STUDIES ON STINGLESS BEES AND THEIR UTILIZATION IN TOMATO (*Lycopersicon esculentum)* Bailey **PRODUCTION**

Thesis

submitted to

NAGALAND UNIVERSITY

in partial fulfillment of requirements for the Degree

of

Doctor of Philosophy

in

Entomology

by

MALAR BUI
Admin. No. Ph - 163/14 Regn. No. 715 (2016-2017)

Department of Entomology,
School of Agricultural Sciences and Rural Development,
Nagaland University, Medziphema Campus - 797106
Nagaland
2019

MENYIR ETE BUI

Dedicated to wife

DECLARATION

I, Mr. Malar Bui hereby declare that the subject matter of this thesis is the record of work done by me, that the contents of this thesis did not form the basis of the award of any previous Degree to me or to the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree to any other University/ Institute.

This is submitted to SASRD, Nagaland University for the Degree of Doctor of Philosophy in Entomology.

Date:
Place:

……..
(MALAR BUI)

…….
Supervisor

NAGALAND UNIVERSITY
Medziphema Campus
School of Agricultural Sciences and Rural Development
Medziphema-797106, Nagaland

Dr. H. K. Singh
Professor
Department of Entomology

CERTIFICATE – I

This is to certify that the thesis entitled "**Studies on stingless bees and their utilization in tomato, *Lycopersicon esculentum* (Bailey) Production**" submitted to Nagaland University in partial fulfillment of the requirements for the degree of Doctor of Philosophy (Agriculture) in Entomology is the record of research work carried out by Mr. Malar Bui, Registration No. 715 (2016-2017) under my personal supervision and guidance.

The result of the investigation reported in the thesis have not been submitted for any other degree or diploma. The assistance of all kinds received by the student has been duly acknowledged.

Dated :
Place :

.....
Dr. H. K. SINGH
Supervisor

NAGALAND UNIVERSITY
Medziphema Campus
School of Agricultural Sciences and Rural Development
Medziphema-797106, Nagaland

CERTIFICATE – II

VIVA VOCE ON THESIS OF DOCTOR OF PHILOSOPHY IN ENTOMOLOGY

This is to certify that the Thesis entitled "**Studies on stingless bees and their utilization in tomato, *Lycopersicon esculentum* (Bailey*)* Production**" submitted by Mr. Malar Bui, Admission No. Ph-163/14, Registration No. 715 (2016-2017) to the NAGALAND UNIVERSITY in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Entomology (Agriculture) has been examined by the student Advisory Committee and the External Examiner on ……...

The performance of the student has been found **Satisfactory /Unsatisfactory.**

Member	Signature
1. Dr. H. K. Singh (Supervisor)	……...…….…….
2. Prof. S. Satpathy (External Examiner)	.....…...……
3. Prof. M. Aleminla Ao	…….…….
4. Dr. G. T. Behere	…….…….
5. Prof. L. Daiho	…….……
6. Dr. A. K. Sahu	…….…….

Head
Department of Entomology

Dean
School of Agricultural Sciences
and Rural Development

ACKNOWLEDGEMENT

I offer my deepest gratitude and thankfulness to our Lord Almighty God-Donyi-Polo, who has showered me with abundant blessings and for enabling me to complete this thesis in good health.

This thesis owes its existence to the support, guidance and prayer of many individuals, friends and my family for which I am eternally grateful.

I sincerely acknowledge the effort of my advisor Prof. H.K. Singh, Department of Entomology, NU: SASRD for his untiring support, help, time, energy and constructive criticism throughout the investigation. He supervised the work with keen interest, provided most valuable, productive suggestions and sound advice at all stages of my work. With heartfelt gratitude, I pay my deepest respect for him personal commitment and kind affectionate attitude towards me.

I owe my special thanks to Dean, Prof A.K. Makar NU: SASRD for providing all the necessary academic stimulus.

I am extremely grateful to the members of my advisory committee Prof. M. Aleminla Ao, HOD, Department of Entomology, DR. G. T. Behere, Principal scientist, Entomology Department ICAR, Shillong, Prof. L. Daiho, Department of Plant Pathology and Dr. A. K. Sahu, Assistant Prof., Department of Agricultural Economics for their sincere help and encouragement during the course of my research work.

I am thankful to staff, Department of Entomology for their co-operation and continuous support in completing this study. I owe special thanks to Dr. Shashidhar Viraktamath, Emeritus Scientist, Department of Entomology, University of Agricultural Sciences, GKVK, Bengaluru-560065, Karnataka for his valuable support in identifying the stingless bee species. I am thankful to Mr. Mhonbemo Ngullie, Assistant Librarian and his staff for providing all the necessary library facilities for the success of my study.

I am extremely Grateful to Mr. Satyajit Singh, Computer Asstt., Library SASRD, Medziphema, Nagaland for helping me in brings out this thesis typing. My immense thanks goes to my friends Walu, Khrieketou Kuotsu and Nchumthung Murry, your helping hands and the valuable time that you have rendered me will always be remembered, thank you so much and God bless you all.

I would also like to acknowledge the help of Dr. Avinash Chauhan, Assistant Scientist AICRP - Honey and Pollinators, SASRD, Medziphema, Nagaland for his valuable suggestions and constant help during the edition of this thesis.

My utmost gratitude to my wife Menyir Ete Bui, who have always been supportive of my work through constant prayers, motivation and financial help.

Date:
Place: (Malar Bui)

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

@	:	at the rate of
°C	:	Degree Celsius
Agric.	:	Agriculture
ANOVA	:	Analysis of Variance
Bull	:	Bulletin
CD	:	Critical Difference
cm	:	Centimetre
DAC & FW	:	Department of Agriculture Cooperation & Farmers Welfare
df	:	Degree of Freedom
E	:	East
Ent.	:	Entomology
et al.	:	co workers
Fig.	:	Figure
FR	:	Foraging rate
FS	:	Foraging speed
FYM	:	Farm Yard Manure
g	:	Gram
ha	:	Hectare
ICAR	:	Indian Council of Agricultural Research
J.	:	Journal
Kg	:	Kilogram
LPG	:	Loose pollen grains
M	:	meter
MSS	:	Mean sum of squares
N	:	North
NCBI	:	National Centre for Biotechnology

N S	:	Non-significant
N U	:	Nagaland University
PE	:	Pollination efficiency
PI	:	Pollination index
Q	:	Quintal
SASRD	:	School of Agricultural Sciences and Rural Development
Sci.	:	Science
Sq. m	:	Square metre
SS	:	Sum of Squares
UV	:	Ultra Violet
Viz.	:	Namely
%	:	Per cent
/	:	per

ABSTRACT

The present investigation on "Studies on stingless bees and their utilization in tomato (*Lycopersicon esculentum*) Bailey production" were conducted in the department of Entomology, SASRD, Nagaland University. The experiments were conducted to investigate stingless bees and their potential for utilization in tomato (*Lycopersicon esculentum*) Bailey production in the states of Nagaland, Meghalaya and Arunachal Pradesh during the year 2016-2017 and 2017-18. The stingless bee colonies were collected from different altitudes of Upper Subansiri district of Arunachal Pradesh and Nagaland. These were identified by traditional and molecular taxonomy. Stingless bees collected from Arunachal Pradesh was identified as *Lepidotrigona arcifera* (Cockrell 1929) and two species of *Tetragonula* genus *i.e. Tetragonula* sp.I and *Tetragonula* sp.II were identified from Nagaland. Due to the highly complex morphological characters, stingless bees collected from Nagaland were identified only up to genus level. Thirty four colonies of *Lepidotrigona arcifera* were successfully propagated / domesticated in new log artificial hives designed in Arunachal Pradesh. Three different hive types were designed, *viz*., Mo = Natural hive (NH), M1= Bamboo hives (BH) and M2=Log artificial hive (LAH). Two hive height (The hives were use at two feet above the ground (Ho) and five feet (H1) above the ground). Wooden hives yielded 665 ml /hive/season as compared to other hive types (bamboo and natural hives). The rate of colony establishment was best in wooden hive followed by natural hive. Bamboo hive failed to establish perennial colony. Wooden hives performed better at both the heights. The pollination potential was observed under greenhouse conditions. Foraging activity of *L. arcifera* indicated that the mean numbers of flowers visited per minute in the greenhouse was 7.7 flowers during the period of 2016-17 & 2017-18 and mean foraging speed (sec./flower) was 8.9 seconds. Present findings indicated that *L. arcifera* visited the tomato flowers. Tomato berry production was statistically significant among the treatments. The production of tomato berry in greenhouse with stingless bees (T1) and without stingless bees (T2) was 1347.1 g/plant and 1330.7 g/plant, respectively. However, the production in the open pollination (T3) was only 1050.1 g/plant. The above experiments have successfully demonstrated the utilization potential of *L. arcifera* in enhancing the production of tomato berry under greenhouse conditions. For improved productivity of tomato crop under greenhouse using *L. arcifera* further trials are required to ascertain the role of stingless bee pollination.

Key words: Stingless bees, *Lepidotrigona arcifera*, hives, taxonomy, molecular, greenhouse, tomato, pollination

CHAPTER - I

INTRODUCTION

INTRODUCTION

Pollination is an important component of the ecosystem service. Adequate pollination results in the increase in food security and an improvement of livelihoods (Costanza *et al.*, 1997). Studies on collection and domestication of feral stingless bee colonies, their identification and use in crop pollination is felt needs for sustainable farming in North East India. North East India, comprises of eight states *viz.* Nagaland, Arunachal Pradesh, Assam, Meghalaya, Mizoram, Manipur, Sikkim and Tripura. These states have wide range of geographical and climatic conditions. Propagation of stingless bee colonies shall lead to preservation of biodiversity by conserving populations of bee species in the Region. Otherwise Bee population might decline owing to human disruption of ecosystems (Watanabe, 1994; Buchmann and Nabhan, 1996; Kearns and Inouye, 1997; Nabhan *et al.*, 1998; Cane and Tepedino, 2001; Villanueva *et al*., 2005; Ghazoul (2005a); Ghazoul (2005b); Steffan-Dewenter *et al.*, 2005 and Biesmeijer *et al*., 2006; Goulson *et al*., 2008).

Bees play a vital role in the pollination of flowering plants resulting in quantity and quality of fruits and seeds production. About 70% of crops that account for 35% of all agricultural production are known to depends on varying extents on pollinators for increased quality and quantity seed and fruit production (Klein *et al*., 2007; Kearns and Inouye, 1997). Logging, bush fires and habitat destruction are the cause of the decline in the pollinator diversity (Kwapong *et al*., 2010). These problems directly affect bee pollination within natural forests resulting in the decline of plant species and animals that depend on fruit and seeds for survival (Kwapong *et al.*, 2010). It has therefore become necessary to identify and conserve the bee species which can be used for pollination in both agricultural and natural vegetation. Therefore, an

exploration needs to be on stingless bees which are in been existence. Like other bees, stingless bees are members of the Class: Insecta, Family: Apidae, Sub family: Meliponinae, Tribe: Meliponini. Meliponini presents several genera of stingless bees. The total number of species within the Meliponini is estimated to be about 400 (Silveira *et al*., 2002). Stingless bees are social insect, living in colony and are present in all tropical and sub-tropical parts of the world.

Due to the overlapping morphological characters, the reliable identification of the stingless bee species is a daunting task even for expert taxonomist. Moreover, very limited molecular data is available in the literature and in the international Gene Bank on stingless bees. Therefore, identification of stingless bee species has mainly been established by traditional taxonomy (morphological features) and biosystematics keys. Some genera of Indian stingless bees can easily be separated using published keys (Sakagami *et al.*, 1990; Michener, 2007). Molecular identification techniques are commonly being used where morphological characters are not useful for distinguishing closely related species (Lee *et al*., 2005). Molecular taxonomy has provided a helping hand to the traditional taxonomists in taxonomically difficult species of insects. For DNA based identification mitochondrial gene cytochrome oxidase subunit 1 (COI) has been widely used in establishment of molecular identity at species level (Hebert *et al.*, 2003). DNA barcoding techniques is one of widely used method of species identification of insects. Molecular identification using COI gene has the advantage of not being limited by morphological polymorphism, sexual form and life stages of the target species (Asokan *et al*., 2011).

Several workers have designed different type of stingless bee hives globally for better honey yield and pollination. Some of examples are; Nogueira-Neto-type hive, the UTOB hive, bamboo hive of Kani tribe and Naga

tribes's stingless bee hives (Sommeijer, 1999; Kumar *et al.*, 2012 and Singh, 2016).

Vegetables are an important component of Indian agriculture due to their productivity, use in diversification, nutritional, medicinal values and value addition has export potential. Vegetable have become essential component of our diet. Apart from fruits, vegetables are the only natural sources of productive food which supply all the nutrients, especially vitamins, minerals, and crude fiber (Wargovich, 2000; Liua *et al.*, 2001). Current vegetable production in India (NHB, 2013) is 162.18 million tons from an area of 9.20 million hectare with productivity level of 17.60 tons/ha. The North Eastern Region of India is producing 40.51 lakhs million tons of vegetable from 3.68 lakhs hectare with productivity level of 11 tons/ha which is slightly lower as compared to national average but availability of vegetables (105 kg per person per year) is comparatively better in the region than national per capita availability of vegetables (93 kg per person per year). Every adult has to consume 250-300 g of vegetable per day. In India, an increase of 2.5% per year in vegetable production is also necessary to feed the increasing population. Present production of vegetable available is only 145 g per capita per day. Where as recommended requirement is 300 g. In India, vegetable grown is 4.8 times more remunerative than cereals and other field crops (NHB, 1998).

Tomato (*Lycopersicon esculentum* Bailey) is one of the important vegetable crops grown in the country which has an area of about 809 thousand hectares and production of 17697 thousand, metric tons with productivity of 21.8 metric ton/ha (Annual Report of Department of Agriculture Corporation & Farmers Welfare, 2017). The production of tomato in North East Region is 572.78 thousand tons from an area of 29.74 thousand hectare with a productivity of 19.2 metric ton/ha (Annual Report of DAC & FW, 2017).

