.33^{e}	7.33^{e}
1700-1800h	0.66^{hi}	4.33^{e}	7.66e	4.66^{f}
CD (0.05)	0.978	1.56	1.215	0.978

Table 13. Number of returning foragers during June, 2022

Time interval	Number/10 minutes			
	Vellayani	Nedumangad	Mariapuram	Navaikulam
0700-0800h	2.33^{g}	0.66^{ef}	3.33^{f}	8.66^{d}
0800-0900h	6.33^{f}	2.00^{c}	6.33^{e}	12.66^{b}
0900-1000h	8.33^{e}	3.66^{b}	10.33^{cd}	13.66^{b}
1000-1100h	11.66^{bc}	0.33^{f}	13.00^{b}	16.33^{a}
1100-1200h	14.33^{a}	1.00^{def}	15.66^{a}	13.66^{b}
1200-1300h	12.66^{b}	4.66^{a}	15.00^{a}	11.33^{c}
1300-1400h	11.33^{bc}	1.66^{cd}	12.00^{bc}	11.33^{c}
1400-1500h	9.66^{d}	1.33^{cde}	10.66^{cd}	9.33^{d}
1500-1600h	8.66^{de}	1.00^{def}	10.33^{cd}	4.66^{e}
1600-1700h	6.33^{f}	1.00^{def}	9.66^{d}	2.66^{f}
1700-1800h	6.33^{f}	0.66^{ef}	6.66^{e}	1.66^{f}
CD (0.05)	1.318	0.932	1.817	1.103

Resin foraging activity during August, 2022 as given in Table 15 varied from 2.66 to 14.66. At Vellayani, peak foraging activity was observed during 1100h-1200h (11.33), 1200h-1300h (11.33) which was on par with 1000h-1100h (10.33), and minimum foraging activity was observed during 0700h-0800h (2.66). Increased foraging activity at Nedumangad was during 1100h-1200h (13.66) and minimum during 0700h-0800h (3.66). Peak foraging activity at Mariapuram was during 1000h-1100h (14.66) and the least foraging activity was observed during 0700h-0800h (3.00). At Navaikulam, the highest foraging activity was during 1000h-1100h (14.66) and the minimum during 1700h-1800h (4.66).

4.2 CORRELATION BETWEEN THE NUMBER OF RETURNING FORAGERS WITH RESIN LOAD AND WEATHER PARAMETERS

The correlation of weather parameters and returning foragers carrying resin load was found to be non-significant among all the locations (Table 16). Correlation study revealed to be a negative correlation in the case of temperature, wind velocity, sunshine hours and was in positive correlation in the case of relative humidity and rainfall. Correlated values of temperature from Vellayani, Nedumangad, Mariapuram, and Navaikulam were-0.321, -0.105, -0.348, and -0.023 respectively. On the other hand, wind velocity values were -0.69, -0.741, -0.185, -0.205, and sunshine hours were -0.576, -0.66, -0.392, -0.309 from Vellayani, Nedumangad, Mariapuram, and Navaikulam respectively. In contrast, values of relative humidity and rainfall were 0.364, 0.449, 0.15, 0 and 0.24, 0.411, 0.543, and 0.335, respectively across all the locations.

Table 14. Number of returning foragers during July, 2022

Time interval	Number/10 minutes			
	Vellayani	Nedumangad	Mariapuram	Navaikulam
0700-0800h	3.66^{g}	1.00^{cd}	3.66^{h}	1.00^{b}
0800-0900h	5.66^{f}	1.33^{c}	9.33^{f}	1.33^{b}
0900-1000h	8.33^{e}	2.33^{b}	14.33^{d}	1.33^{b}
1000-1100h	9.66^{bc}	4.33^{a}	16.66^{c}	3.66^{a}
1100-1200h	10.66^{a}	0.33^{d}	19.66^{b}	1.33^{b}
1200-1300h	10.33^{ab}	1.00^{cd}	21.66^{a}	1.00^{b}
1300-1400h	9.66^{bc}	1.00^{cd}	22.66^{a}	1.33^{b}
1400-1500h	9.33^{cd}	0.66^{cd}	11.66^{e}	1.00^{b}
1500-1600h	9.00^{cde}	0.66^{cd}	13.66^{d}	1.00^{b}
1600-1700h	8.66^{de}	0.66^{cd}	10.66^{e}	1.00^{b}
1700-1800h	5.66^{f}	0.33^{d}	7.33^{g}	0.66^{b}
CD (0.05)	0.932	0.834	1.215	0.722

Table 15. Number of returning foragers during August, 2022

Time interval	Number/10 minutes			
	Vellayani	Nedumangad	Mariapuram	Navaikulam
0700-0800h	2.66^{g}	3.66^{h}	3.00^{g}	8.33^{e}
0800-0900h	4.66^{f}	6.66^{g}	8.66^{de}	9.66^{d}
0900-1000h	6.66^{e}	8.33^{f}	11.66^{bc}	12.66^{b}
1000-1100h	10.33^{ab}	11.66^{bc}	14.66^{a}	14.66^{a}
1100-1200h	11.33^{a}	13.66^{a}	12.66^{b}	11.66^{c}
1200-1300h	11.33^{a}	12.33^{b}	12.33^{bc}	9.66^{d}
1300-1400h	9.33^{bc}	11.33^{cd}	11.33^{c}	9.33^{d}
1400-1500h	8.33^{cd}	10.66^{d}	9.66^{d}	8.33^{e}
1500-1600h	8.00^{d}	9.33^{e}	8.66^{de}	7.33^{f}
1600-1700h	7.66^{de}	8.33^{f}	8.33^{e}	8.33^{e}
1700-1800h	5.33^{f}	6.33^{g}	6.66^{f}	4.66^{g}
CD (0.05)	1.179	0.978	1.179	0.978

Table 16. Correlation between weather parameters and number of returning foragers with resin load

Location ID	Location	Temperature	Relative humidity	Wind speed	Sunshine hours	Rainfall
L1	Vellayani	-0.321	0.364	-0.690	-0.576	0.240
L2	Nedumangad	-0.105	0.449	-0.741	-0.660	0.411
L3	Mariapuram	-0.348	0.150	-0.185	-0.392	0.543
L4	Navaikulam	-0.023	0.100	-0.205	-0.309	0.335

4.3 VOLUME OF RESIN INSIDE THE HIVE

Observations on the volume of resin were recorded during October (2021), March (2022), and August (2022) (Fig. 3). At Vellayani, the volume of resin observed during October were 24000 mm^3, 567000 mm^3 during March, and 945000 mm^3in August. Resin volumes in October, March, and August were 48000 mm^3, 910000 $mm^{3,}$ and 1440000 mm^3 in Nedumangad and 42000 mm^3, 1062500 mm^3, and 1501500 mm^3 in Mariapuram, respectively. Whereas at Navaikulam, resin volumes obtained were 480000mm^3, 990000$mm^{3,}$ and 1386000 mm^3, respectively.

4.4 PHYSICAL PROPERTIES OF PROPOLIS

4.4.1 Colour, texture, and odour of propolis

Propolis samples collected from various locations are represented in the Plate 2. Propolis collected from Nedumangad was dark greyish-reddish brown. Propolis from Mariapuram was moderate olive brown and those from Vellayani and Navaikulam were moderate brown colour. The texture of propolis was sticky at normal temperatures and hard at cold temperatures in all the locations. Odour of propolis was pleasant and aromatic across all the locations (Table 17).

4.4.2 Solubility of propolis

Based on the results obtained, the solubility of propolis varied based on different polarity indices. Propolis was found to have the highest solubility in ethyl acetate, diethyl ether, and hexane. It was moderately soluble in ethanol and least soluble in acetonitrile, methanol, and distilled water (Table 18).

Table 17. Colour, texture and odour of propolis from all locations

Locations	Colour	Texture	Odour
Vellayani	Moderate brown	Sticky at normal temperature and hard at cold temperature	Aromatic
Nedumangad	Dark grayish reddish brown	Sticky at normal temperature and hard at cold temperature	Aromatic
Mariapuram	Moderate olive brown	Sticky at normal temperature and hard at cold temperature	Aromatic
Navaikulam	Moderate brown	Sticky at normal temperature and hard at cold temperature	Aromatic

Table 18. Solubility of propolis in various solvents

Solvent	Polarity index	Vellayani	Nedumangad	Mariapuram	Navaikulam
Distilled water	9.0	+	+	+	+
Acetonitrile	5.8	-	-	-	-
Methanol	5.1	+	+	+	+
Ethyl Acetate	4.4	++	++	++	++
Ethanol	4.3	+	+	+	+
Diethyl ether	2.8	++	++	++	++
Hexane	0.1	++	++	++	++

++ - most of particles dissolved in the solvent, + - some of the particles dissolved in the solvent, – - no apparent dissolution of particles was observed

Vellayani sample

Nedumangad sample

Mariapuram sample

Navaikulam sample

Plate 2. Colour of propolis samples from each location

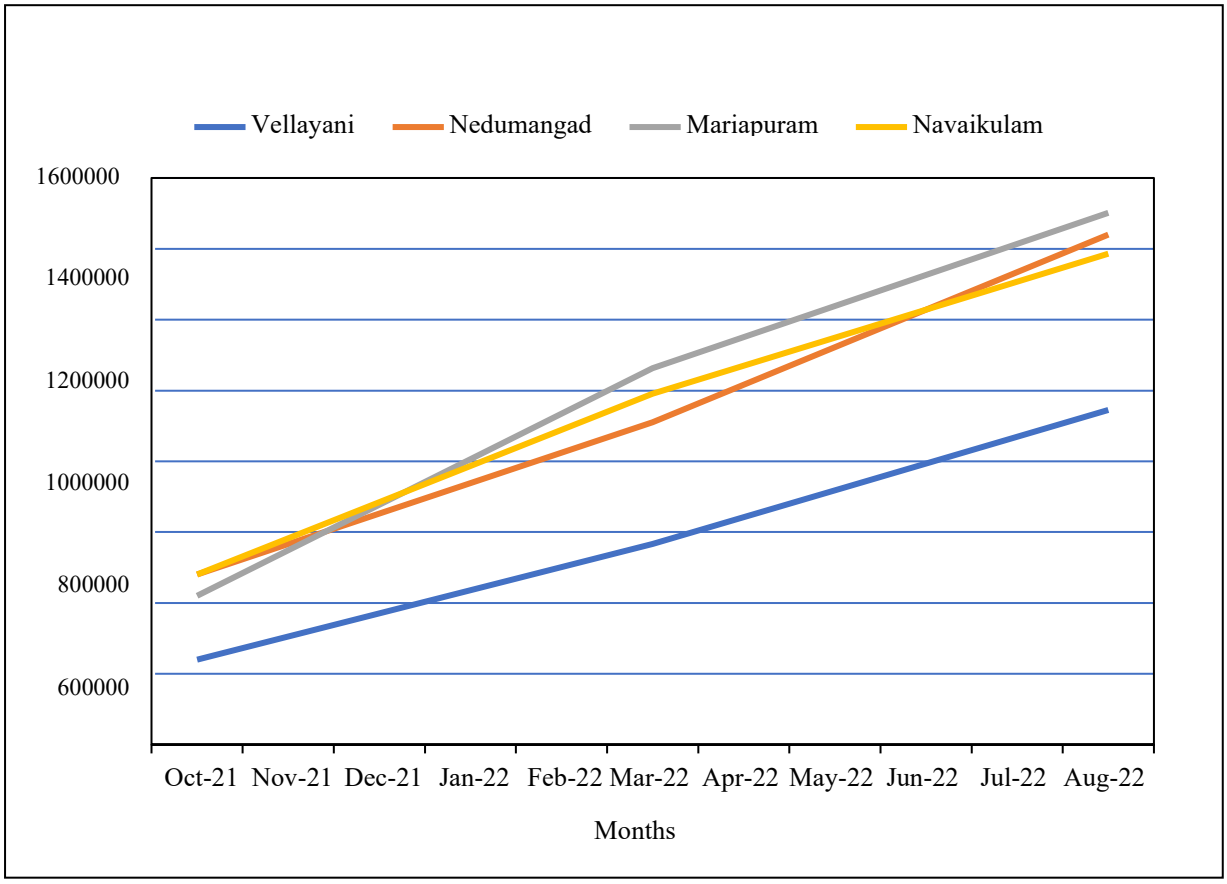


Fig. 3. Volume of resin inside the hive from different locations (in mm^3)