Stingless bees are used as pollinator in greenhouse crops in both temperate and tropical regions, because some of them are buzz pollinator such as *Melipona quadrifasciata* (Sarto *et al*., 2005; Santos *et al.*, 2009). *Nannotrigona perilampoides*,is a small stingless bee and is not a buzz pollinator but pollinate tomato plants effectively under net condition and known to work efficiently under confined condition (Cauich *et al*., 2004; Palma *et al*., 2008b). They are able to vibrate the flower to expel pollen from anthers. Thus, provide better pollination in several crops *viz*. tomato, eggplant etc. Stingless bee use to visit the flowers of crops, forest trees, shrubs and herbs to collect nectar and other plant substances. In doing so, they (pollinator) transfer the matured pollen grains onto receptive stigmas which results in fertilization and eventually fruit and seed production (Momose *et al*., 1998).

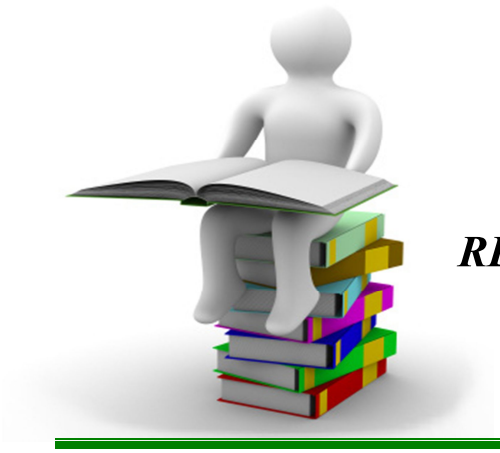
Melipona quadrifasciata and the honey bee *Apis mellifera* have been tested in tomato plots (Santos *et al.*, 2009) to evaluate their effectiveness in crop pollination. Flowers of Tomato plant are poricidial dehiscent. the anthers release pollen through apical pores when shaken (Mc Gregor, 1976; Buchmann, 1983). In open areas, wind is usually sufficient to trigger the pollen release. Thus, effect the self-fertilization (Free, 1993). In the case of greenhouses, successful pollination of cultivated tomato flowers is difficult, hence bee pollination plays an important role in increasing productivity.

In greenhouses, pollination of tomato flowers are commercially employed by using hand held electrical shaker and artificial mechanical vibration. However, this method is expensive and risks damaging the flowers (Banda and Paxton, 1991; Roubik, 1995) although resulting in tomatoes of higher quality than fruits derived from self-fertilization. In addition to such artificial vibration, bees have been used as pollinators to increase the production of greenhouse tomatoes. Bumble bees (Apidae, Bombini) have also been used for pollinating greenhouse tomatoes (Plath, 1925; Banda and Paxton,

1991; Kevan *et al.*, 1991; Asada and Ono, 1997; Dogterom *et al*., 1998; Estay *et al*., 2001; Al-Attal *et al*., 2003).Those bees which are capable of vibrating the anthers of poricidial dehiscent flowers by producing strong thoracic vibration called buzz pollination (Buchmann, 1983). In North India *Bombus haemorrhoidalis* Smith have been employed for greenhouse tomato (Yankit *et al.*, 2018) and cucumber pollination (Chauhan and Thakur, 2014; Kishan *et al*., 2017). However, in North East Region it is not been widely used due to lack of colonies. In recent years, considering needs of tomato to be grown in off-season, need an effective pollinator under protected condition. Therefore, stingless bees can be proven better alternative for tropical and subtropical region of the globe.

Under greenhouse wide range of vegetables and flowers are being grown globally. In India, greenhouse technology started in 1980's and it was mainly used for research activities and now the intensive agriculture is promoted for better productivity. Protected cultivation ensures better yield with high quality produce. Given the necessity of greenhouse for off- season vegetable production and importance of pollinator for assured quantity and quality of fruit and seeds, the present studies aims to investigate and explore the utilization of stingless bee fauna for increase in tomato production with the following objectives;

1) To collect stingless wild bee species from different altitudes of Nagaland and Upper Subansiri district of Arunachal Pradesh.
2) To identify wild stingless bee species by traditional and molecular taxonomy.
3) To study the comparative architectural design of stingless bee hives.
4) To explore the use of stingless bees species for pollination of off- season tomato crops under protected condition.



CHAPTER - II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Stingless bee belongs to order Hymenoptera and family Apidae, the tribe Meliponini, sub tribe Meliponina and these are considered as one of the effective pollinators in glasshouses (Kakutani *et al.*, 1993). Propagation of stingless bee colonies contributes to preservation of biodiversity by conserving populations of species that may otherwise decline owing to human disruption of ecosystems (Cane and Tepedino, 2001). Tomato is one of the commercially important vegetable crops grown both under open and protected conditions. Since tomato crops have export potential, thus, the fruit quality and quantity are major concern for international marketing agencies. Therefore, to ensure the quality and quantity of tomato production under protected conditions, pollination by stingless bee is required. However, tomato production under protected conditions is a limiting factor due to want of an effective pollinator. Since stingless bees are native pollinators, so collection and domestication of stingless bee colonies, their identification and use them in crop pollination is the area of interest for sustainable farming in North East India. The literature relevant to stingless bee species, methods /techniques of colony collection, their comparative architectural design, identification of species by traditional and molecular taxonomy and use of stingless bees colonies for pollination of tomato crop under protected condition, which are with specific reference having direct or indirect bearing on the objectives of this thesis are reviewed in the present chapter. Therefore, in this chapter an attempt has been made to review the available literature which is relevant to the objectives of the present study.

2.1 Stingless bee fauna

Stingless bees are a large group of bees having about 500 described species (Michener, 2013), comprising the tribe Meliponini and sub tribe

Meliponina. They belong to the family Apidae, and are closely related to common honey bees and bumble bees. Meliponines have stingers but has been significantly reduced and cannot be used for defence. Stingless bees have many genera. Some of their genera can be found in most tropical or subtropical regions of the world (Silveira *et al*., 2002). The workers or the females possess weak or vestigial stingers but unable to inflict pain with them. Hence, the term "stingless" is being used to designate the species. Some species have mandibles sufficiently strong to inflict a mild bite, pull hairs or may crawl into ears or nostrils of the intruders. Others emit a caustic liquid from the mouth that in contact with the skin causes intense irritation (Rahman *et al*., 2015).

Rasmussen (2013) studied the distribution of stingless bees throughout the Indian subcontinent and concluded that stingless bees are available in most parts of the Indian subcontinent, except at higher elevation or the drier interior regions. He found nine species of stingless bees in Indian subcontinent: *Lepidotrigona arcifera* (Cockerell), *Lisotrigona cacciae* (Nurse), *Lisotrigona mohandasi* Jobiraj and Narendran, *Tetragonula* aff. *laeviceps* (Smith), *Tetragonula bengalensis* (Cameron), *Tetragonula gressitti* (Sakagami), *Tetragonula iridipennis* (Smith), *Tetragonula praeterita* (Walker) and *Tetragonula ruficornis* (Smith).

Stingless bees are highly evolved social insects and live in a colony with organized system of division of labour. Some species have clusters of as many as 80,000 individuals and other less than 100 (Rahman *et al.*, 2015). Most species do not disturb man and they may be manipulated safely and can be managed at ease in the homestead garden (Rahman *et al*., 2015). Species of stingless bees produce honey around 600-700 g / year (Kumar *et al.*, 2012) and 200-500 g per season (Rahman *et al*., 2015), having high quality medicinal value. Stingless bees proved to be effective pollinators of strawberry, tomato, pepper, citrus fruits, and other crops and adapted well to greenhouse conditions

in different regions around the world (Maeta *et al*., 1992; Sarto *et al*., 2005; Cruz *et al*., 2005; Antunes *et al*., 2007; Santos *et al*., 2009; Giannini *et al*., 2015b). Silveira *et al*. (2002) observed that the flight activity of stingless bee started at relative humidity 80-89% and temperature 17 ^{o}C and 22 ^{o}C. The minimum temperature required for exit of the bee from hive was at 11^{o}C. Nests of stingless bees mostly found on cavities tree trunk, old walls, inside the termite mounds and subterranean cavities (Nogueira-Neto *et al*., 1997; Eltz, 2003; Roubik, 2006). The total number of species is estimated to be about 500 described species worldwide (Michener, 2013).

2.1.1 Stingless Bees of Brazil

Brazil is home to several species of stingless bees belonging to Meliponini, with more than 300 species already identified and probably more yet to be discovered. A total of 244 valid species, and about 89 undescribed forms (species already recognized by the author, but which have not been published yet), affiliated to 29 genera, are recorded for Brazil (Pedro, 2014). They vary greatly in shape, size, and habits, and 20 to 30 of these species have good potential as honey producers (Pedro, 2014). Among many others, species such as *Melipona subnitida* and *Melipona scutellaris* in the northeast of the country, *Melipona quadrifasciata* and *Melipona rufiventris* in the south-southeast*, Melipona compressipes manaosensis* and *Scaptotrigona polistycta* in the north and *Tetragonisca angustula* throughout the country are increasingly kept by small, medium, and large producers.

2.1.2 Stingless Bees of Australia

In Australia, most common group of bees in North tropical and sub tropical Australia are the stingless bees (Heard and Dollin, 2000). They belong to two genera *Trigona* and *Austroplebeia* (Michener, 1990). Six species in *Trigona* (Heterotrigona) occur in Australia (Dollin *et al*., 1997). *Austroplebeia*

only occurs in Australia and they consist about of four species. The Australian stingless bee species are small, measuring less than 4mm in length, black in colour and nest in hollow trees. The most common species kept in Australia are reported to be *Trigona carbonaria* (69%), *T. hockingsi* (20%) and 11% for *T. mellipes* and *T. clypearis*. The various stingless species look quite similar, with the two most common species, *Trigona carbonaria* and *Austroplebeia australis*, displaying the greatest variation, as the latter is smaller and less active.

2.1.3 Stingless Bees of Africa

All African stingless bees (Meliponini) are social. There are six genera (Eardley, 2004) of African stingless bees, *Cleptotrigona, Dactylurina Cockerell, Meliponula Cockerell, Plebeina Moure, Hypotrigona Cockerell* and *Liotrigona moure*, comprising 19 species. In five of the genera (*Dactylurina Cockerell, Meliponula Cockerell, Plebeina moure, Hypotrigona Cockerell* and *Liotrigona moure*) workers collect pollen and nectar from flowers, and in one genus (*Cleptotrigona moure*) they rob pollen and nectar from the nests of other stingless bees. In Kenya, species under domestication include *Meliponula ferruginea, Meliponula bocandei, Meliponula lendliana, Plebeina hildebrandti* and *Dactyrulina schmidti* (Eardley, 2004). Domestication has been envisaged for honey production and as an incentive for forest conservation (Kiatoko *et al*., 2014; Kiatoko *et al*., 2016).

2.1.4 Stingless Bees in Indian Sub continent

Stingless bees belong to the order Hymenoptera under the family Apidae, sub-family Apinae and Tribe Meliponini which has two main genera *viz*. *Melipona* and *Trigona.* About 250 species have been identified throughout the Neotropical and Indo-Burma-Malayan and Australian region of the world. Stingless bees are distinguished from corbiculate Apinae by combination of

reduced fore wing venation and the presence of a jugal lobe in the hind wing. The present state of knowledge on stingless bees of India, their diversity and foraging plants are not clearly known. Bingham (1897) described most of the Indian species under Melipona almost a century ago. There is sporadic information on stingless bees and only recent account was given by Sakagami (1978) and Rasmussen (2013) for continental Asia and Indian subcontinent. Only the two genus, Trigona Jurine and Lisotrigona Moure have long been found in Indian subcontinent (Michener, 2007). According to Rasmussen (2013) all together eight named species of stingless bees are known from the Indian subcontinent *viz*. *Lepidotrigona arcifera* (Cockerell), *Lisotrigona cacciae* (Nurse), *Lisotrigona mohandasi* Jobiraj and Narendran, *Tetragonula laeviceps* (Smith), *Tetragonula bengalensis* (Cameron), *Tetragonula gressitti* (Sakagami), *Tetragonula iridipennis* (Smith), *Tetragonula praeterita* (Walker), and *Tetragonula ruficornis* (Smith) in Indian sub continent.

The distribution pattern of stingless bees in India was studied by Rahman *et al*. (2015). They have reported four species of stingless bees prevalent in South India *viz*. *Tetragonula. iridipennis T. laeviceps* and *Lepidotrigona arcifera*. Whereas *Tetragonula. iridipennis and T. laeviceps* were present in the North West India. The study also revealed that *Tetragonula iridipennis* and *T. laeviceps* were most commonly available species in all selected zone of India. The nesting behaviour of *Tetragonula irridipennis* was found to make nest in timber, whereas *T. bengalensis* makes nest in bamboo and others make in cracks and crevices of stone and mud walls in India. Most of the researchers in India concentrate only on stingless bee biology, morphometry, natural enemies and its pollination biology (Muthuraman and Saravanan, 2004; Danareddi and Viraktamath, 2009; Vijayakumar *et al*., 2013), or species diversity in India (Rahman *et al.*, 2015).

2.1.5 Stingless Bees of North East India

The North-Eastern Region of India, covering a diverse natural habitat with varied topography, climate and forest forms part of the Indo-Burma biodiversity hotspots (Vijayakumar, 2014). Nagaland and Arunachal Pradesh are the hill states of North Eastern India in which, the studies on stingless bee in respect of their domestication and use them in pollination under protected condition in vegetable production are highly essential.