4.5 COMPOSITION OF PROPOLIS

4.5.1 Chemical composition of propolis samples by GC-HRMS

GC-HRMS analysis-based components from Vellayani were glycerine, oxazepam ditms, cycloheptasiloxane, cyclooctasiloxane, cyclodecasiooxane, and d-glucopyranoside. Whereas, from Nedumangad, components identified were cyclohexasiloxane, docosahexaenoic acid, cycloheptasiloxane, 7,8-epoxylanostan-11-ol, cyclooctasiloxane, cyclodecasilooxane, and docosahexaenoic acid. Hexadecenoic acid, ethyl oleate, linoleic acid ethyl ester, propanoic acid, and 7,8- epoxylanostan-11-ol were found from Mariapuram, while from Navaikulam components were hexadecenoic acid, ethyl oleate, linoleic acid ethyl ester, and cyclodecasilooxane (Table 19).

4.5.2 Chemical composition of propolis samples by LC-HRMS

The total number of LC-HRMS analysis-based components from Vellayani was 200 by positive ionization and 85 by negative ionization. In propolis collected from Nedumangad, the number of components analyzed by positive and negative ionization were 136 and 126, respectively. From Mariapuram, it was 201 and 88, while it was 200 and 127 at Navaikulam, respectively. The main chemical classes present in the propolis were acids, fatty acids, steroids, alcohols, amines, amino acids, flavonoids, terpenoids, chalcones, aldehydes, ketones, benzene, coumarin, pterocarpan, ether, and ester.

Table 20 represents the list of major components of propolis identified by LC-HRMS analysis from Vellayani. The major components identified by positive ionisation mode from the Vellayani propolis sample were 20s, 24s-dihydroxydammer-25-en-3-one, isogarcinol, erucamide and glycyrrhizic acid whereas, 2,5-di-tert-butylhydroquinone, coatline a, 4,2'-dihydroxychalcone 4-glucoside, NP-011548, dimethyl-5,5'-[(2-fluoro-1,4phenylene) bis(oxy)] bis (2,2dimethyl pentanoate) were the most abundant negative ionisation compounds.

Table 21 represents the list of major components of propolis identified by LC-HRMS analysis from Nedumangad. 20s,24s-dihydroxydammer – 25 – en – 3 - one, n~2~-(n, n' - dicyclohexylcarbamimidoyl) – n -[4-(hydroxymethyl)phenyl]-3-(2-naphthyl)-l-alaninamide, oleanolic acid, 18-β-Glycyrrhetinic acid, 5,5'-[(2,3'-dipropyl-

Table 19. Components of propolis identified through GC-HRMS analysis

Vellayani		
Sl. No	Components	Chemical formula
1	Glycerine	C3H8O3
2	Oxazepam ditms	C21H27CIN2O2Si2
3	Cycloheptasiloxane	C14H42O7Si17
4	Cyclooctasiloxane	C16H48O8Si8
5	Cyclodecasiooxane	C20H60O10Si10
6	D-Glucopyranoside	C38H62O9
Nedumangad		
Sl. No	Components	Chemical formula
1	Cyclohexasiloxane	C12H36O6Si6
2	Docosahexaenoic acid	C69H9O6
3	Cycloheptasiloxane	C14H42O7Si17
4	7,8-Epoxylanostan-11-ol	C32H4O4
5	Cyclooctasiloxane	C16H48O8Si8
6	Cyclodecasilooxane	C20H60O10Si10
7	Docosahexaenoic acid	C69H134O6
8	9,12,45-Octadecatrienoic acid	C27H52O4Si2
Mariapuram		
Sl. No	Components	Chemical formula
1	Hexadecanoic acid	C18H36O2
2	Ethyl Oleate	C20H38O2
3	Linoleic acid ethyl ester	C20H36O2
4	Propanoic acid	C27H42O4
5	7,8- Epoxylanostan-11-ol	C32H54O4
Navaikulam		
Sl. No	Components	Chemical formula
1	Hexadecanoic acid	C18H36O2
2	Ethyl oleate	C20H38O2
3	Linoleic acid ethyl ester	C20H36O2
4	Cyclodecasilooxane	C20H60O10Si10

4,4'-biphenyldiyl)bis(oxy)]bis(2,2- dimethylpentanoic acid) were the major positive ionisation components and 6-(Dodecylamino)-2-[3-(4-methyl-1-piperazinyl)propyl]-1H-benzo[de] isoquinoline 1, 3(2H) dione, (5alpha, 7alpha, 13alpha, 17alpha, 20S, 23R) – 24 – Hydroxy - 4, 4, 8 – trimethyl–3-oxo21, 23:24, 25-diepoxycholesta-1, 14-dien-7-ylacetate, 3b-Hydroxy-5-cholenoic acid, 20S-Protopanaxatriol, (2beta, 5beta, 21beta)-16[(14beta)-14,15-Dihydroeburnamenin-14-yl]aspidofractinine-21-carboxylic acid, CP 55, 244 were the negative ionisation compounds found from Nedumangad.

Table 22 represents the list of major components of propolis identified by LC-HRMS analysis from Mariapuram. Positive ionisation components from Mariapuram were Glycyrrhizic acid, NP005821, lupeol, ambonic acid, MFCD00045988 whereas, Methyl oleanolate, 6,9,12,15-Docosatetraenoic acid, (3alpha)-3-Hexyl-3-hydroxyandrostan17-one, melliferone estradiol enanthate, anacardic acid were the major negative ionisation components observed.

Table 23 represents the list of major components of propolis identified by LC-HRMS analysis from Navaikulam. Major positive ionisation components were Glycyrrhizic acid, isogarcinol, 20s,24s-dihydroxy-dammer-25-en-3-one, lupeol, NP005821, 5,5'-[(2,3'-dipropyl-4,4'-biphenyldiyl) bis(oxy)] bis (2,2-dimethylpentanoic acid) and (2beta, 5beta, 21beta) - 16 - [(14beta) - 14, 15-Dihydroeburnamenin - 14 - yl] aspido fractinine- 21 – carboxylic acid, (5alpha, 7alpha, 13alpha, 17alpha, 20S, 23R)-24-Hydroxy-4, 4, 8-trimethyl-3-oxo21, 23:24, 25-diepoxycholesta-1,14-dien-7-ylacetate, Methyl oleanolate were the negative ionisation compounds found respectively.

Table 24 represents the list of components of propolis found common in all the locations. The components identified as common in all the locations by positive ionisation were docosahexaenoic acid, octadec-9-ynoic acid, punicic acid, ethyl linoleate coming under the class fatty acids, and steroids. Common components included in the class of flavonoids were bonannione a, thevetiaflavone, and rhamnetin. The terpenoid components included were lupeol, oleanolic acid, b-Amyrenonol, and betulin. The Chalcone compound found common across all the locations was dihydrocordoin whereas hydrocarbon included in the class was (1R,4E,9S)-11,11-Dimethyl-8methylenebicyclo [7.2.0] undecene. The common aminoacid identified was

olomoucine and the common benzophenone was isogarcinol. Alcohols found common across all the locations were 5-[(Z)-Pentadec-8-enyl] benzene-1,3-diol and 2,2,6,6-Tetramethyl-1-piperidinol (TEMPO). Amines comprised octadecanamine, oleamide, (1R)-1-Butyl-9-{1-[(4,6-dimethyl-5-pyrimidinyl) carbonyl] -4-methyl -4- piperidinyl} -N-methyl3,9diazaspiro [5.5] undecane-3-carboxamide and N, N'-Dicyclohexylurea and the compound celestolide belonged to class indanes. Some other common components identified were MFCD00045988, NP-021050 and NP-005821.

Components found common throughout all the locations by negative ionization were 18-β-Glycyrrhetinic acid, 4-[3-(3,5-ditert-butylphenyl)-3-oxoprop1-enyl] benzoic acid, syringic acid, and anacardic acid. Fatty acids and steroids commonly found were 9,10-Dihydroxystearic acid and 16-Hydroxyhexadecanoic acid, ether component identified was cannabidiol dimethyl ether. Flavonoids were 6-Farnesyl-3',4',5,7-tetrahydroxy flavanone, luteolin, quercetin, and sanggenol O. Amino acids found in all locations were N-(Adamantan-1-ylacetyl) glycine and N-Undecanoyl glycine. The commonly found terpenoid was (3R)-hydroxy-beta-ionone and ester was monobutyl phthalate. Alkyl sulfate was dodecyl sulphate and Gancaonin J was the common chalcone found. 2-Hydroxy-1-(4-hydroxyphenyl)-5- methyl-3-hexanone was the common ketone. Commonly found lactone was matairesinol and hydroquinone was 2,5-di-tert-Butylhydroquinone. NP-011548 was the other component identified as common in the different locations.