In North East India five species were reported by Rahman *et al.* (2015) *viz. Tetragonula bengalensis, T. iridipennis, T. ruficornis, T. laeviceps and Lepidotrigona arcifera*.

In Nagaland, Singh (2016) has observed three species *viz*. *Tetragonula irridipenis, T. laviceps* and *Lophotrigona canifrons.* However, later on four stingless bee species were reported from Nagaland *viz*. *Tetragonula iridipennis, T. ventralis, T. laeviceps and Lophotrigona canifrons* and one species from Arunachal Pradesh *i.e. Lepidotrigona arcifera* Cockrell (Anonymous, 2018).

2.2 Location and collection of wild stingless bee colonies

Different researchers described different methods of location and collection of wild stingless bee colonies. According to Kwapong *et al*. (2010) the whole colony of stingless bee could be located and collected from their natural hives and relocated into meliponiaries or colonies are transferred into artificial hives. Later they deduced training manual for stingless bee keepers in which they mentioned that stingless bee feral colonnies might be collected by using Trap nests, which were temporal collecting container that can be used bait and trap swarms of bee colonies.

Similarly, Singh (2016) observed that Naga beekeeper searched the presence of flowering plants, if bees are seen foraging, they would search the colony at the vicinity of foraging area preferably in 0800 h in the summer and 1000 h in winter season. Once hives are spotted they would fell the tree and cut out the portion of hives by saw and transported them to apiary in the evening.

2.3 Identification of stingless wild bee species

Like other insect species, reliable identification of stingless wild bee species is also possible based on morphological characters. Winston and Michener (1977) studied the morphological characters, evolutionary history and the social behaviour of stingless bees and honey bees. This study concluded that, highly eusocial behaviour arose twice in the bees, suggesting an early differentiation and a strong divergence of the stingless bees from the remaining Apidae. They also divided the family Apidae into three subfamilies *viz*., Meliponinae, Apinae, and Bombinae. Moure (1961) described the stingless bees into the tribes Meliponini and Trigonini. Characters differentiating the tribes Trigonini and Meliponini based on their body size, usually small, 2 to 8 mm in length and slender (Trigonini) and usually Meliponi, rather-large (from 8 to 15 mm in length) and robust (Wille, 1979). Sometimes due to the overlapping of morphological characters in between different species of the same genus, reliable identification of insect species is daunting task and in such cases even taxonomist also failed to identify the species correctly (Behere *et al*., 2007). However, due to the advancement of science various molecular techniques have been used to compare the DNA of such taxonomically difficult species. Using DNA barcoding technology, the reliable identification of the taxonomically species is also possible (Hebert *et al*., 2004). DNA barcoding uses the partial fragment of Cytochrome oxidase I gene in mitochondrial genome (Hebert *et al*., 2004).

2.3.1 Identification of stingless bee species by traditional taxonomy (morphological features) and biosystematics keys

The few genera of stingless bees from the Indian region can easily be separated using published keys (Sakagami *et al.*, 1990; Michener, 2007). However, the species-level identification is not trivial. A complex genus of stingless bees is Tetragonula, with more than 30 described species and no global identification key (Rasmussen 2008). Specimens of Tetragonula from the Indian subcontinent were treated as a single species in the key by Sakagami (1978). Older keys, such as those of Bingham (1897) and Schwarz (1939), cannot reliably be used for species identification. Lastly, sometimes external nest features such as nest entrances are the most diagnostic field traits observed, distinguishing closely related species (Camargo and Pedro, 2003, 2004; Rasmussen, 2004).

Vijayakumar (2014) dissected the feral nests of *Lepidotrigona arcifera.* Adult worker bees from these feral nests were collected and various key morphological characters were analyzed and identified based on previous literature. Within the subgenus Lepidotrigona the "*ventralis*" species group varied based on the smaller body size from other species in the subgenus.

Ndungu *et al.* (2017**)** used combined morphological features, morphometrics and molecular methods in the identification of stingless bees. They reported that morphometrics alone could not be used to identify all the stingless bees, when the species identity is completely unknown. By morphometry they could not distinguish *M. ferruginea* reddish brown and *M. ferruginea* black collected from two different locations as two morphs were similar in size.

The identification of stingless bees can be established with the sight of nest entrance, nest architecture and nesting site (Roubik, 2006). For example,

Ductylurina schmidti and the two *Meliponula ferruginea* morphs had very similar morphometry, but their nesting behavior is distinct. *Dactylurina schmidti* constructs external nest on tree branches while *M. ferruginea* reddish brown nests in mud walls and trees and *M. ferruginea* black nests in trees (Eardley, 2004; Nkoba *et al.*, 2012). DNA barcoding revealed cryptic genetic variation within the two morphs.

2.3.2 Description of stingless bee identification traits

Rasmussen (2013) reported that *Lepidotrigona arcifera* is holotype specimen and it can be identified with diagnostic dense tessellation on head and thorax and densely plumose hairs on the margin of mesoscutum. Tetragonula species of the "*iridipennis*" species group are characterized by having a dark mesoscutum with four distinct hair bands separated by broad glabrous interspaces, and by their smaller body size, with the forewing length, including tegula, measuring 3.0 to 4.3 mm. In case of *Lepidotrigona ventralis* (Smith) and *Lepidotrigona flavibasis*, first species can be recognized by the two lateral and separate dark spots on the otherwise pale metasomal tergum 1 while the second species can be recognized by the semi-circle of black integument, partly enclosing the basal depression on the otherwise pale tergum 1. *L. flavibasis* can also be recognized by the apical metasomal terga brownish to blackish and the fore and middle tibiae with blackish hairs on the external surface (Schwarz, 1939). Members of *Tetragonula* aff. *laeviceps* (Smith 1857) usually have a mesoscutum almost as evenly banded with hair as the "*iridipennis*" group, but these species are larger, with forewing length, including tegula, measuring between 4.2 and 4.8 mm. Sakagami (1978) referred to this species group as the taxonomically most difficult group within Tetragonula.

Vijayakumar (2014) reported that worker bee of *Lepidotrigona arcifera* had usually a black spot on each side of the other-wise pale tergite of abdomen

and the mesonotum usually enclosed by a border of short thick scale like or tomentose yellowish to whitish hairs. Hair fringes of hind tibia as well as hairs on mid and hind tibiae and basitarsi were brown to blackish. The hairs on posterior margin are very thick and short and simple.

Rahman *et al.* (2015) reported that *Tetragonula iridipennis* (Smith, 1854) had head and mesosoma black, metasoma brownish; antenna brownish, legs brownish black and their head devoid of pubescence, mesosomal pleuron with brownish short keirotrichia. *Tetragonula bengalensis* (Cameron, 1897) entirely black, head black, Mesosoma black and metasoma jet black. Pubescence generally whitish brown; head with brownish pubescence; antennae brownish; Legs black with dark brown pubescence, tarsus with brownish keriotrichia.

Tetragonula laeviceps (Smith 1857) general colouration black, metasoma brownish, wings brownish, hyaline beyond stigma, antenna deep brownish in base and light in the apex; legs: trochanter brownish black, tibia black, femur black metatarsus brownish black in base and brownish tarsomere. Head with sparse hairs; mesosomal hairs in the pleurite; metasoma very sparsely distributed hairs; legs: trochanter with long bristles like hairs, tibial spur present, femur without spur, metatarsus with rows of keriotrichia and tarsus with keriotrichia.

Tetragonula ruficornis **(**Smith 1870**)** head brownish; mesosoma brownish black; metasoma black; legs: trochanter, femur and tibia deep brown, tarsus brownish at base and light in apex. Head with hairs on the cervix, mesosoma with pleural spur and metasoma without hairs; legs: trochanter without spur, tibia with bristle like keriotrichia, femur without spur, tarsus with rows of keriotrichia, metatarsus with sparsely distributed keriotrichia. legs testaceous; penicillum testaceous.

Nayak and Prakash (2017) identified the stingless bee, *Tetragonula* spp. of India at the species level using the morphological characters described by Sakagami (1978) and Rasmussen (2013).

2.3.3 Molecular characterization of stingless bee species using mitochondrial cytochrome oxidase subunit I (COI) gene

Due to the extreme morphological similarities within or between species of stingless bees the identification of stingless bee species just based on either external or internal morphological characters is a daunting task. However, molecular techniques have provided some additional help to the taxonomist. Molecular characterization using different markers like mitochondrial genes (for example, Cytochrome oxidase I gene) or nuclear genes (Internal Transcribed Spacer Region I and II) have provided more clear picture on speciation.

Alves (2006) had applied RAPD method molecular characterization on *Tetragonisca angustula angustula* and *T. a. fiebrigi*. He reported that the two bee species were separated with genetic distance values, which were in accordance with that of Nei (1978), who reported it as 0.23 and between the two *T. a. fiebrigi* populations was 0. 0507.

Cytochrome oxidase I (COI) gene has been widely used as standard barcoding gene for identification of insect species at molecular level (Hebert *et al*., 2004; Behere *et al*., 2007).

DNA barcoding has recently been applied to bees that have shown reliably distinguish between different species (Sheffield *et al*., 2009).

Integrating DNA barcoding with morphology has also previously helped in resolving taxonomically difficult groups of bees and other organism (Gibbs, 2009; Packar *et al.*, 2009).

Koch (2010) reported that the taxonomy of the western Malagasy stingless bees (*Liotrigona* spp.) has been complicated by the high degree of morphological similarity between the described species. However, species boundaries of the taxonomically difficult taxa were successfully resolved by application of combined morphology and DNA barcoding analysis. The different species of *Liotrigona* genus were extremely similar in their morphology. Morphologically, *Liotrigona bitika, L. madecassa L. kinzelbachi sp,* and *L. mahafalya* were not clearly differentiated. By application of COI sequence, data as independent additional evidence were shown as four clusters to be genetically distinct taxa.

Stuchi *et al.* (2012) reported that with the use of molecular markers *viz*. EST-1, EST-2 and EST-3, *Tetragonisca fiebrigi* and *T. angustula* were identified as two distinct species.

Manger (2015) reported that the molecular characterization using COI gene revealed significant nucleotide polymorphism among ten *Bactrocera* spp. in Meghalaya.

Sreejith and Sebastian (2015) studied the molecular evolution of green leaf hopper, *Nephotettix virescens* using COI gene. The genetic diversity studies revealed that *N. virescens* populations from Kerala showed 96% similarity with *N. virescens* populations from Orissa.

Kuotsu (2016) developed DNA barcode images of 32 insect species observed in rice and maize under Meghalaya condition, which could be used as diagnostic guide for identification of insect species at both morphological and molecular level.

Ndungu *et al.* (2017) by using DNA barcoding showed clear separation between *Meliponula ferruginea* reddish brown and *M. ferruginea* black into two separate clades. The genetic distance between *M. ferruginea* black

collected from Kakamega and *M. ferruginea* black from Mwingi was low (1.4%), within expected species variation for barcode data based on COI for most animals (Hebert *et al.*, 2003) and in accordance with the barcode gap estimate for within species genetic variation (<1.6%). The genetic distance between *M. ferruginea* black and *M. ferruginea* reddish brown collected from Kakamega, in sympatry, was 97.3%.

The molecular studies of two different species of bumble bees *Bambus haemorrhoidalis* and *B.rufofaciatus* showed similarity index of 62% (Chauhan *et al.*, 2017).

2.4 Nest architecture of stingless bees

The presence of nesting sites is essential for survival, maintenance and reproduction of stingless bees (Hubbell and Johnson, 1977; Batista *et al*., 2003; Eltz *et al*., 2003; Roubik, 2006). Although their nesting habits are variable, most of the stingless bee species build their nests in pre-existing cavities of trees, in termite and ant nests, or in other hollow spaces in the ground. They build their nests in several substrates, such as subterranean cavities, tree trunks, rock crevices, brick walls, abandoned termite nests, arboreal ant nests, subterranean chambers abandoned by ants (Campos, 1987, Kerr *et al*., 1996; Roubik, 1983; 2006). Few species build their nests in exposed positions (Schwarz, 1948; Michener, 2000). The main building material is cerumen, a mixture of bee wax and plant resins. Extensive use is also made of batumen, a mixture of mud, plant resins, animal faeces *etc*. (Rasmussen and Camargo, 2008). The nest entrance of stingless bees varied in shape, length, and colour. For example, nest entrance of *Heterotrigona itama* is funnel shape, whereas, in *Geniotrigona thoracica* is round mount-like shape (Syafrizal and Yusuf, 2014; Kelly *et al*., 2014). The common structure of stingless bee nests consists of entrance tunnels, brood cells, food storages (honey and pollen cells), cerumen and batumen layers (Sakagami *et al*., 1983; Starr and Sakagami, 1987;

Michener, 2007; Boongird, 2011; Erniwati, 2013). The narrow nest entrance of *Melipona* and other genera allows the nest to be defended by one or only a few guards positioned in the mouth of the entrance tube and variation of nest entrances related to defence and foraging activities of stingless bees (Biesmeijer *et al*., 2005).

Within the nest the brood chamber is always clearly separated from the area of food storage. There are two cell types: brood cells and storage pots (Vijayakumar, 2014). Storage pots are in most species several times larger than the brood cells and pots containing honey are generally intermixed with those that contain pollen. However, pots with honey are sometimes grouped at the periphery of the storage compartment, whereas pollen pots may be found near the brood chamber. Most species arrange their brood cells in single-layer horizontal combs. The pile of horizontal combs is surrounded by a series of sheets of cerumen. The brood section is enveloped by multiple layers of membranes of cerumen which is called the involucrum and is important for temperature control in the nest (Kwapong *et al*., 2010). Brood cells are arranged horizontally in most species or in a cluster, however in others such as Dactylurina cells are vertically from the storage compartment. Brood cells in a cluster arrangement were not surrounded by an involucrum. This allows them to fit into irregularly shaped cavities (Sommeijir, 1999).