Table 20. Major components of propolis from Vellayani identified by LC-HRMS analysis

Positive ionization				Negative ionization			
Sl No.	Components	Chemical formula	Relative abundance (%)	Sl No.	Components	Chemical formula	Relative abundance (%)
1	20S,24S-dihydroxydammer-25-en-3-one	C30 H50 O3	14.96	1	2,5-di-tert-Butylhydroquinone	C14 H22 O2	16.42
2	Isogarcinol	C38 H50 O6	9.21	2	Coatline A	C21 H24 O10	11.35
3	Erucamide	C22 H43 N O	7.01	3	4,2'-Dihydroxychalcone 4-glucoside	C21 H22 O8	5.93
4	Glycyrrhizic acid	C30 H46 O4	6.17	4	NP-011548	C18 H34 O3	5.00
5	Testosterone decanoate	C29 H46 O3	5.82	5	Dimethyl 5,5'-[(2-fluoro-1,4-phenylene)bis(oxy)]bis(2,2- dimethyl pentanoate)	C22 H33 F O6	4.23
6	b-Amyrenonol	C30 H48 O2	3.03	6	Asiatic acid	C30 H48 O5	2.38
7	(2S,3R)-3,5-Dihydroxy-2-(4-hydroxyphenyl)-3,4-dihydro-2H chromen-7-yl β-D-glucopyranoside	C21 H24 O10	2.80	7	1-{8-[2-(1-Benzoyl-4-phenyl-4- piperidinyl) ethyl]-8-azabicyclo[3.2. 1]oct-3-yl}-1,3-dihydro-2Hbenzimidazol-2-one	C34 H38 N4 O2	2.34
8	Lupeol	C30 H50 O	2.68	8	Prebarbigerone	C24 H26 O6	2.15
9	[(3E)-4,8-Dimethyl-3,7-nonadien-1-yl] benzene	C17 H24	2.21	9	(1aR,4E,7aS,10aS,10bS,1a'R,4'E,7a'S, 10a'S,10b'S)-5,5'-[1,3- Propanediylbis(1H-1,2,3-triazole-4,1- diylmethylene)]bis(1a-methyl-8-methylene 2,3,6,7,7a,8,10a,10 boctahydrooxireno[9,10]cyclo-deca[1,2- b]fura n-9(1aH)-one)	C37 H46 N6 O6	2.10
10	NP-005821	C30 H46 O3	2.12	10	Nonyl [(2R)-5-hydroxy-3-oxo-4- palmitoyl-2,3-dihydro-2-furanyl]acetate	C31 H54 O6	1.87

Table 21. Major components of propolis identified through LC-HRMS from Nedumangad

Positive ionization				Negative ionization			
Sl No.	Components	Chemical formula	Relative abundance (%)	Sl No.	Components	Chemical formula	Relative abundance (%)
1	20S,24S-dihydroxy dammer-25-en-3-one	C30 H50 O3	19.37	1	6-(Dodecylamino)-2-[3-(4-methyl-1-piperazinyl)propyl]-1Hbenzo[de]isoquinoline-1,3(2H)-dione	C32 H48 N4 O2	19.82
2	N~2~-(N,N'-Dicyclohexylcarbamimidoyl)-N-[4-(hydroxy methyl)phenyl]-3-(2-naphthyl)- L-alaninamide	C33 H42 N4 O2	18.77	2	(5alpha,7alpha,13alpha,17alpha,20S, 23R)-24-Hydroxy-4,4,8-trimethyl-3-oxo21,23:24,25-diepoxycholesta-1,14- dien-7-yl acetate	C32 H46 O6	13.68
3	Oleanolic acid	C30 H48 O3	16.04	3	20S-Protopanaxatriol	C30 H52 O4	9.87
4	18-β-Glycyrrhetinic acid	C30 H46 O4	8.65	4	(2beta,5beta,21beta)-16-[(14beta)- 14,15-Dihydroeburnamenin-14- yl] aspidofractinine-21-carboxylic acid	C39 H46 N4 O2	5.47
5	5,5'-[(2,3'-Dipropyl-4,4'-biphenyldiyl)bis(oxy)]bis(2,2-dimethylpentanoic acid)	C32 H46 O6	5.83	5	CP 55,244	C26 H42 O3	5.14
6	b-Amyrenonol	C30 H48 O2	2.17	6	Cervonoyl ethanolamide	C24 H36 O3	5.12
7	5-[(Z)-Pentadec-8-enyl] benzene-1,3-diol	C21 H34 O2	2.02	7	Cardanolide	C23 H36 O2	5.11
8	(22E)-24nor-cholesta-1,4,22-trien-3-one	C26 H38 O	1.70	8	3-Oxo-4,6-choladienoic acid	C24 H34 O3	2.83
9	Isofraxidin	C11 H10 O5	1.68	9	Anacardic acid	C22 H36 O3	2.01
10	Prednisone	C21 H26 O5	1.46	10	16-Hydroxyhexadecanoic acid	C16 H32 O3	1.95

Table 22. Major components of propolis identified through LC-HRMS from Mariapuram

Positive ionization				Negative ionisation			
Sl No.	Components	Chemical formula	Relative abundance (%)	Sl No.	Components	Chemical formula	Relative abundance (%)
1	Glycyrrhizic acid	C30 H46 O4	12.36	1	Methyl oleanolate	C31 H50 O3	10.53
2	NP-005821	C30 H46 O3	11.03	2	6,9,12,15-Docosatetraenoic acid	C23 H38 O2	10.37
3	Ambonic acid	C31 H48 O3	6.05	3	Melliferone	C30 H44 O3	9.33
4	Lupeol	C30 H50O	5.70	4	Estradiol enanthate	C25 H36 O3	4.17
5	MFCD00045988	C20 H38 O3	4.65	5	Anacardic acid	C22 H36 O3	3.69
6	5-[(8Z)-8-Heptadecen-1-yl]-1,3-benzenediol	C23 H38 O2	3.15	6	(2R)-2-[(3S,4S)-3-[(4-{3-[4- (Cyclo propyl oxy)benzyl]-1-ethyl-1Hpyrazol-5-yl}-1-piperidinyl) methyl]-4-(3- fluoro phenyl)-1-pyrrolidinyl]-3- methylbutanoic acid	C36 H47 F N4 O3	3.47
7	Oleanolic acid	C30 H48 O3	3.00	7	Cannabidiol dimethyl ether	C23 H34 O2	3.43
8	Betulin	C30 H50 O2	2.58	8	Glochidone	C30 H46 O	2.15
9	20S,24S-dihydroxy dammer-25-en-3-one	C30 H50 O3	2.28	9	Docosapentaenoic acid	C22 H34 O3	2.07
10	(6E,10E,14E,18E)-7,11,15,19,23-Pentamethyl-3-methylene1,6,10,14,18,22-tetracosahexaene	C30 H48	2.14	10	6-Farnesyl-3',4',5,7- tetrahydroxyflavanone	C30 H36 O6	1.96

Table 23. Major components of propolis identified through LC-HRMS from Navaikulam

Positive ionization				Negative ionization			
Sl No.	Components	Chemical formula	Relative abundance (%)	Sl No.	Components	Chemical formula	Relative abundance (%)
1	Glycyrrhizic acid	C30 H46 O4	10.31	1	(2beta,5beta,21beta)-16-[(14beta)- 14,15-Dihydroeburnamenin-14- yl]aspidofractinine-21-carboxylic acid	C39 H46 N4 O2	36.20
2	Isogarcinol	C38 H50 O6	8.14	2	(5alpha,7alpha,13alpha,17alpha,20S, 23R)-24-Hydroxy-4,4,8-trimethyl-3-oxo21,23:24,25-diepoxycholesta-1,14- dien-7-yl acetate	C32 H46 O6	5.50
3	20S,24S-dihydroxydammer-25-en-3-one	C30 H50 O3	7.63	3	Methyl oleanolate	C31 H50 O3	5.19
4	Lupeol	C30 H50 O	5.25	4	1-{8-[2-(1-Benzoyl-4-phenyl-4- piperidinyl)ethyl]-8-azabicyclo[3.2. 1]oct-3-yl}-1,3-dihydro-2Hbenzimidazol-2-one	C34 H38 N4 O2	4.74
5	NP-005821	C30 H46 O3	4.79	5	4-[(1E)-3-{5-(Adamantan-1-yl)-4-[(2-methoxyethoxy)methoxy]-2- pentylphenyl}-3-oxo-1-propen-1- yl]benzoic acid	C35 H44 O6	3.75
6	5,5'-[(2,3'-Dipropyl-4,4' biphenyldiyl)bis(oxy)]bis (2,2- dimethylpentanoic acid)	C32 H46 O6	4.44	6	CP 55,244	C26 H42 O3	3.74
7	NP-011548	C18 H34 O3	2.88	7	6,9,12,15-Docosatetraenoic acid	C23 H38 O2	3.26
8	Ursolic acid	C30 H48 O3	2.87	8	NP-011548	C18 H34 O3	3.20
9	6,6'-[1,4-Phenylenebis(carbonylimino)]bis(1-pentylquinolinium)	C36 H40 N4 O2	2.63	9	(3alpha)-3-Hexyl-3-hydroxyandrostan17-one	C25 H42 O2	1.81
10	3-Cyclohexyl-1-[2-(3-methoxyphenyl)ethyl]-1-{[4'-(1-piperazinyl)-2-biphenylyl]methyl}urea	C33 H42 N4 O2	2.56	10	Cardanolide	C23 H36 O2	1.80

Table 24. Components of propolis found common across different locations

Positive ionisation		Negative ionization	
Classes	Components	Classes	Components
Fatty acids and sterouids	Docosahexaenoic acid; octadec-9-ynoic acid; Punicic acid; Ethyl Linoleate	Acids	18-β-Glycyrrhetinic acid; 4-[3-(3,5-ditert-butylphenyl)-3-oxoprop 1-enyl]benzoic acid; Syringic acid; Anacardic acid
Aminoacids	Olomoucine	Fatty acids and sterouids	9,10-Dihydroxystearic acid; 16-Hydroxyhexadecanoic acid
Flavanoids	Bonannione A; Thevetiaflavone; Rhamnetin	Aminoacids	N-(Adamantan-1-ylacetyl)glycine; N-Undecanoylglycine
Terpenoids	Lupeol; Oleanolic acid; Betulin; b-Amyrenonol	Flavanoids	6-Farnesyl-3',4',5,7-tetrahydroxyflavanone; Luteolin; Quercetin; Sanggenol O
Chalcones	Dihydrocordoin	Terpenoids	(3R)-hydroxy-beta-ionone
Ketone	20S,24S-dihydroxy dammer-25-en-3-one; 5,7-Dihydroxy-2-(4-hydroxy-3- methoxy phenyl)-6-(3-methyl-2-buten-1- yl)-2,3-dihydro-4H-chromen-4-one; 1,1,1-Trifluoro-2-octadecanone	Chalcones	Gancaonin J; Orotinichalcone
Alcohol	5-[(Z)-Pentadec-8-enyl] benzene-1,3- diol; 2,2,6,6-Tetramethyl-1-piperidinol (TEMPO)	Ketone	2-Hydroxy-1-(4-hydroxyphenyl)-5- methyl-3-hexanone
Others	MFCD00045988; NP-021050; NP-005821	Others	NP-011548
Benzophenone	Isogarcinol	Hydroquinones	2,5-di-tert-Butylhydroquinone
Hydrocarbon	(1R,4E,9S)-11,11-Dimethyl-8-methylenebicyclo[7.2.0]undec-4-ene	Alkyl sulfate	Dodecyl sulfate
Indanes	Celestolide	Lactone	Matairesinol
		Benzenes	Helilupolone
		Ester	Monobutyl phthalate
		Ether	Cannabidiol dimethyl ether

DISCUSSION

5. DISCUSSION

Although propolis is with a wide range of biological activities like antibacterial, anti-inflammatory, antiulcer, antioxidant, anticancer activities, etc., and has extensive application in modern medicine, it was not utilized properly in comparison with other honeybee products. In this context, the present study was based on resin foraging, the origin, and the composition of propolis. The results of the study are discussed below.