Martins *et al.* (2004) reported about the use of tree species stingless bees for nesting and also explained the way of taking measurement of the nest. The external perimeter of trees and trunks was measured through which the diameter could be calculated. In case a tree or trunk was not evenly cylindrical, two measurements were taken, one at its smallest and one at its biggest perimeter, together rendering an average perimeter value. Length of trunks was measured as well and these lengths delineated indirectly the dimension of the nest they hooded as trunks were always cut off near the top and bottom end of

nests. In the cases where a colony was transferred from its trunk to a rational hive, it was possible to measure the internal trunk diameter and precise length of the nest. This consequently made it possible to calculate the volume a colony occupied.

2.4.1 Nesting sites of stingless bee

Camargo (1970) reported and considered that the nesting site as the main limiting factor for population growth in stingless bees. Stingless bee habitat preferences do indeed have a genetic basis (Jaenike and Holt, 1991). Nests being notable point to the colonial life of social insects play a major role in providing physical protection against environmental perturbations (Nayak *et al.*, 2012).

In north-eastern Brazil, mostly tree trunks were kept by Meliponinae beekeepers for stingless bee (Martins *et al.*, 2004). They observed that nearly 13 per cent of observed nests were in living trees in the field, sheltering seven species of stingless bees with 227 nests in 12 tree species. More than 75% of stingless bees were found in two tree species being *Caesalpinia pyramidalis* (Caesalpiniaceae, 41.9%) and *Commiphora leptophloeos* (Burseraceae, 33.9%). Furthermore, all bee species nidify in *C. pyramidalis*. A great part of the nests in trunks were of *Melipona subnitida*, (N = 130) of which 50% was found in *C. leptophloeos* and 22.3% in *C. pyramidalis*. *M. asilvai* was predominantly found in *C. pyramidalis* (92.3%).

Carvalho *et al*. (2014) reported about the new nidification of stingless bee that the *Melipona subnitida* usually make nests in hollow spaces in trees. However, the nest of this bee have also regularly found inside living arboreal termite nests of *Constrictotermes cyphergaster* Silvestri. Thus, nesting habit of *M. subnitida* is an adaptation to shortage of pre-existing cavities in trees locally.

Suriawanto *et al*. (2017) conducted a survey on nesting sites of *Tetragonula fuscobalteata.* They reported that the highest number of *Tetragonula fuscobalteata* colonies were found in wooden wall (74 colonies), followed by stone cavity (40 colonies), brick wall (31 colonies), bamboo (6 colonies), and iron cavity (4 colonies).

2.4.2 Nest orientation

It has been reported by Nayak *et al*. (2012) that nests orientation of stingless bee within eight directions *viz*. north, south, east, west, northeast, northwest, south east, and southwest. North direction was most preferred direction of the nesting. Greenish and black colour hives were most preferred by *Trigona iridipennis,* a stingless bee species.

2.4.3 Nest height from ground

Nayak *et al*. (2012) also studied the nesting heights of Trigona. They reported that the nesting elevations offered by Trigona above ground level showed very distinct preference of 47% between an elevation range of 11–15 ft from the ground point , while between 0–5 ft and 6–10 ft of ranges only 28% of nests were found .

2.4.4 Internal nest architecture of stingless bee

Vijayakumar (2014) dissected the feral nests of *Lepidotrigona arcifera* and explained the nest architecture for *L. arcifera* from North East India. The internal part of the nest covered with cerumen. The internal nest divided into two major parts: brood chamber and storage pots. The brood chamber located at centre of the nest, which were covered with involucrum. Internal tunnel directly connected to external entrance tube from the brood chamber. The brood cells arranged in regular horizontal combs. The larval brood cells were rounding, darker and larger than pupal cells which were pale in colour. New

cells were brown in colour but older turned yellow. Workers and males emerged from similar cells in the same combs. Queen cells positioned at the margin of combs elliptically. The food storage pots (honey pots and pollen pots) were usually found at the top and bottom of the nest. Pollen and honey pots were larger in size. The pollen pots were closer to the brood chamber.

2.5 Different artificial hives of stingless bees

2.5.1 Nogueira-Neto-type hive

Sommeijer (1999) has described the Nogueira-Neto-type hive for stingless bee rearing in which the food pots are constructed in a shallow tray that ensures the bees construct only one layer of pots in that chamber. The hive allows for the unobstructed vertical development of the brood chamber.

2.5.2 The UTOB hive

Sommeijer (1999) has explained the dimension of UTOB hive designed and developed by Utrecht University, Tobago that brood chamber having 11 cm length, 13 cm width, 13 cm height and honey chamber 40 cm length, 13 cm width and 7 height cm for stingless bees especially for *Melipona fovoso.* This hive provides better extraction of honey from the colony without disturbing/damage the brood in *M. fovoso.*

2.5.3 Bamboo hive of Kani tribe

Kumar *et al*. (2012) reported that the Kani tribe of Tamil Nadu, India used bamboo hives having 30-35 cm diameter and 80-85 cm length for stingless bee colony, especially for *Trigona iridipennis*. The bamboo stem were split into two halves and then tied with the help of rope.

2.5.4 Naga tribes's stingless bee hives

Singh (2016) has reported that the beekeeper of Naga tribes of Nagaland, India used traditional hives, which are not scientifically standardized. He mentioned in his report that bee keepers uses Log hive, bee box and rectangular wooden box. Reportedly, log hives having 43-123 cm long and 11-38 cm in diameter were used at different district of the Nagaland for *Tetragonula iridipennis* and *T. Laviceps.*

2.5.5 Modern stingless bee hives

Seven different types of wooden hives have been developed for better domestication of *Tetragonula iridipennis* in Nagaland (Anonymous, 2018).

2.6 Foraging behaviour of stingless bees

Foraging activities of bees assessed through direct observations of the flow of workers from nests. The quantity and quality of resources (pollen, nectar/water or water, resin, mud and garbage) that entered and/or left the nests were recorded every 10 minutes during each hour from 0600 h to 1800 h (Bartelli *et al*., 2014). A single flower patch is often exploited by several bees and bee species simultaneously (Boogert *et al*., 2006). Stingless bee prefers white or yellow flowers (Cortopassi-Laurino *et al.*, 1991). They prefer small flowers (Wille *et al.*, 1983) and dense inflorescences (Roubik *et al.*, 1990). Stingless bee stick to floral constancy *i.e*. worker on a trip usually visits only one plant species (Ramalho *et al*., 1994).

Bhambura (1958) recorded that *Melipona* sp. started collection of pollen from watermelon at 0830 h and their activity reached the peak at 1030 h. Inoue *et al*. (1985) observed the colonies of *Trigona itami*, *Trigona moorei* and *T. minangkabau* with populations of 5000, 2000, and 2600 made about 7000, 2400, and 1200 flights per day, respectively.

Rao and Suryanarayana (1988) stated that *A. carena* was the principal pollinating insect and was found to be efficient pollinator of water mellon than *A. florea* and *T. iridipennis*.

Heard and Hendrikz (1993) reported that the flight activity of stingless bees depends on species, population of colonies and availability of resources. They recorded that 10,000 workers of a hive of *Tetrgonula carbonaria* made about 20,000 flights per day. Similarly, Kakutani *et al.* (1993) reported that a newly established hive of *T. minangkabau* with only 350 workers made only about 700 flights per day, showing the strong positive relationship between hive population and flight activity.

Eswarappa (2001) reported that among the honey bees, maximum time spent in collection of pollen was by *A. florea* (14.63 s), followed by *T. iridipennis* (12.89 s), *A. cerana* (7.59 s), *A. mellifera* (6.77 sec.) and the lowest in *A. dorsata* Fab.

Prakash (2002) observed that the time spent by *A. florea* in collection of nectar from cucumber flowers of both the sexes was found to be maximum (305.93 sec. on pistillate and 276.68 sec on staminate flower), followed by *T. iridipennis* (286.61 sec on pistillate and 271.99 sec on staminate flower), *A. mellifera* (37.47 sec on pistillate and 34.00 sec on staminate flower) and *A. cerana* (38.12 sec on pistillate and 35.31 sec on staminate flower). The lowest time spent was recorded in *A. dorsata* (3.52 sec on pistillate and 31.44 sec on staminate flower). He also reported that among the honey bees, maximum time spent in collection of pollen from cucumber was found in *A. florea* (13.49 sec), followed by *Tetragonula iridipennis* (11.44 sec).

Ciar *et al.* (2013) preformed an experiment to find the foraging behaviour of stingless bees (*Tetragonula biroi friese*) in respect of distance, direction and height of preferred food source. They placed feeders at 1m

(which is the shortest distance considered in all experiments) apart from the hives within 13 meters experiment area was found the most preferred distance by the bees. Similarly, the bees forage to the nearest food sources, and usually forage to the food source in the neighbourhood. They concluded that 1m from the ground was the most preferred height of the bees. The feeders with 1m height from the ground have the shortest distance from the beehive at which the hive opening can easily be discovered by scouts/foragers. For the direction, they observed that bees did not have consistent preferred direction. The choice of feeder varies depending on current environmental conditions (such as wind speed and direction) or might be due to non-apparent behaviour (such as random search). He observed Area-Restricted Searching Strategy and Marginal Value Theorem in the foraging activities of *T. biroi*. Bees tend to maximize the time spent in an abundant area but when food sources are depleting they choose another feeding site which is usually in the nearest abundant neighbour (Ciarl *et al.*, 2013).

2.7 Use of stingless bees for pollination under protected condition

Most crop plants depend on pollination for fruit and seed set. For many crops, insects are the main pollination vector (with the main exception of grains, which are wind or self-pollinated). It has been estimated that about 30% of human food is derived from bee-pollinated crops (Kearns and Inouye, 1993). In Latin America, stingless bees (Apidae, Meliponini) have received increasing attention as crop pollinators during the past few years. These highly eusocial bees live in perennial colonies, are easily domesticated and show various behavioural traits (such as recruitment of foragers (Lindauer and Kerr, 1960), high flower constancy, great diet-breadth, and easy adaptation to new plant species that make them promising candidates as pollinators of commercial crops (Roubik *et al.*, 1986; Ramalho *et al*., 1994; Nogueira-Neto, 1997; Heard, 1999).

The potential of stingless bees for crop pollination is enhanced by the ability to transfer colonies into artificial hives. These hives can be propagated (Heard, 1988; Nogueira-Neto, 1997 ; Roubik, 1995) so that growers do not need to rely on natural populations. Hives can also be transported where needed for pollination or for hive strengthening. Hives may be opened for extraction of honey, inspection, feeding, or requeening if necessary and for treatment against natural enemies (Nogueira-Neto, 1997).

A wide variety of bee species are known to be efficient and effective pollinators of many crops. Tomato (*Lycopersicon esculentum*, Solanaceae) flowers are self-compatible but need animal or wind pollination to set fruit due to low nectar production and pollen is released from poricidal anthers upon vibration (Free, 1993). Tomato is one of the most widely grown vegetable crops in the world, and is commonly produced in greenhouses (Benton, 1998). Over the last several decades the management of some other pollinators has been developed which have proven to be much more efficient than the honey bee for certain crops. Examples include Nomia, Osmia, Megachile (for alfalfa), bumble bees (for crops of the Solanaceae family; tomatoes and brinjal), flies, and more recently, stingless bees (Free, 1993; Heard, 1999). In tomato flowers pollen is released through vibration ('buzzing') of their poricidal anthers. Bees produce these vibrations by shivering the indirect flight muscles and anther buzzing has been observed in many bee species, including bumble bees and stingless bees of the genus Melipona (Buchmann, 1995). In 1999, the first detailed review on the role of stingless bees in crop pollination appeared (Heard, 1999). He reported that stingless bees are effective and important pollinators of nine crops, and that they contribute to pollination in 60 other species out of the 90 crop species they were found visiting. Over the past years, several new studies on stingless bee pollination appeared. After the review by Heard (1999) there was a clear trend towards a more experimental approach using enclosures such as bags, cages and greenhouses (Biesmeijer and Slaa,

2004; Nieh, 2004). These inter-specific differences allow for selection of the most appropriate stingless bee for a given crop species and crop breeding system (greenhouse, open field, *etc*.). Commercial pollination with stingless bees has not been developed yet. Nevertheless, several biological features make stingless bees strong candidates for commercial pollination services. Stingless bees are true generalists, collecting nectar and pollen from a vast array of plants (Heithaus, 1979 a, 1979 b; Roubik, 1989). Many stingless bee species have proven to forage well in enclosed areas and under adequate climatic conditions they forage year round. This makes them especially suitable for off season production of crops in green houses. Most species of stingless bees have a foraging range smaller than that of the honey bee, which may enhance foraging efficiency in confined spaces (Visscher and Seeley, 1982; Seeley, 1985; Katayama, 1987; Kakutani *et al.*, 1993). Stingless bees have many advantages; they are generally less harmful to humans and domesticated animals. They are able to forage effectively in glasshouses (Kakutani *et al.*, 1993). Propagation of colonies contributes to preservation of biodiversity by conserving populations of species that may otherwise decline owing to human disruption of ecosystems (Inoue *et al.*, 1984). Colonies are rarely able to abscond, as the old queen is flightless (Inoue *et al*., 1984); and they are resistant to the diseases and parasites of honey bees (Delfinado-Baker *et al.*, 1989). Two studies have reported on the pollination effectiveness of *Melipona quadrifasciata* for tomato grown in greenhouses in Brazil. Stingless bees have as yet not been commercially bred on a large scale for pollination purposes, although they have been shown to have great potential for the pollination of many crops, including avocado, coconut, coffee, guava, and mango and rose apple grown in open culture, as well as for strawberry, sweet pepper and tomato kept under greenhouse conditions (Heard, 1999; Slaa *et al*., 2006).