5.1 RESIN FORAGING BEHAVIOUR

5.1.1 Plants visited by stingless bees for resin

Propolis is made up by combining resinous substances from exudates of diverse plants with their mandibular and wax gland secretion. Bees used multiple resin sources, whereas relative quantities of different resins varied among sites. When the botanical origin was investigated, it appears that they favor some plants over others indicating a highly selective forage strategy. Various potential resin sources *viz*., *M. indica*, *A. heterophyllus*, *A. altilis*, *A. occidentale*, *G. xanthochymus, H. brasiliensis*, *G. cambogia*, *A. araucana*, *A. hirsutus* and *M. peltate* were identified across the four different locations. Resins from different sources act synergistically when combined may benefit bees by augmenting the protective function of propolis against multiple antagonists. The foraging bees located the natural or artificial wound from resin sources including cut tree branches, fruit stalks, fruit surfaces, etc., and collected the sticky resin with their mandibles, packed into small balls in their hind legs.

Roopa *et al.* (2017) also found stingless bees collected latex or resin from four different plant species, including the cut branches of mango, the cut branches and fruit stalk of jack fruit, the stem, bark, and cut branches of drumstick, as well as the leaf lamina of periwinkle. In a study conducted in Kerala by Vazhacahrickal and Jose (2018), mango trees, jack trees, tapioca, cashews, drumsticks, banyan trees, fig trees, and sunflowers were identified as resin sources of stingless bees.

Alvarez *et al.* (2013) identified resin sources of propolis by *Tetragonula biroi*, such as *M. indica, A. heterophyllus,* etc. Also, as per Georgieva *et al*. (2019) and Mulyati

et al. (2020) *M. indica* was the botanical source of stingless bee propolis. Marisa and Salni, (2012) discovered the origin of propolis from *P. indicus* and *A. communis. G. mangostana* was identified as a resin source of stingless bee propolis (Sanpa *et al.,* 2015; Ishizu *et al*., 2018).

A. occidentale was recorded as a botanical source of Brazilian red propolis of *A. mellifera* by Silva *et al.* (2008) and *A. angustifolia* as a resin source by both Bankova *et al*. (1999) and Giovanini de *et al.* (2021).

5.1.2 Visitation at resin wounds

An artificial wound was made in the dominant resin producing plants. Wounds were graded from 1 to 4 based on resin flow and the foraging activities were recorded on daily basis. Based on that, *M. indica* was with the highest grade '4', it was found that the species visited with only 11.53 bees per wound on a daily basis whereas, *G. cambogia* of grade '3' was with more foragers of 16.71. It was found that there was no association between the number of bees visited and the size of the wound, which was similar to the observations by Leonhardt and Bluthgen (2009). There may be other characteristics like quantity of resin that can influence bees' foraging decisions. Unloading and processing resin without getting stuck in it was a laborious task for the bees. They took 10 to 15 minutes for completing resin foraging on the identified resin sources with maximum time on *A. occidentale* at 15 minutes, and least time on *A. araucana* at 10 minutes. Moreover, the duration of the collection depends on the quantity and consistency of the resin.

It was found that foraging started on the 1st or 2nd day of wound initiation, attained peak on the 4th-16th day, and stopped foraging on the 14th-23rd day in various sources. Similar findings carried out by Leonhardt and Bluthgen (2009) revealed that the number of foragers at naturally occurring resin wounds remained constant throughout the observation period; it was observed that bees discovered artificially induced wounds within 1-2 days.

5.1.3 Resin foraging activity of *T. travancorica*

The proportion of workers retuning with resin varied considerably during different seasons between locations. As per Fig. 4, resin foraging started after 0700h and declined after 0600h in all the locations. High resin foraging activity was found from 0900h to 1600h in all the locations throughout the observation**.** The resin foraging was seen during the warm part of the day when the resin is still soft and readily available for collection. Peak foraging activity was noticed at Vellayani, Nedumangad, Mariapuram, and Navaikulam from 1100h to 1200h with 9.55, 9.98, 10.79, and 11.25 returning foragers with resin load/hive/10minutes. Least foraging was observed from 0700h to 0800h with 1.70, 1.70, 1.82, and 1.87 number of foragers and also in case of 1700h to 1800h. In accordance with the above study, Wicaksono *et al*. (2020) found the lowest number of stingless bees *Lepidotrigona terminata Smith* were departing and returning to the nest at 0700h to 0800h and 1600h to 1700h, while peak hours were from 1000h to 1200h. Another similar finding by Saravanan and Alagar (2007), recorded that after 0900h and evenly dispersed throughout the day, resin foraging increased, but it decreased after 1600h. Similar study by Vijayan *et al.* (2018), found that resin foragers started during 0700h to 0800h, foraging activity reached its peak between 0800h and 1100h, reduces to a minimum during 1700h to 1800h. Additionally, on sunny days, foraging for resins was usually observed between 1000h and 1530h as resins were more malleable at higher temperatures (Alfonsus, 1933; Meyer, 1956; Nyeko *et al*., 2002).

As per Fig. 5, in all the locations, it was observed that after December month there was a sudden decline in resin foraging activity, i.e., during January, February up to March and the foraging started increasing afterward. As the honey flow season was from January to March, there was a considerable increase in nectar and pollen foragers which may be the cause for reduced resin foragers during this period in all the locations. In accordance with Crane (1990), he discovered that, in temperate regions, resin foragers always found resin from May to November and also found seasonality in resin collection and propolis use. According to reports, late summer (end of June) to autumn, when the honey flow is significantly diminished, was when the resin is most regularly gathered (Alfonsus, 1933; Meyer, 1956; Crane, 1990). Similar results were also noted

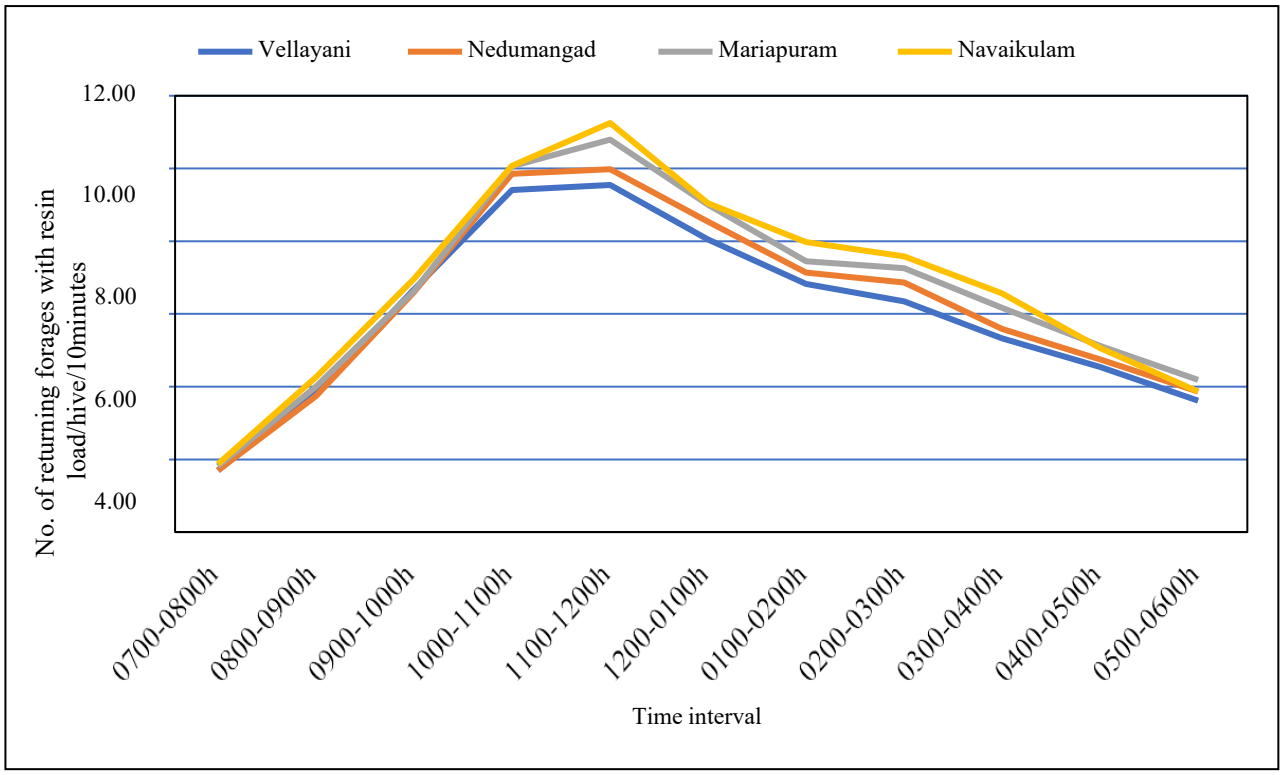


Fig. 4. Resin foraging activity of *Tetragonula travancorica* at different hours

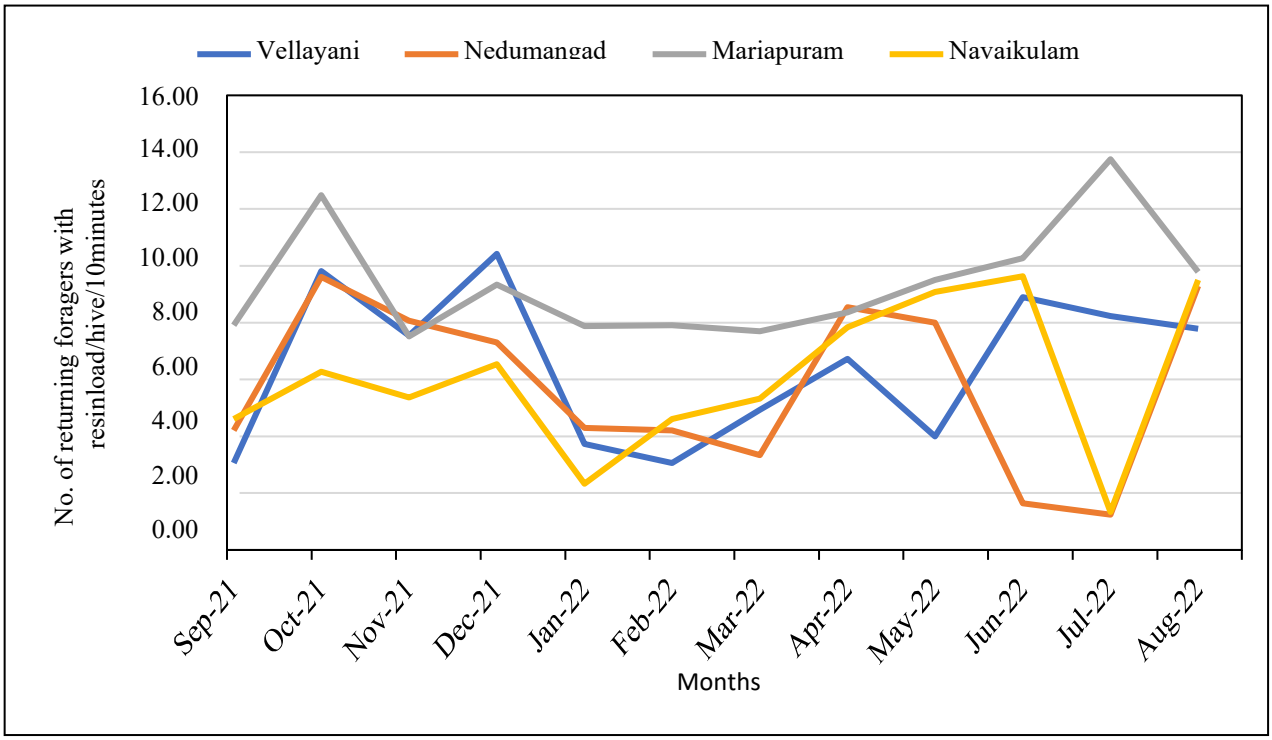


Fig. 5. Resin foraging activity of *Tetragonula travancorica* during different months

by Devanesan *et al.* (2002) who found that foraging activity peaked in July and was at its lowest in December and January.

5.2 CORRELATION BETWEEN THE NUMBER OF RETURNING FORAGERS WITH RESIN LOAD AND WEATHER PARAMETERS

The correlation of weather parameters and returning foragers carrying resin load was found to be non-significant among all the locations. In the present study, correlation results were revealed to be a negative correlation in the case of temperature, wind velocity, and sunshine hours. Whereas, it was a positive correlation in the case of relative humidity and rainfall. So, the correlated values of temperature and weather parameters were -0.321, -0.105, -0.348, and -0.023 from Vellayani, Nedumangad, Mariapuram, and Navaikulam respectively. Also, wind velocity varied from -0.741 to -0.185, and sunshine hours ranged from -0.66 to -0.392. Whereas, in the case of relative humidity, ranged from 0.01 to 0.449, across each location, and rainfall observation varied from 0.24 to 0.543. This was found in accordance with the study conducted by Pereira *et al.* (2009), who delineated that temperature and output of propolis were not shown to be correlated, while rainfall showed a correlation in comparison. According to Bastos *et al.* (2011), they noticed a spike in the number of resin foragers on *B. dracunculifolia* during the rainy season. Whereas, in contrary to the above results, Wicaksono *et al*. (2020) reported that temperature and light levels were positively connected with bees' flight behaviours, this may be due to differences in climatic conditions and other environmental factors.

5.3 PHYSICAL PROPERTIES OF PROPOLIS

5.3.1 Colour, texture, and odour of propolis from all locations

In this present study, colour of propolis from Nedumangad was dark greyish reddish brown in colour, that from Mariapuram was moderate olive brown and those from Vellayani and Navaikulam were moderate brown. The variation of propolis colour may be depending on its age and the plant sources. In the studies by Ghisalberti (1979) and Kuropatnicki *et al*. (2013), colour of propolis was identified as light cream, green, red, brown, or black propolis. The present research found that the texture of propolis was sticky at normal temperatures and hard at cold temperatures in all the locations and

this was in accordance with the study of Ghisalberti (1979), Kuropatnicki *et al*. (2013), and Wagh, (2013). Also, the odour of propolis was pleasant and aromatic across all the locations and similar observations were recorded by Ghisalberti, (1979), Kuropatnicki *et al*. (2013), and Wagh, (2013).

5.3.2 Solubility of propolis

As per our study, propolis was found to have the highest solubility in ethyl acetate, diethyl ether, and hexane, moderate solubility in ethanol, and the least solubility in acetonitrile, methanol, and distilled water. In accordance with this, Mendoza *et al*. (2011) also found similar results in the solubility study of propolis.

5.4 COMPOSITION OF PROPOLIS

Propolis contains more than 300 components, including phenolic aldehydes, polyphenols, amino acids, steroids, and inorganic compounds. However, the high variation in the chemical composition of propolis was noticed in samples from different locations according to the diverse plant sources. This remarkable compositional chemical diversity among propolis indicates that bees may not only yield from a diversity of resin producing plants species, but also from this compositional diversity.

5.4.1 GC-HRMS analysis of propolis

Eventhough propolis samples were chemically distinct among different regions, based on GC-HRMS analysis, cycloheptasiloxane and cyclooctasiloxane were found common in samples collected from Vellayani and Nedumangad. Whereas, hexadecenoic acid, ethyl oleate, and linoleic acid ethyl ester were identified from Mariapuram and Navaikulam samples. 7,8-epoxylanostan-11-ol was found in propolis from both Nedumangad and Mariapuram. While, cyclodecasiloxane was found in almost all locations except Mariapuram. Other components were, from Vellayani glycerine, oxazepam ditms, d-glucopyranoside, cyclohexasiloxane from Nedumangad and propanoic acid from Mariapuram. Linoleic acid, ethyl oleate, hexadecanoic acid, and propanoic acid were the important components from our study as reported by (Ramnath *et al.,* 2015) and additionally, heptasiloxane, octasiloxane, 7,8, Epoxylanostan were similar components reported by them. The presence of

hexadecenoic acid was a characteristic of both *A. heterophyllus* and *M. indica*, which are the identified botanical sources from our study. This was in accordance with study of Peng *et al*., 2013 and Muchiri *et al*., 2012, respectively. Propanoic acid and ethyl oleate were found in *M. indica*, one of the major botanical sources of our study was in line with (Reddy and Reddy, 2009) and (Pino, 2012) respectively. Linoleic acid was found in *M. indica* and *A.altilis* and conformed with the investigation of (Abdalla *et al.,* 2007) and (Aremu *et al.,* 2017).

5.4.2 LC-HRMS analysis of propolis

5.4.2.1 Acids and phenolic acids

Phenolic acids or phenol-carboxylic acids, the diverse group of secondary plant metabolites are one of the major bioactive constituents of propolis. The most abundant phenolic acids that were predominately detected in the identified locations were 18-β-Glycyrrhetinic acid, syringic acid, and anacardic acid, suggesting a common origin of propolis. 18-β-Glycyrrhetinic acid was identified to be the major component of propolis from Vellayani, with 6.13%, Nedumangad (8.64%), Mariapuram (16.71%) and Navaikulam (10.28%). 5,5'-[(2,3'-Dipropyl-4,4'- biphenyldiyl) bis(oxy)] bis (2,2-dimethyl pentanoic acid) was the main constituent of propolis from Nedumangad (5.62%) and Navaikulam (4.43%). Glycyrrhizic acid (12.12%) and (2beta,5beta,21beta)-16-[(14beta)-14,15-Dihydroeburnamenin-14yl] aspido fractinine -21-carboxylic acid (30.94%) were the acids detected in highest quantity from Mariapuram, and Navaikulam, respectively. Similarly, Aliyazıcıoglu *et al.* (2013) also reported syringic acid from propolis, and anacardic acid was reported by Silva *et al*. (2008) and Popova *et al.* (2013). Researchers identified many compounds typically found in resinous exudates of *M. indica* and *A. occidentale.* This may be due to the presence of anacardic acid and it is in conformity with the report of Popova *et al.* (2021) and Viswalingam and Emerson Solomon, (2013), and syringic acid by Ajila and Rao, (2013) and Quintana, (2021) respectively. *Glycyrrhiza glabra* L. was found to be another expected botanical source due to the presence of 18-β-Glycyrrhetinic acid and Glycyrrhizic acid in higher amount which was in agreement with the study by Morana *et al*. (2002). These substances identified exhibit various biological actions of propolis

including antibacterial, antifungal, antiviral, cytotoxic, antioxidant, anti-inflammatory, etc. 18β-Glycyrrhetinic acid had anti-inflammatory activity and antioxidant properties (Kowalska and Kalinowska-Lis, 2019), syringic acid had hepatoprotective, neuroprotective, anti-inflammatory, antibacterial, anti-oxidant, and anti-endotoxic properties (Srinivasulu *et al.,* 2018) and anacardic acid had anti-inflammatory and antinociceptive actions (Gomes Junior *et al.,* 2020).

5.4.2.2 Flavanoids

Flavonoids, a group of secondary polyphenolic compounds found in plants are the major constituents of propolis. Major compounds found in all the locations included bonannione A, thevetiaflavone, rhamnetin, luteolin, and 6-Farnesyl-3',4',5,7-tetrahydroxyflavanone. 6-Farnesyl-3',4',5,7- tetrahydroxyflavanone was the major component of Mariapuram. As per our study, quercetin, kaempferide, isorhamnetin, rhamnetin, naringenin, and kaempferol were some of the flavonoids included, similar reports were found by Walker and Crane (1987) and Marcucci (1996). Similar findings were stated by Sarıkahya *et al.* (2021) who detected flavanoids such as naringenin, dihydrokaempferol, and quercetin. In this study, *M. indica* was identified as the probable source of propolis due to rhamnetin, kaempferol, and quercetin and was in accordance with Matheyambath *et al.* (2016). *Macaranga triloba* (Thunb.) Müll.Arg. may be the probable botanical source of propolis due to the presence of 6-farnesyl-3′,4′,5,7-tetrahydroxyflavanone which was similar to findings done by Zakaria *et al*. (2012). Also, few studies found the medicinal properties possessed by flavonoids. Bonannione A had antibacterial activity (Wang *et al.,* 2001), thevetiaflavone had anticancer activity and antioxidant activity by Shahrajabian *et al.* (2021) and Zhang *et al.* (2018). Quercetin had antioxidant, and anticancerous activity (Baghel *et al.,* 2012), rhamnetin possesses anticancer, antiviral, anti-inflammatory, antiviral, and antibacterial activity (Medeiros *et al*., 2022), kaempferol with antioxidant, nephroprotective and hepatoprotective effects (Tlili *et al*., 2017). Quercetin and kaempferol had anti-inflammatory, antimicrobial, antioxidant, and anticoagulant activities. Al-Rajhi *et al.* (2022) and Omisore *et al.* (2005) reported antioxidant properties in quercetin.

5.4.2.3 Terpenoids

Terpenoids, the diverse group of secondary plant metabolites were the most abundant volatile components of propolis. They contribute to the characteristic resinous odour of propolis which determines the quality of propolis. The major terpenoids found common in all the locations were lupeol, oleanolic acid, betulin, β-amyrenonol, and (3r)-hydroxy-beta-ionone. 20S,24S-dihydroxydammer-25-en-3-one, was found to be the foremost constituent of propolis collected from Vellayani, (14.89%), Nedumangad (19.35%) and Navaikulam (7.61%) whereas at Mariapuram, ambonic acid (5.92%) was the major one. Asiatic and ursolic acid were some other important terpenoids detected from samples of Vellayani and Navaikulam, respectively. Lupeol, an important terpenoid from propolis revealed by Furukawa *et al*. (2002), Pereira *et al*. (2003), de Carvalho *et al*. (2020), and Ipav *et al.* (2022). Accordingly, betulin was reported from propolis by Talla *et al.* (2017), Alenezi *et al.* (2022), and Afata and Dekebo (2022). Oleanolic acid is another important terpenoid reported by Tamfu *et al.* (2020). Omar *et al*. (2017) also reported ambonic acid from stingless bee propolis. The likely plant source of propolis may be *M. indica* due to the presence of lupeol, ursolic acid, and ambonic acid which is in conformity with the findings of Saleem (2009) and Pujirahayu *et al.* (2019). *G. glabra* and *Psidium guajava* L. are other probable source due to the presence of β-Amyrenonol and Asiatic acid which was earlier reported by Zhu *et al.* (2018) and Rishika and Sharma (2012), respectively. Numerous studies revealed different biological activities of propolis like antibacterial, antifungal, antiviral, cytotoxic, antioxidant, and anti-inflammatory, etc, Lupeol with anti-inflammatory, and anticancer activity (Saleem, 2009) oleanolic acids combat multiple sclerosis, ulcerative colitis, metabolic diseases, diabetes, hepatitis, and many malignancies (Sen, 2020), betulin possessing anti-inflammation, anti-HIV, anti-cancer, and anti-malarial properties (Alakurtti, 2006), β-Amyrenonol exhibiting anti-proliferative and anti-inflammatory properties (Reed *et al.,* 2017) are some among them. Asiatic acid possesses anti-inflammatory, anticancer, anti-cancer, anti-diabetic, hypertension protection, anti-rheumatoid, arthritis, wound healing, brain improvement and neuroprotective impact, stomach ulcer prevention, cardio protection, anxiolytic activity,

and venous hypertension improvement properties (Hashim, 2013), while oleanolic acid and ursolic acid had anti-tumor and anti-hepatitis activities (Zhang *et al.*, 2013).