Cauich *et al*. (2004) observed pollination efficiency of *N. perilampoides* on greenhouse tomatoes in Subtropical México. He reported that although this species is not a buzz-pollinator but it effectively pollinated tomato plants grown in netted cages (4m × 4m × 3.5 m, one colony for 40 plants). Pollination by *N. perilampoides* was as effective as mechanical vibration in terms of percentage fruit set, number of seeds per fruit and fruit weight. However, tomato flowers that did not receive any pollination treatment more than half did set fruit. Fruits produced without a pollination treatment had significantly less seeds than fruits produced after mechanical vibration or bee pollination, but fruit weight did not significantly differ among the treatments.

Santos *et al*. (2004a) compared pollination effectiveness *of Melipona. quadrifasciata* and *Apis mellifera* (each species in a 86 m^2 greenhouse), and found that tomatoes were bigger, heavier and had more seeds following pollination by *M. quadrifasciata* compared to *A. mellifera.*

Santos *et al*. (2004b) reported that *Scaptotrigona aff. depilis* and *N. testaceicornis* effectively pollinated greenhouse cucumber in Brazil, resulting in a higher fruit production, higher fruit weight and a higher percentage of perfect fruits compared to the control, where no pollinators were present.

Sarto *et al*. (2005) found that pollination of tomato by *M. quadrifasciata* (six colonies for 700 plants in a 234 m^2 plastic greenhouse, 3 m high) resulted in equal fruit quality (size and shape) compared to hand pollination or bee plus hand pollination. However, bee pollinated fruits contained 11% less seed compared to hand pollination, possibly due to the low temporal overlap in foraging activity and stigma receptivity.

CHAPTER - III

MATERIALS AND METHODS

MATERIALS AND METHODS

Various materials and methods employed for studying the stingless bees and their utilization in tomato (*Lycopersicon esculentum*) Bailey production are presented in this chapter.

3.1 Experimental Site

The experiments for present investigation on stingless bees and their utilization in tomato (*Lycopersicon esculentum*) Bailey production were carried out during 2016-2018 in the Insect Molecular Laboratory, Division of Crop Protection, ICAR Research Complex for North East Hill Region, Umiam, Meghalaya and in the field at Arunachal Pradesh and School of Agricultural Sciences and Rural Development, Nagaland University Campus Medziphema, India.

3.1.1 Geographical location and experimental sites

The ICAR Research Complex for North East Hill Region is situated at Umiam, Meghalaya, 24°41’21’’ N and 91°55’25’’ E at an altitude of 1010 meter. Molecular analysis to ascertain the species level identity of stingless bee specimens was carried out in Insect Molecular Laboratory, Division of Crop Protection, ICAR Research Complex (Plate 1).

The present study on collection of wild stingless bee colonies, domestication and standardization of hives and use of stingless bee in tomato pollination under protected condition were carried out in the Darin farm, Riporijo, Bui village, under Gusar circle of Upper Subansiri district of Arunachal Pradesh during 2016-2017 and 2017-18. Riporijo is situated at 28°4’16’’ N and 94°12’4’’ E at an altitude of 424 meter (Plate 1).

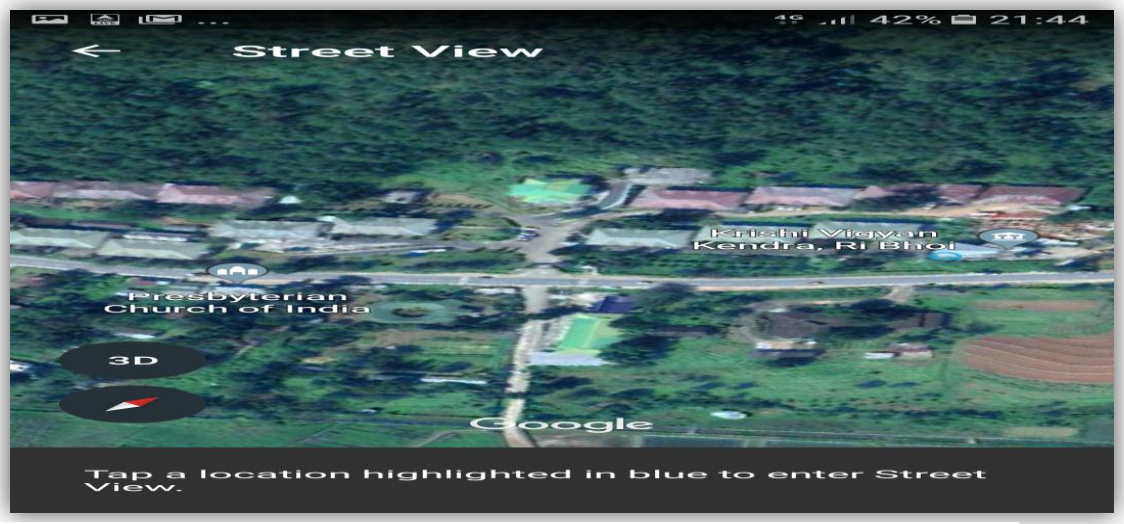


Plate 1a: ICAR Meghalaya

Plate 1b: Darin Farm, Arunachal Pradesh

Plate 1c: SASRD, Medziphema Nagaland

Plate 1: Different locations of experiment at Meghalaya, Arunachal Pradesh and Nagaland

The collection of stingless bee samples and evaluation of data and interpretation of statistical analysis at School of Agricultural Sciences and Rural Development, Nagaland University Campus Medziphema, India, which is situated at 25°45'53'' N and 93°53'04'' E at an altitude of 310 meter (Plate 1).

3.2 Experimental Details

3.2.1 Collection of wild stingless bee colony from forest having different altitude

3.2.1.1 Collection of colonies in Arunachal Pradesh

Visual survey was conducted in the July – December 2015 intensively at Bui, Uli, Nguki, Bulo, Bator Riddi and Segi villages. Location of survey area in Upper Subansiri district Arunachal Pradesh (Plate 2).

Eighteen numbers of wild stingless bee colonies were collected from different locations and altitudes from Bui, Uli, Bator, Nguki, Bulo and Riddi village of Upper Subansiri district Arunachal Pradesh during 5th March 2016 to 25th March 2016.

All the 18 colonies were introduced into new hives and placed in previously tagged shed house. Thirteen colonies were absconded out of 18 colonies. Thus another 13 colonies were collected and re-introduced in absconded hives in March 2017.

3.2.1.2 Stingless bee colonies from Nagaland

Three colonies were collected from SASRD Medziphema Nagaland: White SASRD, colony, black SASRD colony and underground SASRD colony. These colonies were collected for traditional and molecular taxonomy for species identification. Internal colony structure, honey production and their

Plate 2a: Survey area locating the Leyarijo, Segi of Bui and Riddi village

Plate 2b: Survey area locating the Nguki Bui and Bator village

Plate 2: Google earth showing the sites of survey for feral stingless bee colony

uses in pollination were not studied. Only external features such as nest entrances were observed and recorded, as they are one of the most diagnostic field traits for distinguishing closely related species.

3.2.2 Method of wild stingless bee colony collection

Based on previously surveyed, location of feral stingless bee habitat was identified at the forest. Stingless bee nested trees were felled by hand sawing machine mostly at morning time. Once tree was secured on the ground by taking the entrance tunnel as a mark of the centre of the colony, the fallen tree was sectioned into cylindrical pieces. Later on both the end of the cylindrical hive having colony inside was closed and transported in experiment site in the evening after 1730 h.

3.2.3 Construction of shed house

Two types of shed house were constructed in the month of February 2016 for placing the collected stingless bee colonies which were

i) Poly shed type
ii) Thatch shed type

In poly shed type, the roof was enclosed with U V sheet, while other one was roofed with thatch. There were two sheded house for each type: Poly shed-1 and poly shed-2; Thatch shed-1 and thatch shed house-2 (table 3.1 and plate 3). Ten meters length for both the shed houses-1 and 8 m in case of shed house-2 were kept. The heights of the rack were adjusted at 2 feet and 5 feet above the ground in poly and thatch respectively.

Plate 3a: Thatch shed

Plate 3b: Thatch shed

Plate 3c: Poly shed

Plate 3: Different types of shed houses

Table 3.1: Type of shed house

Sl. No.	Type of shed house			Length (m)	Number of hives placed (2m apart)
01	Shed house	Poly shed	Poly shed-1	10	5
			Poly shed-2	8	4
		Thatch shed	Thatch shed-1	10	5
			Thatch shed-2	8	4
02		**Total**	**4**	**-**	**18**

3.2.4 Construction of different hives

Different designs of hives were used in an exploration for the scope of easy handling for monitoring the internal growth of the colonies without much disturbance or injury to developing colony in the hives.

Three types of hives were designed for stingless bees, which are: Mo: Natural hive (NH), M1: Bamboo hive (BH) and M2: Wooden hive (LAH). Each type of hive have six subtypes *viz*., Natural Hives (NH1, NH2, NH3, NH4, NH5 and NH6); Bamboo Hive (BH1, BH2, BH3, BH4, BH5 and BH6) and Wooden Hive (LAH1, LAH2, LAH3, LAH4, LAH5 and LAH6). All the hives were of different dimensions and shape (Table 3.2). In LAH hives, three designs were used; Split type, Side open type and Top open type. In case of Bamboo hives, middle part split type and both end open type were used. However, Natural hives were kept as whole colony habitat (plate 4).

Table 3.2: Dimension and shape of hives used during the years 2016-17 and 2017-18

2017			2018		
Name of hive	**Dimensions (LXBXH) (cm)**	**Shape of cavity**	**Name of hive**	**Dimensions (LXBXH)**	**Shape of cavity**
NH1	30 x 7.0 x 4.0	Irregular	**NH1**	30 x 7.0 x 4.0	Irregular
NH2	18 x 6.0 x 5.09	Irregular	**NH2**	18 x 6.0 x 5.09	Irregular
NH3	17 x 7.0 x 5.04	Irregular	**NH3**	17 x 7.0 x 5.04	Irregular
NH4	18 x 8.5 x 6.04	Irregular	**NH4**	18 x 8.5 x 6.04	Irregular
NH5	24 x 10 x 7.0	Irregular	**NH5**	24 x 10 x 7.0	Irregular
NH6	20 x10 x 5.07	Irregular	**NH6**	20 x10 x 5.07	Irregular
BH1	80 x 7.5	Cylinderical	**BH1**	80 x 7.5	Cylinderical
BH2	45 x 6.5	Cylinderical	**BH2**	45 x 6.5	Cylinderical
BH3	80 x 7.5	Cylinderical	**BH3**	80 x 7.5	Cylinderical
BH4	45 x 6.5	Cylinderical	**BH4**	45 x 6.5	Cylinderical
BH5	82 x 8.0	Cylinderical	**BH5**	82 x 8.0	Cylinderical
BH6	82 x 8.0	Cylinderical	**BH6**	82 x 8.0	Cylinderical
LAH 1	45 x 7.0 x 5.0	Cuboid	**LAH 1**	99 x 11 x 5.87	Cuboid
LAH2	60 x 18 x 7.0	Cuboid	**LAH2**	60 x 16 x 5.0	Cuboid
LAH3	100 x 10 x 5.0	Cuboid	**LAH3**	60 x 16 x 5.0	Cuboid
LAH4	45 x 7.0 x 5.0	Cuboid	**LAH4**	98.8 x 10.8 x 6.0	Cuboid
LAH5	40 x 18 x 8.0	Cuboid	**LAH5**	40 x 18 x 8.0	Cuboid
LAH6	40 x 18 x 8.0	Cuboid	**LAH6**	40 x 18 x 8.0	Cuboid

*NH=Natural hive; BH=Bamboo hive; LAH= Wooden hive

Plate 4a: Bamboo hives

Plate 4b: Natural hive

Plate 4c:Wooden Hive- Split type

Plate 4d: Wooden hive- Side open type

Plate 4e: Wooden hive-top

Plate 4: Different type of hives for stingless bee rearing

3.2.5 Placement of hive with live colony at experimental site

The new brood chambers were inoculated in new hives. While, inoculating it was ensured that pollen and honey were not contaminated with brood chambers. New hives were made sure that they are free of any insect pests infestation. After inoculation hives were closed, tied with rope in case of split type and bamboo hives and sealed all the crevices by slurry, mixture of cow dung, clay soil and wax of stingless bee. Hives with inoculated brood were placed in shed house at 2 m apart in all shed houses, which were previously tagged in the shed houses.

Five colonies of stingless bees were kept at poly shed-1 and four colonies in poly shed-2 at 2 meters apart and all the colonies placed under the thatch shed were above 5 feet from the ground. Five and four colonies were placed in thatch shed-1 and thatch shed-2 respectively (plate 5).

3.3 Identification of stingless bees

3.3.1 Identification of feral stingless bee species by traditional taxonomy

For identification of species through traditional taxonomy stingless bee specimens were collected from all the hives (Plate 6). Ten worker bees from each hive were collected at the entrance tunnel of the hive by sweeping net. Specimens were preserved in 99% ethanol and laboratory analysis was carried out at ICAR, Umiam, Meghalaya.

3.3.1.1 Preparation of specimens

For biosystematics work, stingless bee species stored in 99% ethanol were removed from vial and were kept at room temperature for one hour. The specimens were removed carefully from the tubes with the help of forceps or camlin brush and placed on blotting paper to drain out the excess ethanol. The specimens were kept on blotting paper for 30 minutes to drain out and

Plate 5a: Hives placed in thatch shed (H1) house

Plate 5b: Hives placed in poly shed (Ho) house

Plate 5: Stingless bee hives placed under different shed houses

evaporate the excess ethanol. The individual specimens were mounted using insect pin (11mm) for further photograph (photomicrograph) in the Laizes electron microscope at ICAR, Umiam, Meghalaya. Head and thorax, abdomen hind and forelegs, wings of the stingless bee of all the specimens were photographed. Rest specimens were preserved 100% ethanol in 10 ml screw cap vials for molecular analysis.

3.3.1.2 Identification of stingless bee species using taxonomic keys

The individual mounted specimens were observed under microscope and identified morphologically based on established taxonomic keys (Rasmussen, 2013). The identified species were then labeled along with their locations, name of collector and date of collection. The identified specimens after labeling were kept inside the insect box. The insect boxes and the vials of each identified species were deposited along with voucher numbers at Insect Museum of Entomology section of Crop Protection Division, ICAR Research Complex for NEH Region, Umiam, Meghalaya.

3.3.1.3 Procedure for morphological identification

Apparatus and procedures used to prepare the specimen.

3.3.1.3.1 Apparatus

- Stereoscopic microscope
- Light source
- 90 mm diameter Petri dishes
- Camlin and Forceps brush

3.3.1.3.2 Preparation procedure

- Workstation was cleaned and cleared of all flies before commencing.
- Adjusted chair height and microscope, and turned on the light source

- Carefully placed the stingless bee into a plastic Petri dish when examining more than one bee at one time.

3.3.1.3.3 Identification

Following were the key features used for the morphological diagnosis of adult male stingless bee:

- Wing morphology and infuscation
- Overall colour and colour patterning of scutum and abdomen
- Presence and colour of thoracic vittae (vitta is a band or stripe of colour).

3.3.1.3.4 Confirmation of identified species from taxonomist

The specimens of stingless bees were initially sent to Insect Identification Service, National Pusa Collection, Division of Entomology, Indian Agricultural Research Institute (IARI), New Delhi, however the taxonomic identification service was not available for stingless bees specimens at IARI. Later on all the species were taxonomically identified by Dr. Shashidhar Viraktamath, Emeritus Scientist, Department of Entomology, University of Agricultural Sciences, GKVK, Bengaluru-560065, Karnataka.

3.3.2 Identification using molecular taxonomy / DNA barcoding

3.3.2.1 Extraction of DNA

In order to extract the DNA from ethanol preserved specimens, the specimens were removed from the individual vials separately with the help of sterilized forceps and specimens were air dried on sterilized blotting paper for an hour at ambient temperature (*i.e.* room temperature). This has allowed all the ethanol to evaporate from the specimens. DNA was extracted from whole adult specimens by Phenol: Chloroform modified protocol of Behere *et al.*

(2007) from those specimens, which were different in morphological features. Specimens having similar morphological characters were not analysed.

Modified Phenol: Chloroform protocol (Behere *et al*., 2007)

Reagents used

- Homogenization buffer: pH 8.0 (store at 4^{o}C)
- 0.1M NaCl
- 0.2M Sucrose
- 0.01M EDTA
- 0.03M Tris base

Lysis buffer: pH 9.2

- 0.025 M EDTA
- 2.5% SDS
- 0.5M Tris buffer

Potassium acetate (8M)

TE buffer:

- 10mM Tris HCl
- 1mM EDTA

Protocol for DNA extraction

1. Whole stingless bee adults was crushed using sterilized micro pestles in 300 □l of homogenization buffer and pestles were washed with additional 150 □l of homogenization buffer.
2. 150 □l of lysis buffer was added in each tube and vortex vigorously.
3. Tubes were incubated for 30 minute at 65 ºC on hot plate.

4. After incubation, 80 □l of Potassium Acetate (8M) was added and vortex for 1 minute.
5. Tubes were incubated at 4 ºC (in the refrigerator) for 30 minute.
6. After incubation at 4 ºC, tubes were centrifuged at 12000 rpm for 10 minute in refrigerated centrifuge at 4 ºC.
7. The supernatant from each tube were taken out and transferred in new labelled tube.
8. Phenol: Chloroform Iso amyl alcohol (25:24:1) was added in 1:1 proportion and the tubes were vortex vigorously for 1-2 minutes.
9. Tubes were centrifuged at 12000 rpm for 10 minutes in refrigerated centrifuge at 4 ºC.
10. Aqueous phase was taken carefully into new tubes and 10 □l of Potassium Acetate (8M) was then added in each of the tubes and mixed gently.
11. A double (1:2) volume of 100% ice cold ethanol was added in each tube and individual tubes were gently inverted few times.
12. All the tubes were kept at 20 ºC overnight for precipitation of DNA.
13. After overnight incubation, the tubes were centrifuged in refrigerated centrifuge or at 4 ºC at 1200 rpm for 10 minute.
14. The supernatant in each tube was decanted and the pellets were washed with 70% ice cold ethanol and centrifuge at 1200 rpm for 10 minute in refrigerated centrifuge or at 4 ºC.
15. The pellets were air dried for 10 minutes at room temperature and re-suspended in 1X TE buffer and subsequently stored in -20 ºC until further use.

Qualitative and Quantitative estimation of DNA

DNA extracted from all the identified specimens by Phenol: Chloroform method were subjected to qualitative and quantitative estimation using Nano

drop. 1 □l of DNA was used from each sample for this purpose and accordingly the DNA was diluted for PCR reactions. The absorbance was recorded at 260 and 280 nm.

3.3.2.2 Determination of *Wolbachia* (Bacterial symbiont) infection

The bacterial species in the genus *Wolbachia* are gram negative □-proteo bacteria, the members of family Rickettsiales are most ubiquitous obligate intracellular symbionts present in Arthropods. *Wolbachia* are able to manipulate many biological and ecological aspects of insects including evolution by cytoplasmic incompatibility. Several studies suggested that *Wolbachia* infections can be transferred horizontally between different hosts and has been considered as driving force for mitochondrial-nuclear discordance in many insect systems. *Wolbachia* and mitochondrial DNA transferred maternally and both are linkage disequilibrium. Hence spreading of *Wolbachia* is also results to the spreading of mutations in mitochondrial genes and their frequency. Hence it is a pre-requisite to test for the infection of *Wolbachia* in study insect species before the characterization of mitochondrial genes. The testing for *Wolbachia* infection can be done by *Wolbachia* coat protein genes specific primers. Two pairs of *Wolbachia* genes specific primers *viz.*, wspF and wspR and Wol16SF and Wol16SR were used.

PCR amplification of *Wolbachia* genes in stingless bee species

Material and Equipment used

- PCR Machine (Eppendorf Mastercycler® nexus)
- Refrigerated centrifuge
- Vortex machine
- Pipettors and tips

- Sterile disposable micro centrifuge tubes (1.5 ml and 200 □l capacity)
- Surgical gloves

Reagents used:

- PCR Master mix (2X)
- Primers (Forward and Reverse)
- DNA
- Molecular Biology Grade water

Table 3.3: List of primers used for detection of *Wolbachia* infection

Name of Primer	Primer sequence (5' to 3')	Primer length	Reference
WOL16SF	CGGGGGGAAAAATTTATTGCT	21 bp	O' Neil *et al*. (1992)
WOL16SR	AGCTGTAATACAGAAAGTAAA	21 bp	
wspF	CATACCTATTCGAAGGGATAG	21 bp	Murthy *et al.* (2011)
wspR	AGATTCGAGTGAAACCAATTC	21 bp	

PCR reaction

PCR reaction was carried out in a total volume of 10 □l by using 2 □l of template DNA (40-60ng), 5 □l PCR master mixes (2X), 0.5 □l each of forward and reverse primers. PCR profile had initial denaturation of one cycle at 94 ºC for 2 minutes followed by 35 cycles 94 ºC at 1 minute, 50 ºC for 45 seconds and 72 ºC for 1 minute with one cycle of final extension at 72 ºC for 5 minutes and samples were hold at 10 ^{0}C.

Gel electrophoresis and gel documentation

Material and Equipment used

- Gel electrophoresis Unit
- Gel documentation system
- Microwave oven
- Pippets and tips
- Nitrile gloves

Reagents used

- 1X TAE buffer
- Agarose gel
- 6X Loading dye
- DNA molecular weight marker (100 bp ladder)
- Ethidium bromide staining solution

After the completion of PCR amplifications of all the samples, the success of PCR amplification was tested on 1.5% agarose gel. For staining the PCR fragments, a 2 □l of Ethidium bromide was pre mixed in 1.5% agarose gel just before the solidification and mixed thoroughly. The separation of PCR fragments were undertaken at 160 V for 20 minutes. In addition to the master mix sample, 100 bp molecular ladder/marker was also loaded to determine the PCR product size. After completion of electrophoresis, the images of gels were visualized under UV light illuminator and images were documented in Gel Documentation system (Carestream Gel Logic 212 Pro).

3.3.2.3. Characterization of stingless bee species using mitochondrial Cytochrome oxidase subunit I (COI) gene

Polymerase Chain Reaction (PCR) Amplification

Material and Equipment used

- PCR Machine
- Refrigerated centrifuge
- Vortex machine
- Pipettors and tips
- Sterile disposable micro centrifuge tubes (1.5 ml and 200 □l capacity)
- Surgical gloves

Reagents used are same as described under section 3. 3. 2. 2.

Table 3.4: Primer sequences for mitochondrial COI gene

Name of Primer	Primer sequence (5' to 3')	Primer length	Reference
Lep-F1	ATTCAACCAATCATAAAGATATTGG	25 bp	Hebert *et al.* (2004)
Lep-R1	TAAACTTCTGGATGTCCAAAAAATCA	26 bp	
LCO	GGTCAACAAATCATAAAGATATTGG	25 bp	Folmer (1994)
HCO	TAAACTTCAGGGTGACCAAAAAATCA	26 bp	

PCR Profile and PCR reactions

Separate PCR reaction was carried out for LepF1/LepR1 and LCO/HCO primer pairs. Both primer pairs target the same region of COI gene. For testing the success of PCR amplification, PCR reaction was carried out in a total volume of 10 □l by using 2 □l of template DNA (40-60 ng), 5 □l PCR master mixes (2X), 0.5 □l each of forward and reverse primers.

Temperature profile for amplification of mitochondrial COI gene

Name of Cycle	Temperature (°C)	Duration	Number of Cycles
Initial denaturation	94	2.0 min	01
Denaturation	94	30 seconds	05
Annealing	45	40 seconds	
Extension	72	1.0 min	
Denaturation	94	30 seconds	35
Annealing	51	40 seconds	
Extension	72	1.0 min	
Final Extension	72	10.0 min	01

Gel electrophoresis and gel documentation

PCR reaction was carried out in a total volume of 10 □l by using 2 □l of template DNA (40-60 ng), 5 □l PCR master mixes (2X), 0.5 □l each of forward and reverse primers. The gel electrophoresis and gel documentation were similar as described in section 3. 3. 2. 2.

Bioinformatics Analysis

Sequencing analysis

All the samples were analyzed in sequence analysis software Staden Package (Staden *et al*., 2000). All the sequences were also checked manually within the software for accuracy. The messy 5' and 3' ends of sequences were trimmed for all the sequences. The Single Nucleotide Polymorphism (SNP) if any within different individuals of same species were detected.

BLASTN analysis

All the analyzed sequences were subjected to BLASTN search in online portal of National Centre for Biotechnology Information (NCBI)

(http://www.ncbi.nlm.nih.gov/). The nucleotide blast (nr) option was used for BLASTN search. The species 99-100% homology was considered as similar species.

Nucleotide and protein sequence alignment

Multiple sequence alignment of all the nucleotide sequences was performed in the Clustal W software (Thompson *et al*., 1997). All the sequences were also translated into protein under invertebrate genetic code. Protein sequences were also aligned using Clustal W software. The synonymous and non-synonymous nucleotide substitutions in between sequences were also determined in Clustal W software.

Determination of nucleotide compositions in partial COI gene

The composition of nucleotide in partial COI sequence of all the stingless bee species was determined in Clustal W software. The composition of AT and GC% was also determined at first, second and third positions of codons.

Submission of nucleotide sequences to NCBI

The representative sequence of partial COI gene of each species identified in this study was deposited to NCBI and accession numbers of all the submitted sequences were obtained.

3.4 Comparative study on architectural design of stingless bee hives

For comparative study on architectural design of stingless bee hives. Three types of hive were designed *viz*. Mo: Natural hive (NH); M1: Bamboo hive (BH); M2: Wooden hive (LAH). Detail of hives given at 3.2.4 (construction of hives).

For stingless bee domestication, comparative studies on hive architectural designs were used. The feral colonies were inoculated into the

newly designed hives and placed in previously tagged spots in the shed house and then allowed them to establish freely. External features such as nest entrances were observed and recorded by taking their dimension (Table 3.5 and plate 6) at the time of their honey harvest. Entrance funnels being one of the most diagnostic field traits for distinguishing closely related species. Internal nest structure was viewed during honey harvest. Honey and pollen chamber of *Lepidotrigona arcifera* were measured.

This experiment was conducted to see whether three treatments *i.e*. Natural hive (NH); Bamboo hive (BH); Wooden hive (LAH) were significantly different for successful establishment of stingless bee colonies and also to find out the interaction effect between treatments (factor A) and the height of the hive placement in shed house (factor B).

For statistical analysis, the data were observed and recorded under following parameters for two years w. e. f. 5th March, 2016 to 6th April, 2018 consecutively.

i) Establishment and growth of colony (days).

ii) Size of honey and pollen chamber (cm^3).

iii) Honey production (ml)/year.

iv) Durability and portability of hive (months).

Data were analysed statistically as per statistical software IBM SPSS 16.0 (2007).

Table 3.5: Entrance funnel length (cm) of bee colonies under different hive height placement

Funnel length (cm)	Height (H_0=2ft)									Height (H_1=5ft)								
	HoMo			HoM_1			HoM_2			H_1Mo			H_1M_1			H_1M_2		
2017	NH3	NH6	NH2	BH1	BH6	BH2	LAH3	LAH6	LAH4	NH4	NH1	NH5	BH5	BH4	BH3	LAH1	LAH5	LAH2
	3.6	3.4	3.6	2	2.2	2.1	3	3.4	2.8	4	3.4	3.6	4	2.1	1.8	2.6	4	3
2018	4	3.3	4.2	2.2	1.8	2.3	4.1	4.3	4	4.1	3.1	3.8	3	2.1	1.7	4.4	4.3	4
Total	7.6	6.7	7.8	4.2	4	4.4	7.1	7.7	6.8	8.1	6.5	7.4	7	4.2	3.5	7	8.3	7
Mean	3.8	3.3	3.9	2.1	2	2.2	3.5	3.8	3.4	4.05	3.2	3.7	3.5	2.1	1.75	3.5	4.1	3.5

Legend: Mo=Natural hive (NH); M1=Bamboo hive (BH); M2=Log artificial hive (LAH).