5.4.2.4 Fatty acids and steroids

Fatty acids and steroids, a class of secondary metabolites forms the waxy nonpolar parts of propolis. The major fatty acids and steroids found common in each location were docosahexaenoic acid, octadec-9-enoic acid, punicic acid, 16-hydroxy hexadecanoic acid, and ethyl linoleate. The major fatty acid found from Vellayani was testosterone decanoate (5.82%), while, 3-Oxo-4,6-choladienoic acid (2.83%) was the main constituent from Nedumangad. Methyl oleanolate was the main component found in Mariapuram (10.53%) and Navaikulam (5.19%). 6,9,12,15-Docosatetraenoic acid and 16-Hydroxyhexadecanoic acid were some other components detected from Mariapuram and Nedumangad respectively. Pant *et al.* (2022) identified docosahexaenoic acid from propolis, while octadec-9-enoic acid, a major acid, was revealed by Zahra *et al.* (2021) and Burgut (2022), while Fayaz *et al.* (2017) identified punicic acid, Muema, (1987) found 16-hydroxy hexadecanoic acid from propolis. *Punica granatum* L. was the likely source of punicic acid and was in agreement with the report of Filho, (2014). Normal brain function was influenced by docosahexaenoic acid (Horrocks and Yeo, 1999), while Punicic acids have a broad range of biological qualities, such as anti-diabetic, anti-obesity, anti-proliferative, and anticarcinogenic effect against several types of cancer (Aruna *et al.* 2016).

5.4.2.5 Amino acids

Amino acids are another group of components included, which is made up of amino and carboxylic acid groups. The major amino acids found common in all locations were olomoucine, N-(Adamantan-1-ylacetyl) glycine, and N-Undecanoyl glycine. Leucine, adenine, and guanine were other components found. El Hady and Hegazi (2002) and Marcucci *et al*. (1996) reported leucine from the propolis sample. Numerous medicinal uses were reported for amino acids. Adenine was effective against HIV, HBV, CMV, and other virus-infected diseases (Wang *et al.,* 2015), guanine had neuroprotective properties, neuroinflammation, oxidative stress, and excitotoxicity

(Bettio *et al*., 2016) and leucine was reported for treatment on sarcopenia or type 2 diabetes (Van Loon, 2012).

5.4.2.6 Chalcones

Chalcones are another secondary metabolite which is an α, β-unsaturated ketone. The predominant chalcones found throughout all the locations were dihydrocordoin and gancaonin J. Coatline A (11.35%) was the major chalcone from Vellayani. Other important chalcones were bartericin c, 4,2',6'-trihydroxy-3,4'-dimethoxychalcone, orotinichalcone, derrichalcone, etc. *Pterocarpus marsupium* Roxb. was the botanical source of Coatline A (NCBI, 2023).

5.4.2.7 Amines, ketones, alcohols

Some other constituents are found in amines, ketones, and alcohols. Among amines, major components found common were octadecanamine, oleamide, and N, N'-Dicyclohexylurea. N~2~-(N, N'-Dicyclohexyl carbamimidoyl)- N-[4- (hydroxy methyl)phenyl]-3-(2-naphthyl)- L-alaninamide (18.77%) was the major component from Nedumangad and erucamide (7.01 %) from Vellayani. Oleamide was found to be effective in the treatment of inflammatory diseases, especially for peripheral inflammatory disorders, such as atopic dermatitis (Naumoska *et al.,* 2020).

The 20S, 24S - dihydroxy dammer-25-en-3-one with Vellayani (14.96%), Nedumangad (19.37%), Mariapuram (2.28%), and Navaikulam (7.63%) was the major ketone detected. Also, 2,5-di-tert-Butyl hydroquinone (16.42%) and 6-(Dodecylamino)-2-[3-(4-methyl-1-piperazinyl)propyl]-1Hbenzo[de]isoquinoline-1,3 (2H)-dione (19.82%) were the other major components identified from Vellayani and Nedumangad, respectively. "20S,24S-dihydroxydammer-25-en-3-one" and 1,1,1-Trifluoro-2-octadecanone were common ketones found from each location.

The major alcohol was 5-[(8Z)-8-Heptadecen-1-yl]-1,3- benzenediol (3.15%) from Mariapuram. Whereas, 5-[(Z)-Pentadec-8-enyl] benzene-1,3-diol and 2,2,6,6-Tetramethyl-1-piperidinol (TEMPO) were components common in each location.

5.4.2.8 Sugars

Nectar and honey or plant mucilages or hydrolyzed flavonoid glycosides in propolis are thought to be the sources of sugar. Taxifolin 3-galactoside, fucose, and 2-deoxyglucose were some of the major sugars identified. Fucose possessed anti-cancer, anti-inflammatory, anti-viral, and anti-microbial activities (Xiao *et al.,* 2022) whereas antioxidant, antibacterial, and anti-inflammatory of 2-deoxyglucose was reported by (León-Annicchiarico *et al*., 2015).

5.4.2.9 Other components

Isogarcinol was one of the major components under the class benzophenones. Among the compounds isolated from propolis, Isogarcinol was reported as a constituent of *G. mangostana* and was in line with the study of Fu *et al*. (2014) and *G. cambogia* in accordance with (Shukla *et al*., 2014). Isogarcinal had anticancerous, antimicrobial anti-inflammatory activities, and antioxidant activities (Schobert and Biersack, 2019). Norswertianolin, a xanthone was found in *M. indica* and it was in line with the report (Ngui, 2018).

The dominant resin sources identified from our study were *M. indica, A. heterophyllus,* A. *altilis, A. occidentale, G. cambogia, G. xanthochymus* and *A. araucana.* When, the characterisation studies of propolis were carried out, several components were detected with the functional groups such as acids, fatty acids, steroids, alcohols, flavonoids, terpenoids, etc. The bioactive principles will provide advance understanding of this significant bee product, provide fresh perspectives on their qualities, and raise awareness of their potential use in contemporary medicine.

SUMMARY

6. SUMMARY

Propolis is a natural resinous mixture produced by honey bees from substances collected from various plant species which comprises more than 300 constituents. Though propolis had a broad range of medicinal properties such as antibacterial, anti-inflammatory, antiulcer, antioxidant, hepatoprotective, and anticancer activities, it is the most underutilized beehive product. Hence this investigation entitled "Origin and composition of stingless bee propolis' were conducted during 2020-2022 for studying major botanical resin sources, resin foraging rate, and composition of propolis.

The Stingless bees visited plants and trees within a 100m radius of the beehive to acquire resin from a range of sources. Based on that, resin-producing trees like the Mango tree (*Mangifera indica* L.), Jack tree (*Artocarpus heterophyllus* Lam.), Breadfruit tree (*Artocarpus altilis* Parkinson), Cashew tree (*Anacardium occidentale* L.), Cambodge tree (*Garcinia cambogia* Syn.), False mangosteen (*Garcinia xanthochymus* Hook.), Monkey puzzle tree (*Araucaria araucana* Molina) were the identified botanical sources of stingless bee.

Resin-producing sources were graded from 1-4, based on the resin flow from the wounds placed. Accordingly, *M. indica,* with a resin-covered area of 5.4 cm^2 of grade (4) was the highest grade. While *A. araucana* and *G. xanthochymus* grade (2) was the lowest. The remaining sources identified were graded (3). According to the total number of bees visited per wound daily: *G. cambogia* with 16.71 foragers per day was recorded with the highest bee foraging activity, while *A. araucana* with 2.17 foragers per day was recorded as the lowest population. It was found that resin foraging activity begins on the 1^{st} or 2nd day of wound initiation, attained peak during the 4^{th}-16^{th} day, and stopped foraging on the 14^{th}-23^{rd} day on different sources.

The time spent by a stingless bee to complete resin foraging varied from 10 to 15 minutes. The maximum time taken in *A. occidentale* (15 minutes) was followed by *A. altilis* (14 minutes) and while the least time taken was in the case of *A. araucana* (10 minutes).

Observations on the number of returning foragers with resin load from September 2021 to August 2022 revealed that resin foraging activity commenced after

0700h and reduced after 1800h from each of the locations. High resin foraging activity was from 0900h to 1600h in all the locations throughout the observation**.** Peak foraging activity was noticed from 1100h to 1200h in all the locations. During honey flow season, i.e, from January to March there was a sudden decline in resin foraging activity and afterward, it started increasing. The correlation of weather parameters and returning foragers carrying resin load was found to be non-significant among all the locations.

Propolis collected from Nedumangad was dark greyish-reddish brown, Mariapuram was moderate olive brown, and Vellayani and Navaikulam were moderate brown. The texture of propolis was sticky at normal temperatures, and hard at cold temperatures in all the locations and the odour was aromatic in every location.

The main chemical classes present in the propolis by LC-HRMS and GC-HRMS analysis were acids, fatty acids, steroids, alcohols, amines, amino acids, flavonoids, terpenoids, chalcones, aldehydes, ketones, benzene, coumarin, pterocarpan, ether, and ester. The major component identified by positive ionisation from Vellayani (14.96%) and Nedumangad (19.37%) was 20S,24S-dihydroxydammer-25-en-3-one. Whereas, Glycyrrhizic acid was the key component in Mariapuram (12.36%) and Navaikulam (10.31%) samples. The main components identified by negative ionisation from Vellayani, Nedumangad, Mariapuram, and Navaikulam were 2,5-di-tert-Butylhydroquinone, 6-(Dodecyl amino)-2-[3-(4-methyl-1-piperazinyl) propyl]-1Hbenzo[de]isoquinoline-1,3(2H)-dione, Methyl oleanolate, and 2beta,5beta,21beta)-16-[(14beta)-14,15-Dihydroeburnamenin-14-yl] aspido fractinine -21- carboxylic acid, respectively.

The components identified as common in all the locations by positive ionisation from the class fatty acids, and steroids were docosahexaenoic acid, octadec-9-ynoic acid, punicic acid, and ethyl linoleate. Common flavonoids included were bonannione a, thevetiaflavone, and rhamnetin. Whereas, lupeol, oleanolic acid, b-Amyrenonol, and betulin were common terpenoids in each location. Chalcone found common was dihydrocordoin, the common amino acid identified was olomoucine and benzophenone was isogarcinol. Alcohols found common across all the locations were 5-[(Z)-Pentadec-8-enyl] benzene-1,3-diol and 2,2,6,6-Tetramethyl-1-piperidinyl (TEMPO). Amines comprised of octadecanamine, oleamide, (1R)-1-Butyl-9-{1-[(4,6-dimethyl-5-

pyrimidinyl) carbonyl] -4-methyl -4- piperidinyl} -N-methyl3,9diazaspiro [5.5] undecane-3-carboxamide and N, N'-Dicyclohexylurea.