Plate 6a and 6B: Measurement of entrance funnel in LAH at height H1

Plate 6c: Entrance funnel at LAH hive

Plate 6d: Entrance funnel at Ho

Plate 6e: Entrance funnel

Plate 6f: Entrance funnel at LAH

Plate 6g: Entrance funnel

Plate 6h: Entrance funnel at H1

Plate 6: Measurement of entrance funnels of different hives at Ho and H1

3.4.1 Establishment and growth of colony (days)

After inoculation of brood chamber along with involucrum in a new improved hive, in how many days and months it took to establish full strength colony was observed at the time of honey harvest.

3.4.2 Size of honey and pollen chamber (cm^3)

The size of the honey and pollen chamber was recorded by measuring the dimensions of the chamber precisely during honey extraction on completion of one year. In case of those, which were absconded, the data were recorded on the next day from the day of absconding.

3.4.3 Honey production (ml) /year

After the extraction of honey, the quantity ml/hive/year were recorded and subjected to compare. In case of those, which were absconded, the data were recorded on the next day from the day of absconding.

3.4.4 Durability and portability of Hives

The durability of different hives were measured in term of non cracking/ split into aperture, enable easy handling and required less maintenance to know which type of hives last longer without trivial maintenance and care off were the basis of durable assessments in term of months.

3.4.5 Absconded colonies

Under daily routine, regular site inspection were conducted. When less activity of foraging bees were observed in the hives, those were marked and designed as the colonies which were absconding.

3.4.6 Statistical analysis

The average of data collected was statistically analysed for various parameters. The data were then statistically analysed by using two way factor ANOVA.

3.5 To explore the use of stingless bee species for pollination of off- season tomato crop under protected condition.

3.5.1 Experimental Detail

The experiment was carried out at Darinrijo, under Dumporijo circle, Upper Subansiri district of Arunachal Pradesh to use a stingless bee species for pollination of off-season tomato crops under protected condition. Two naturally ventilated poly /greenhouses (100 m^2) were constructed and two stingless bee colonies were introduced in greenhouse (T1). The other greenhouse was kept as control (no pollinator was introduce, T2). Similarly, crop was grown under open condition for treatment T3. Whether introduced stingless bee colonies in greenhouse generated an effective pollination to improve fruit quality and quantity were studied. All agronomical practices were kept same in three different conditions.

3.5.2 Treatment details

i) Greenhouse with stingless bees T1
ii) Greenhouse without stingless bees T2 (control)
iii) Open pollination assigned as T3.

3.5.3 Experimental Design

I) Crop : Tomato.
II) Variety : Megha-1
III) Treatments : 3 (three)

IV) Design of Experiment : Completely Randomized Design

V) Number of Replication : 7 (Seven)

VI) The plot size : 2 m x 1.5 m

VII) Total no. of plot : 21

VIII) Spacing : 50 cm x 25cm

IX) No. of plant/plot : 18 plants

X) Area of a greenhouse : 100 m^2

XI) Type of greenhouse : Naturally ventilated low cost greenhouse

3.5.4 Crop raising and date of hive placement

Tomato seedlings were raised in the nursery. The nursery site was chosen where potato, brinjal, pepper and other solanaceous crops were not grown in the last three years. Seeds were sown on 16th October 2016 and seedlings were later thinned to 7 cm in rows so as to ensure sturdy seedlings.

The seedlings were uprooted with a ball of soil at 4 - 6 leaves stage and one month old seedling were transplanted at evening in well prepared pits in each plot. Pits were added with FYM @ one wheelbarrow of manure in per meter^2 in all three treatments (plate 13). Seedlings were planted at a spacing of 50 (row) × 25 (plant) centimeters at pencil thick and approximately 15 cm tall. Tomato plants were staked individually by central string method, installed for proper growth and fruit setting. All suckers were removed by hand as soon as they appear. When tomato fruit formed, the leaves below their truss were removed. Pruned the plant in the afternoon when the cells were flaccid and elastic to avoid breakage of the stem. Plants were irrigated in the morning and evening. Weeding was manually operated to keep the crops free from weeds for light and nutrient competition. Tomato berries were harvested at seven days interval w.e.f. 8th March 2017.

Date of first flowering on 16th December 2016. Two colonies of stingless bee (*Lepidotrigona arcifera*) were introduced/ placed in greenhouse

on 5th January 2017 at 10% flowering in each replication. The hives were brought into greenhouse at night and hang at the centre of green house at 1 meter height from the ground (Cier *et al*., 2013). Stingless bee a medium colony (Hilario *et al.*, 1999) was placed in greenhouse. Containers with water, mud and cerumen (alternative source for plant resin) were kept on the top of the hives.

3.5.5 Temperature and Humidity

The temperature and relative humidity inside the greenhouse was recorded with a digital thermo-hygrometer. Mean temperature and humidity was worked out from data recorded at 0500 h -1700 h daily during 1st January-28th February 2017 and 2018 (Table 3.6).

3.5.6 Study on the effect of bee pollination

In order to investigate and examine, whether *Lepidotrigona arcifera* (Cockrell 1929) is an effective pollinator of tomato in naturally ventilated greenhouse. An experiment was conducted to find the effect of bee pollination in enhancing the yield, the foraging behaviors of stingless bee were observed within the greenhouse with the following parameters:

3.5.6.1. Foraging rate of the *Lepidotrigona arcifera* on tomato

Five tomato plants were randomly selected from each plot from seven replications in the greenhouse and were tagged and labelled. Foraging rate observations were recorded by counting the number of flowers visited by each bee per minute, using stop watch.

3.5.6.2 Foraging speed

Foraging speed was recorded using stop watch in terms of the time spent by the forager on each flower (in second).

Table 3.6: Daily temperature and humidity during January and February 2016-17 and 2017-18

2017				2018			
Date and month of observation	Temperature (^{0}C)		Mean relative humidity (%)	Date and month of observation	Temperature (^{0}C)		Mean relative humidity (%)
	Max.	Min.			Max.	Min.	
1/1 2017	17.3	14.5	83.0	1/1/2017	17.5	14.0	78.0
2/1 2017	18.6	11.5	79.0	2/1 /2017	17.3	11.4	76.1
3/1 2017	19.6	12.0	78.0	3/1/2017	18.2	11.9	76.0
4/1 2017	17.3	11.0	81.5	4/1 /2017	17.9	12.0	78.0
5/1 2017	17.5	11.4	78.0	5/1 /2017	22.3	12.1	73.0
6/1 2017	18.9	11.8	78.3	6/1 /2017	17.5	11.0	77.0
7/1 2017	16.5	10.6	84.0	7/1 /2017	21.6	14.4	78.5
8/1 2017	19.7	13.0	80.0	8/1 /2017	21.8	12.5	66.5
9/1 2017	22.0	13.4	81.0	9/1 /2017	17.5	10.0	82.0
10/1 2017	16.9	11.9	84.0	10/1 /2017	17.6	11.6	76.0
11/1 2017	20.4	13.0	76.7	11/1 /2017	19.0	13.2	77.0
12/1 2017	18.8	12.9	81.2	12/1 /2017	16.8	11.0	77.0
13/1 2017	19.9	11.7	79.4	13/1 /2017	23.6	11.5	74.0
14/1 2017	17.8	12.1	79.0	14/1 /2017	18.9	12.0	73.0
15/1 2017	16.8	11.9	85.0	15/1 /2017	16.4	10.0	82.0
16/1 2017	18.8	12.8	74.0	16/1 /2017	25.8	13.0	65.5
17/1 2017	17.9	12.0	78.5	17/1 /2017	18.1	12.0	78.0
18/1 2017	18.7	11.3	80.0	18/1 /2017	18.0	11.9	86.0
19/1 2017	21.8	12.6	73.0	19/1 /2017	17.9	12.0	79.0
20/1 2017	21.5	12.1	72.0	20/1 /2017	17.7	12.6	77.4
21/1 2017	17.7	11.5	79.5	21/1 /2017	21.6	13.0	77.0
22/1 2017	18.8	12.0	74.1	22/1 /2017	25.3	14.3	64.5
23/1 2017	20.0	12.3	71.0	23/1 /2017	19.9	12.0	75.0
24/1 2017	20.1	11.0	76.7	24/1 /2017	22.7	13.1	72.0
25/1 2017	21.7	10.0	78.5	25/1 /2017	18.6	12.8	80.5
26/1 2017	19.7	12,9	74.3	26/1 /2017	19.2	11.8	78.9
27/1 2017	17.7	12.0	83.5	27/1 /2017	18.1	12,8	85.0
28/1 2017	21.8	12.4	72.0	28/1 /2017	22.6	11.9	72
29/1 2017	16.4	11.5	86.0	29/1 /2017	19.1	12.8	73.0
30/1 2017	15.6	12.2	87.0	30/1 /2017	16.2	11.0	86.0
31/1 2017	17.9	12.5	75.2	31/1/ /2017	18.9	12.4	83.0
1/2/2018	17.5	14.0	78.0	1/2//2018	17.7	14.5	80.0
2/2/2018	17.3	11.4	76.1	2/2//2018	18.6	11.5	78.0
3/2/2018	18.2	11.9	76.0	3/2//2018	19.6	12.0	77.0
4/2/2018	17.9	12.0	78.0	4/2//2018	18.3	11.0	78.5
5/2/2018	22.3	12.1	73.0	5/2//2018	22.5	11.4	75.0
6/2/2018	23.5	12.6	74.0	6/2//2018	22.9	11.8	74.3

7/2/2018	21.6	14.4	78.5	7/2//2018	22.5	10.6	76.0
8/2/2018	21.8	12.5	66.5	8/2//2018	19.7	13.0	80.0
9/2/2018	17.5	10.0	82.0	9/2//2018	22.0	13.4	81.0
10/2/2018	17.6	11.6	76.0	10/2//2018	17.9	11.9	78.0
11/2/2018	19.0	13.2	77.0	11/2//2018	20.4	13.0	76.7
12/2/2018	16.8	11.0	77.0	12/2//2018	18.8	12.9	81.2
13/2/2018	23.6	11.5	74.0	13/2//2018	19.9	11.7	79.4
14/2/2018	29.5	13.0	60.5	14/2//2018	18.8	12.1	77.0
15/2/2018	30.3	13.1	59.5	15/2//2018	23.8	11.9	74.0
16/2/2018	22.5	12.5	70.0	16/2//2018	18.8	12.8	75.0
17/2/2018	18.1	12.0	78.0	17/2//2018	17.9	12.0	78.5
18/2/2018	18.0	11.9	86.0	18/2//2018	18.7	11.3	80.0
19/2/2018	17.9	12.0	79.0	19/2//2018	21.8	12.6	73.0
20/2/2018	17.7	12.6	77.4	20/2//2018	21.5	12.1	72.0
21/2/2018	21.6	13.0	77.0	21/2//2018	17.7	11.5	79.5
22/2/2018	18.6	11.7	80.0	22/2//2018	18.8	12.0	74.1
23/2/2018	19.9	12.0	75.0	23/2//2018	20.0	12.3	71.0
24/2/2018	22.7	13.1	72.0	24/2//2018	23.1	11.0	76.0
25/2/2018	18.6	12.8	80.5	25/2//2018	21.7	10.0	77.5
26/2/2018	19.2	11.8	78.9	26/2/2018	19.7	12,9	74.3
27/2/2018	18.1	12,8	81.0	27/2//2018	18.7	12.0	80.5
28/2/2018	22.6	11.9	72	28/2/2018	20.8	12.4	73.0

3.5.6.3 Diurnal abundance of *Lepidotrigona arcifera*

Diurnal abundance of the *Lepidotrigona arcifera* visiting tomato flowers at different hours of the day was recorded *i.e*. No. of bees/m^2/10 minute at time interval of one hour from 0600 h to 1700 h during 6th January – 31st January, 2017 and 2018 using the stop watch.

3.5.6.4. Initiation and cessation time of the *Lepidotrigona arcifera*

Within the greenhouse, colony performance of stingless bee was observed. To determine initiation time of *Lepidotrigona arcifera*, the point of the time at which do they come out from the hives in the morning for foraging was recorded. The initiation time of the bee was observed from 0500 h. It was determined by visual observation. The cessation time, by which foragers return to hive completely in the evening, was observed up to 1700 h.

3.5.6.5 Loose pollen grains

The number of the loose pollen grains adhering to the body of stingless bee foragers was determined by capturing the foraging bees and killing immediately in measured quantity (2 ml) 70% alcohol. Nectar foragers were captured by means of forceps. The hind legs of the pollen foragers were amputated before killing in alcohol.

3.5.6.6 Pollination Index

The pollination index of stingless bees for the following attributes was calculated using the formula as per Bohart and Nye (1960).

P.E. = (FR + FS + LPG) X RA

Where FR is the Foraging Rate

FS is the Foraging Speed

LPG is the Loose Pollen Grain

RA is the Relative Abundance

3.5.7 Monitoring of pollination by inspecting flowers for bruising

When stingless bee landed in tomato flowers upon which carefully observation were recorded whether bees perform sonication (buzz pollination) or not. Any bite sign left on the flower cone by the bee were recorded instantly. There was no observation of any sign of bite bruises on the tomato flower cones of tagged plants.