Components found common throughout all the locations by negative ionisation were 18-β-Glycyrrhetinic acid, 4-[3-(3,5-ditert-butylphenyl)-3-oxoprop1-enyl] benzoic acid, syringic acid, and anacardic acid. Fatty acids and steroids were 9,10-dihydroxy stearic acid and 16-Hydroxyhexadecanoic acid, the ether identified was cannabidiol dimethyl ether. Flavonoids were 6-Farnesyl-3',4',5,7- tetrahydroxy flavanone, luteolin, quercetin, and sanggenol O. Amino acids found in all locations were N-(Adamantan-1-ylacetyl) glycine and N-Undecanoyl glycine. Terpenoid was (3R)-hydroxy-beta-ionone whereas ester was mono butyl phthalate. 2-Hydroxy-1-(4-hydroxyphenyl)-5- methyl-3-hexanone was the common ketone.

When the GC-HRMS analysis was carried out cycloheptasiloxane and cyclooctasiloxane were the common components found both in Vellayani and Nedumangad. Whereas, from Mariapuram and Navaikulam, Hexadecenoic acid, ethyl oleate, and linoleic acid ethyl ester were the common components identified. 7,8-epoxylanostan-11-ol was found in Nedumangad and Mariapuram, while cyclodecasiloxane was found in almost all the locations except Mariapuram.

The present investigation identified various botanical resin sources and found that *M. indica* and *G. cambogia* with maximum stingless bees foraging. The foraging activity was found high from 0900h to 1600h in all the locations throughout the year. When the chemical characterization studies of propolis were carried out, several components were identified with functional groups like acids, fatty acids, steroids, alcohols, flavonoids, terpenoids, etc.

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7. REFERENCES

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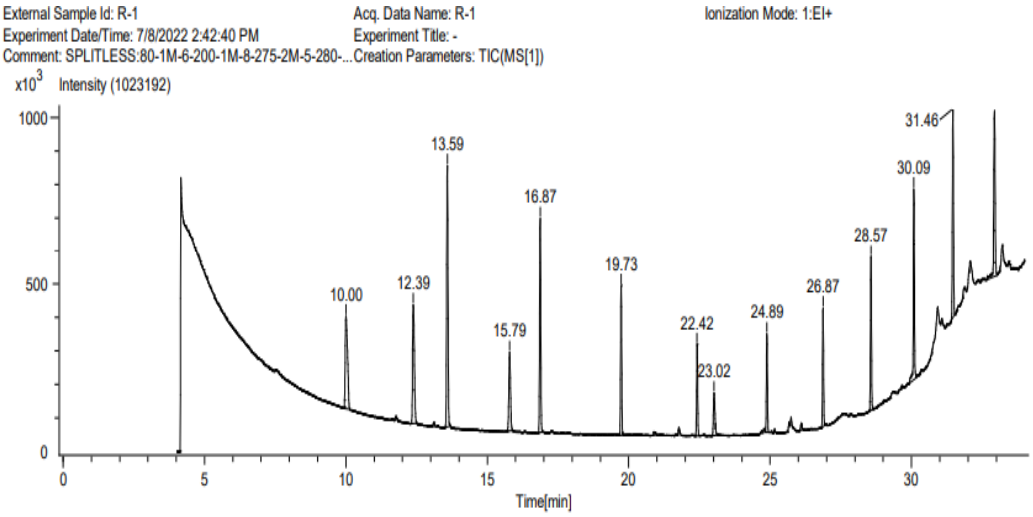
APPENDICES

APPENDICES

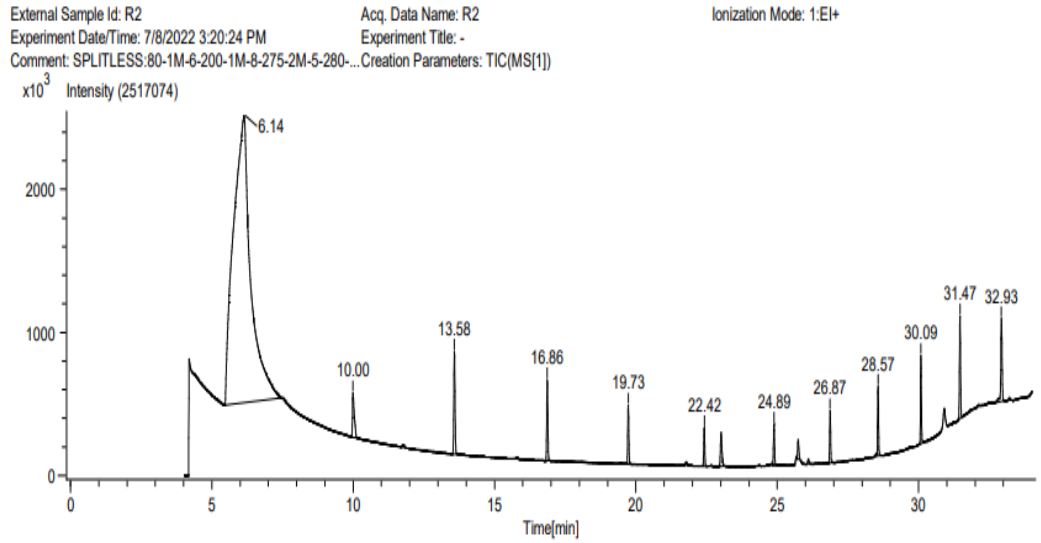
Appendix-I

GC-HRMS analysis from Vellayani



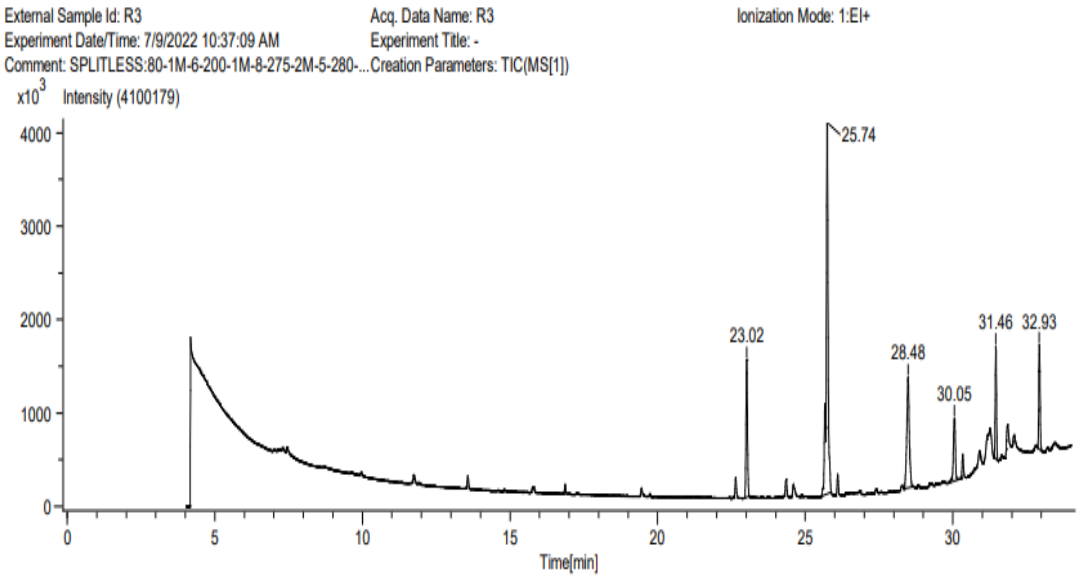
Appendix-II

GC-HRMS analysis from Nedumangad



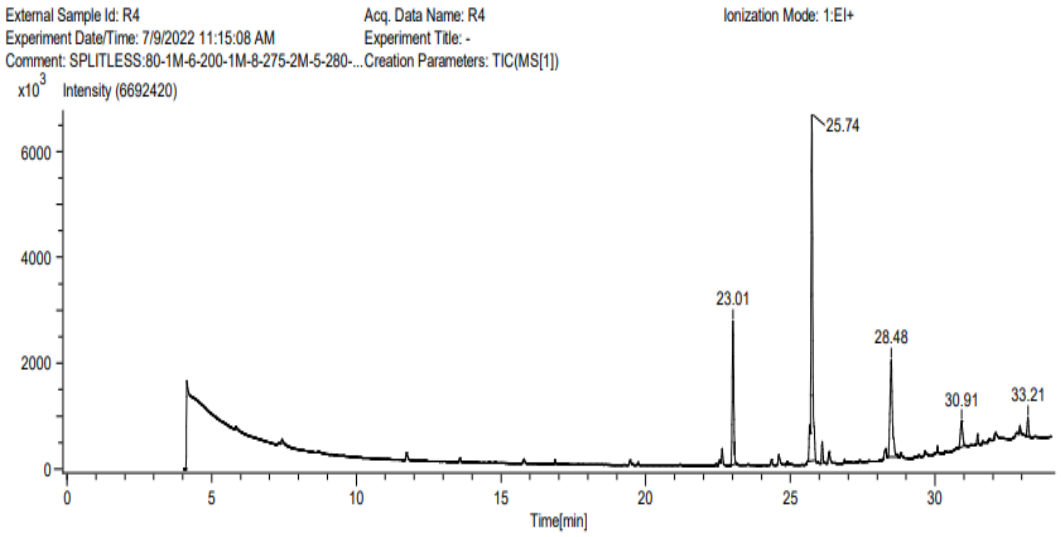
Appendix-III

GC-HRMS analysis from Mariapuram



Appendix-IV

GC-HRMS analysis from Navaikulam



ORIGIN AND COMPOSITION OF STINGLESS BEE PROPOLIS

By

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(2020-11-053)

ABSTRACT

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ABSTRACT

The present investigation entitled “Origin and composition of stingless bee propolis” was conducted at the Department of Agricultural Entomology, College of Agriculture, Vellayani during 2020-2022. The objective of the study was to assess the resin-foraging behaviour of stingless bees, the origin of the resin, and the characterization of bee propolis.

Four locations *viz*., AICRP on Honey Bees and Pollinators, Vellayani, and apiaries in Nedumangad, Mariapuram, and Navaikulam were selected for the study. Resin sources, foraging rate, physical characteristics, and major components of propolis were studied.

The trees and plants were observed within up to a 100m radius of the bee hive for identifying the resin source. Plants identified were Mango tree (*Mangifera indica* L.), Jack tree (*Artocarpus heterophyllus* Lam.), Breadfruit tree (*Artocarpus altilis* Parkinson), Cashew tree (*Anacardium occidentale* L.), Cambodge tree (*Garcinia cambogia* Syn.), False mangosteen (*Garcinia xanthochymus* Hook.), Monkey puzzle tree (*Araucaria araucana* Molina).

Based on the resin flow and wounds present, plants were grouped into 4 grades from 1-4. The number of bees visiting the wound was also counted. *M. indica* (4) and *G. cambogia* (3) were recorded with maximum stingless bees foraging per day. *A. araucana* and *G. xanthochymus* came under grade 2 with minimum stingless bees foraging per day.

Resin foraging activity was found high from 0900h to 1600h in all the locations throughout the observation. Peak foraging activity was noticed from 1100h to 1200h at Vellayani, Mariapuram, and Nedumangad, Navaikulam. There was no significant correlation between weather parameters and resin foraging rate when correlation studies were conducted.

Propolis from all the locations possess pleasant aromatic smells and was sticky in texture at normal temperatures and hard at cold temperatures. The colour of propolis from Vellayani and Navaikulam was moderate brown. It was dark grayish-reddish brown and moderate olive brown in Nedumangad and Mariapuram respectively.

LC-HRMS and GC-HRMS analysis revealed that the main chemical classes present in the propolis were acids, fatty acids, steroids, alcohols, amines, amino acids, flavonoids, terpenoids, chalcones, aldehydes, ketones, benzene, coumarin, pterocarpan, ether, and ester. The predominant components identified in the propolis of Vellayani and Nedumangad were 20S, 24S-dihydroxy dammer-25-en-3-one, whereas it was Glycyrrhizic acid in Mariapuram and Navaikulam. The various components found common throughout the locations were syringic acid, and ellagic acid (acids), punicic acid, 9,10-dihydroxystearic acid, and phloionolic acid (steroids and fatty acids), thevetiaflavone, luteolin, and quercetin (flavonoids), octadecanamine and oleamide (amines), ursolic acid and oleanolic acid (terpenoids), 5-[(z)-pentadec-8-enyl] benzene-1,3-diol (alcohols), dihydrocordoin and orotinichalcone (chalcones).

Thus, this study identified different botanical resin sources and found *M. indica* and *G. cambogia* with maximum stingless bees foraging. High foraging activity was observed from 0900h to 1600h in all the locations throughout the year. Several components were identified with functional groups like acids, fatty acids, steroids, alcohols, flavonoids, terpenoids, etc. from the propolis when the chemical characterization studies were carried out.

സംഗ്രഹം

2020-2022 കാലയളവിൽ വെള്ളായണി കാർഷിക കോളേജിലെ കീടശാസ്ത്ര വിഭാഗത്തിൽ "ചെറുതേനീച്ച പ്രോപോളിസിന്റെ ഉത്ഭവവും ഘടനയും" എന്നതിനെ കുറിച്ച് ഗവേഷണം നടത്തി. ചെറുതേനീച്ചകളുടെ മരക്കറ ശേഖരണ സ്വഭാവം, മരക്കറ ഉത്ഭവം, തേനീച്ച പ്രോപ്പോളിസിന്റെ സ്വഭാവം എന്നിവ വിലയിരുത്തുക എന്നതായിരുന്നു ഈ പഠനത്തിന്റെ ലക്ഷ്യം.

വെള്ളായണി, നെടുമങ്ങാട്, മരിയാപുരം, നാവായിക്കുളം എന്നിവിടങ്ങളിലെ തേനീച്ചക്കൂടുകളാണ് പഠനത്തിനായി തിരഞ്ഞെടുത്തത്. മരക്കറ സ്രോതസ്സുകൾ, ശേഖരണ നിരക്ക്, ഭൗതികമായ സവിശേഷതകൾ, പ്രോപോളിസിന്റെ പ്രധാന ഘടകങ്ങൾ എന്നിവയെ കുറിച്ച് പഠനം നടത്തി.

തേനീച്ചക്കൂടിന്റെ 100 മീറ്റർ ചുറ്റളവിൽ മരക്കറയുടെ ഉറവിടം തിരിച്ചറിയുന്നതിനായി മരങ്ങളും ചെടികളും നിരീക്ഷിച്ചു. മാവ് (മാഞ്ജിഫെറ ഇൻഡിക്ക എൽ.), പ്ലാവ് (ആർട്ടോകാർപസ് ഹെറ്ററോഫില്ലസ് ലാം.), ശീമപ്ലാവ് (ആർട്ടോകാർപസ് ആൾട്ടിലിസ് പാർക്കിൻസൺ), കശുമാവ് (അനാകാർഡിയം ഓക്സിഡന്റേൽ എൽ.), കുടംപുളി (ഗാർസീനിയ കംബോജിയ സിങ്കോസ്), രാജാപുളി (ഗാർസീനിയ സാന്തോചൈമസ് ഹുക്ക്.), മങ്കി പസിൽ മരം (അരൗക്കറിയ അരുകാന മൊലിന) തുടങ്ങിവയാണ് പ്രധാന മരക്കറ സ്രോതസ്സുകൾ.

മരക്കറ ഒഴുകിന്റെയും മുറിവുകളുടെയും അടിസ്ഥാനത്തിൽ, സസ്യങ്ങളെ ഒന്ന് മുതൽ നാല് വരെ ഗ്രേഡുകളായി തിരിച്ചിരിക്കുന്നു. മുറിവ് സന്ദർശിച്ച തേനീച്ചകളുടെ എണ്ണവും കണക്കാക്കി. മാവ് (4), കുടംപുളി (3) എന്നിവയിൽ പ്രതിദിനം ഏറ്റവും കൂടുതൽ ചെറുതേനീച്ചകൾ മരക്കറ തേടുന്നതായി രേഖപ്പെടുത്തിയിട്ടുണ്ട്.

നിരീക്ഷണത്തിലുടനീളം എല്ലാ സ്ഥലങ്ങളിലും രാവിലെ ഒമ്പത് മുതൽ വൈകുന്നേരം നാല് വരെ മരക്കറ ശേഖരിക്കുന്ന പ്രവർത്തനം ഉയർന്നതായി കണ്ടെത്തി. അതിനുപുറമെ വെള്ളായണി, മരിയാപുരം, നെടുമങ്ങാട്, നാവായിക്കുളം എന്നിവിടങ്ങളിൽ ഏറ്റവും അധികം മരക്കറ ശേഖരിക്കൽ നടക്കുന്നത് രാവിലെ പതിനൊന്നിനും ഉച്ചക്ക് പന്ത്രണ്ടിനും ഇടയിലുള്ള സമയത്താണ്. പരസ്പര ബന്ധ പഠനങ്ങൾ നടത്തിയപ്പോൾ കാലാവസ്ഥാ ഘടകങ്ങളും മരക്കറ ശേഖരിക്കുന്ന നിരക്കും തമ്മിൽ കാര്യമായ ബന്ധമില്ല എന്ന് മനസിലായി.

എല്ലാ സ്ഥലങ്ങളിൽ നിന്നുമുള്ള പ്രോപോളിസിന് സുഖകരമായ സൌരഭ്യവാസനയുണ്ട്, അതുപോലെ പ്രോപ്പോളിസിന്റെ ഘടന സാധാരണ താപനിലയിൽ ഒട്ടിപ്പിടിക്കുന്നതും തണുത്ത താപനിലയിൽ കാഠിന്യമേറിയതുമാണ്. വെള്ളായണി, നാവായിക്കുളം എന്നിവിടങ്ങളിൽ നിന്നുള്ള പ്രോപ്പോളിസിന്റെ നിറം മിതമായ തവിട്ടുനിറമായിരുന്നു. എന്നാൽ നെടുമങ്ങാടും മരിയാപുരത്തും നിന്നുള്ള പ്രോപോളിസിന് യഥാക്രമം കടും ചാര-ചുവപ്പ് കലർന്ന തവിട്ടുനിറവും മിതമായ ഒലിവ് തവിട്ടുനിറവുമാണ്.

ആസിഡുകൾ, ഫാറ്റി ആസിഡുകൾ, സ്റ്റിറോയിഡുകൾ, ആൽക്കഹോൾ, അമിനുകൾ, അമിനോ ആസിഡുകൾ, ഫ്ലേവനോയ്ഡുകൾ, ടെർപെനോയിഡുകൾ, ചാൽകോണുകൾ, ആൽഡിഹൈഡുകൾ, കീറ്റോണുകൾ, ബെൻസീൻ, ആൽകഹോൾ, കൗമാരിൻ, ടെറോകാർപാൻ, എസ്റ്റർ എന്നിവയായിരുന്നു പ്രോപോളിസിലെ പ്രധാന രാസവിഭാഗങ്ങൾ. വെള്ളായണിയിലെയും നെടുമങ്ങാട്ടിലെയും പ്രോപ്പോളിസിൽ കണ്ടെത്തിയ പ്രധാന ഘടകങ്ങൾ 20 എസ്, 24 എസ് - ഡൈ ഹൈഡ്രോക്സി ദാമ്മേർ - 25 - എൻ - 3 - ഓൺ ആയിരുന്നു, അതേസമയം മരിയാപുരത്തും നാവായിക്കുളത്തും ഗ്ലൈസിർഹിസീക് ആസിഡ് ആയിരുന്നു. സിറിഞ്ചിക് ആസിഡ്, എലാജിക് ആസിഡ് (ആസിഡുകൾ), പ്യൂനിസിക് ആസിഡ്, 9,10-ഡൈഹൈഡ്രോക്സിസ്റ്റിറിക് ആസിഡ്, ഫ്ളോയോനോളിക് ആസിഡ് (സ്റ്റിറോയിഡുകൾ, ഫാറ്റി ആസിഡുകൾ), തൈവെറ്റിയാഫ്ലവോൺ, ലുട്ടിയോലിൻ, കെർസെറ്റിൻ (ഫ്ലേവനോയ്ഡുകൾ), ഒക്ടാഡെക്കാന ഒലിമൈഡ് (അമിനുകൾ), ഉർസോളിക് ആസിഡ്, ഒലിയാനോളിക് ആസിഡ് (ടെർപെനോയിഡുകൾ), ഡൈഹൈഡ്രോകോർഡോയിൻ, ഓറോട്ടിനിചാൽക്കോൺ (ചാൽകോണുകൾ) എന്നിവയായിരുന്നു എല്ലായിടത്തും പൊതുവായി കാണപ്പെടുന്ന വിവിധ ഘടകങ്ങൾ.

അങ്ങനെ, ഈ പഠനം വ്യത്യസ്ത മരക്കറ സ്രോതസ്സുകൾ തിരിച്ചറിയുകയും, മാവ്, കുടംപുളി എന്നിവയിൽ പരമാവധി ചെറുതേനീച്ചകളെ കണ്ടെത്തുകയും ചെയ്തു. വർഷം മുഴുവനും പഠനം നടന്ന എല്ലാ സ്ഥലങ്ങളിലും വർഷം മുഴുവനും രാവിലെ ഒമ്പത് മുതൽ വൈകുന്നേരം നാല് വരെ ഉയർന്ന മരക്കറ

ശേഖരണം നിരീക്ഷിക്കപ്പെട്ടു. രാസ സ്വഭാവ പഠനങ്ങൾ നടത്തിയപ്പോൾ പ്രോപോളിസിൽ നിന്നും ആസിഡുകൾ, ഫാറ്റി ആസിഡുകൾ, സ്റ്റിറോയിഡുകൾ, ആൽക്കഹോൾ, ഫ്ലേവനോയ്ഡുകൾ, ടെർപെനോയിഡുകൾ തുടങ്ങിയ രാസവിഭാവങ്ങളുടെ സാന്നിദ്ധ്യം തിരിച്ചറിഞ്ഞു.