3.5.8 Tomato Berry Harvest schedule and yield

Tomato berries were harvested at seven days interval w.e.f. 8th March 2017. The fully ripened tomato berries were harvested from 5 tagged plants from seven plots (replication).

3.5.8.1 Yield per plant

The tender fruit harvested at different dates from tagged plant are weighed (kg) and recorded till the plant senescence completely.

3.5.8.2 Yield per plot

Yield per plot was determined by multiplying the average yield per plant with the number of plants per plot. The yield per plot was expressed in kilogram (kg).

3.5.8. 3 Yield per hectare

The yield per hectare has been calculated by multiplying the yield of one plant with the number of plants in one hectare. The data obtained have been expressed in quintals and data are subjected to statistical analysis.

3.5.9 Statistical analysis

All the data collected were subjected to statistical analysis by using statistical software IBM SPSS 16.0 (2007). Data obtained was analyzed by using one-way Analysis of Variance (ANOVA).

3.5.10 Unusual observation during experimental period

Two colonies of stingless bee were introduced into the greenhouse at 10% flowering. The colonies collapsed after 93 days from date of placement. After few days of initiation of activity in the hive placed in the greenhouse, the

worker bees started grouping at polythene sheet without making flight on the flowers and later they were eaten by ants. It was observed that bees flow from the hive; fly away through naturally ventilated space of the greenhouse. The sign of the collapsed hives were externally visible as the entrance funnel were shrank and closed. Secondly honey was leaking through crevices from absconding hives. This behaviour still needs to be investigated in *Lepidotrigona arcifera* for utilizing these bees for pollination under protected conditions.

CHAPTER - IV

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

The present investigations entitled, "Studies on stingless bees and their utilization in tomato (*Lycopersicon esculentum*) Bailey production" were conducted during the year 2016-18. The results obtained during the course of investigation pertaining to various objectives and their results have been discussed in this chapter under following heads:

4.1 Collection and relocation of wild stingless bee colony from having different altitudes

4.1.1 Collection of colonies in Arunachal Pradesh and Nagaland

Thirty four colonies of wild stingless bees were collected during 2016-18 by the whole colony collection technique. All the colonies were collected from their natural habitat and were relocated into artificial hives successfully as per Kwapong *et al*. (2010) (Table 4.1, 4.2 and 4.3). They deduced the training manual regarding the collection of feral whole colony of stingless bee from their natural habitat and relocated them at meliponiaries.

Similarly, Singh (2016) observed that Naga beekeepers collect the feral colonies from wild trees and cut out the portion of hives by saw and transported them to apiary in the evening and re-established in their houses.

4.2 Construction of different hives

Among three types of hive designed, *viz*. Mo: Natural hive (NH); M1: Bamboo hive (BH); M2: Wooden hive (LAH) for stingless bee domestication (Table 4.4). Singh (2016) has reported that the beekeeper of Naga tribes of Nagaland, India used traditional hives, log hives having 43-123 cm long and

11-38 cm in diameter at different districts of the Nagaland for *Tetragonula iridipennis* and *T. laviceps.*

Wooden hive (LAH) having side open and top open type was found best followed by Natural hives. Bamboo hives and LAH split types were worst in term of duration of bee stays in the hives and honey production with the scope of easy handling for monitoring the internal growth of the colonies without much disturbance or injury to developing colony in the hives (Table 4.5).

4.3 Hive height placement at experimental site

Hives placed at two different heights *i.e.* 2 and 5 feet above the ground in shade houses at 2 m apart were not significantly different among three different hive types in relation to colony establishment and honey production (Table 4.6). However, the hive height placement was affected on durability of the hives.

Table 4.1: Collection of feral stingless bee colonies from Anurachal Pradesh in 2016-17

Sl. No.	Date of colony collection	Name of hive/colony	Location and altitudes	Circle/District and State	Name of collector (s)
1	25/3/2016	NH 2	Uli village area and 1000 m	Gusar circle, Upper Subansiri District ,Arunachal Pradesh	ShriTakak Riddi and Shri T. Uli
2	5/3/2016	NH1	Bui village area and650 m	Gusar circle, Upper Subansiri District ,Arunachal Pradesh	Shri Malar Bui and ShriTakak Riddi
3	7/3/2016	NH3	Takte Bui and 650 m	Gusar circle, Upper Subansiri District ,Arunachal Pradesh	Shri Takak Riddi
4	13/3/2016	NH4	Bui village area and 680 m	Gusar circle, Upper Subansiri District ,Arunachal Pradesh	Shri Malar Bui and Shri Takak Riddi
5	20/3/2016	NH5	Bui village area and 655 m	Gusar circle, Upper Subansiri District ,Arunachal Pradesh	Shri Malar Bui and Shri Takak Riddi
6	8/3/2016	NH6	Denri, Nguki village and 815 m	Gusar circle, Upper Subansiri District ,Arunachal Pradesh	Shri Malar Bui and Shri Takak Riddi
7	17/3/16	LAH1	Bator village and 652 m	Gusar circle, Upper Subansiri District ,Arunachal Pradesh	Shri Takak Riddi and Mr. Tashi Bui
8	5/3/2016	LAH3	Bulo Village area and 950 m	Gusar circle, Upper Subansiri District ,Arunachal Pradesh	Shri Malar Bui and ShriTakak Riddi
9	11/3/16	LAH4	Riddi village and 516m	Gusar circle, Upper Subansiri District ,Arunachal Pradesh	Shri Malar Bui and ShriTakak Riddi
10	15/3/16	LAH2	Segi area and 760 m	Gusar circle, Upper Subansiri District ,Arunachal Pradesh	Shri Malar Bui and ShriTakak Riddi
11	7/3/2016	LAH5	Takte Bui and 650 m	Gusar circle, Upper Subansiri District ,Arunachal Pradesh	Shri Takak Riddi and Mr. Tashi Bui
12	17/3/16	LAH6	Bator village and 650 m	Gusar circle, Upper Subansiri District ,Arunachal Pradesh	Shri Takak Riddi
13	5/3/2016	BH1	Bulo Village area and 945 m	Gusar circle, Upper Subansiri District ,Arunachal Pradesh	Shri Malar Bui and Shri Takak Riddi

14	13/3/2016	BH2	Bui village area and 649 m	Gusar circle, Upper Subansiri District ,Arunachal Pradesh	Shri Malar Bui and Shri Takak Riddi
15	8/3/2016	BH3	Denri,Nguki village and 813 m	Gusar circle, Upper Subansiri District ,Arunachal Pradesh	Shri Malar Bui and Shri Takak Riddi
16	6/3/2016	BH4	Segi area and 800 m	Gusar circle, Upper Subansiri District ,Arunachal Pradesh	Shri Malar Bui andShriTakakRiddi
17	9/3/2016	BH5	Kumdi,Bui village and 555 m	Gusar circle, Upper Subansiri District ,Arunachal Pradesh	Shri Malar Bui and Shri Takak Riddi
18	8/3/2016	BH6	Selok rijo Uli village and760 m	Gusar circle, Upper Subansiri District ,Arunachal Pradesh	Shri Malar Bui and Shri Takak Riddi

Legend: i) Mo=Natural hive (NH), M_1 =Bamboo hive (BH) and M_2 =Log artificial hive (LAH).

Table 4.2: Collection of feral stingless bee colonies from Anurachal Pradesh in 2017-18

Sl. No.	Date of colony collection	Name of hive/colony	Location and altitudes	Circle/District and State	Name of collector (s)
1	22/3/2017	NH 2	Uli village area and 1000 m	Gusar circle, Upper Subansiri District, Arunachal Pradesh	Shri Takak Riddi and Shri T. Uli
2	21/3/2017	NH3	Takte Bui and 659 m	Gusar circle, Upper Subansiri District, Arunachal Pradesh	Shri Takak Riddi and Mr. Durga
3	21/3/2017	NH6	Denri, Nguki village and 810 m	Gusar circle, Upper Subansiri District, Arunachal Pradesh	ShriTakak Riddi and Durga
4	3/4/17	LAH1	Bator village and 652 m	Gusar circle, Upper Subansiri District ,Arunachal Pradesh	Shri Takak Riddi and Mr. Nyope Bator
5	26/3/2017	LAH3	Bulo Village area and 950 m	Gusar circle, Upper Subansiri District, Arunachal Pradesh	Shri Kogom Bulo and ShriTakak Riddi
6	10/3/17	LAH4	Riddi village and 516 m	Gusar circle, Upper Subansiri District, Arunachal Pradesh	Shri Malar Bui and Shri Takak Riddi
7	4/4/17	LAH2	Segi area and 760 m	Gusar circle, Upper Subansiri District, Arunachal Pradesh	Shri Pentum Bui and Shri Takak Riddi
8	23/3/2017	BH1	Bulo Village area and 949 m	Gusar circle, Upper Subansiri District, Arunachal Pradesh	Shri Kogom Bulo and Shri Takak Riddi
9	6/4/2017	BH2	Bui village area and 649 m	Gusar circle, Upper Subansiri District, Arunachal Pradesh	Shri Tayom Bui and Shri Takak Riddi
10	24/3/2017	BH3	Denri, Nguki village and 813m	Gusar circle, Upper Subansiri District, Arunachal Pradesh	Shri Tabe Nguki and Shri Takak Riddi
11	22/3/2017	BH4	Segi area and 800 m	Gusar circle, Upper Subansiri District, Arunachal Pradesh	Shri Durga and Shri Takak Riddi
12	25/3/2017	BH5	Kumdi, Bui village and 555 m	Gusar circle, Upper Subansiri District ,Arunachal Pradesh	Shri Pepak Bui and Shri Takak Riddi
13	24/3/2017	BH6	Selok rijo, Uli village and 766 m	Gusar circle, Upper Subansiri District, Arunachal Pradesh	Shri Malar Bui and Shri Takak Riddi

Table 4.3: Collection of feral stingless bee colonies from Nagaland

Sl. No.	Name of colony	Characteristic of colony	Location & altitude	Circle/District and State	Remark(s)
1.	White SASRD	Arboreal type and has white in colour entrance tube	SASRD & 452m	Medziphema/Dimapur, Nagaland	Internal colony structure, honey production and their uses in pollination were not investigated and observed from the samples collected in Nagaland. Only their identification by traditional taxonomy and molecular analysis were done.
2.	Black SASRD	Arboreal in nesting habit and has black colour entrance gate, devoid of funnel shape.	SASRD& 452m	Medziphema/Dimapur, Nagaland	
3.	Underground SASRD	Nest in underground and has black entrance tube.	SASRD& 452m	Medziphema/Dimapur ,Nagaland	

*SASRD= School of Agricultural Sciences and Rural Development

Table 4.4: Different types of hives designed for *Lepidotrigona arcifera*

Sl. No.	Type of Hive	2017		2018	
		Shape of hive	Name of hive	Shape of hive	Name of hive
1	M_0	NH1	Irregular	NH1	Irregular
		NH2	Irregular	NH2	Irregular
		NH3	Irregular	NH3	Irregular
		NH4	Irregular	NH4	Irregular
		NH5	Irregular	NH5	Irregular
		NH6	Irregular	NH6	Irregular
2	M1	BH1	Cylinder	BH1	Cylinder
		BH2	Cylinder	BH2	Cylinder
		BH3	Cylinder	BH3	Cylinder
		BH4	Cylinder	BH4	Cylinder
		BH5	Cylinder	BH5	Cylinder
		BH6	Cylinder	BH6	Cylinder
3	M2	LAH1	Cuboid	LAH1	Cuboid
		LAH2	Cuboid	LAH2	Cuboid
		LAH3	Cuboid	LAH3	Cuboid
		LAH4	Cuboid	LAH4	Cuboid
		LAH5	Cuboid	LAH5	Cuboid

Legend: Mo=Natural hive (NH); M1=Bamboo hive (BH); M2=Log artificial hive (LAH)

Table 4.5: Effect of different hive designs on domestication of *Lepidotrigona arcifera*

Sl. No.	Type of Hive	2017		2018	
		Number of days bees stayed	Honey (ml)	Number of days bees stayed	Honey (ml)
1	M_0	300.16	253.68	290.33	335.16
2	M1	131.16	0.367	40.00	0.217
3	M2	254.00	200.28	366.16	615.00

Legend: Mo=Natural hive (NH); M1=Bamboo hive (BH); M2=Log artificial hive (LAH)

Table 4.6: Effect of different heights on domestication of *Lepidotrigona arcifera*

Sl. No.	Type of hive	Height (ft)	2017		2018	
			Honey (ml)	Durability (months)	Honey (ml)	Durability (months)
1	M_0	H_0	253.00	9.88	335	10.38
		H1	241.25	7.60	404.52	8.76
2	M1	H_0	0.367	5.43	0.217	5.71
		H1	106.68	8.9	0.433	10.23
3	M2	H_0	200.28	12.11	615.00	10.81
		H1	211.16	12.16	221.02	12.33
$CD=_{0.05}$			NS	NS	NS	NS

4.4 Identification of stingless bee wild species through traditional taxonomy and using molecular taxonomy / DNA barcoding

4.4.1 Specimens from Arunachal Pradesh

During the present studies, the collected stingless bee species were identified based on the taxonomical keys developed by Rasmussen (2013). Their morphological characters (Table 4.7), *viz.* colour of head, presence of thoracic vittae, color and orientation of antennae, overall colour pattern of scutum and abdomen were conformity with the report of Rasmussen (2013). Present observation revealed that head and thorax of the specimen are with dense tessellation. Dense plumose hair present at the mesoscutum, hind wing with six hamuli (Plate 7). It was also confirmed that all the stingless bee colonies collected from the Arunachal Pradesh was *Lepidotrigona arcifera* (Cockrell 1929) and three species/colonies collected from SASRD Nagaland were identified as *Tetragonula* sp.I (Black SASRD and white SASRD) and *Tetragonula* sp.II (underground SASRD).

Table 4.7: Morphological features of stingless bee species of Arunachal Pradesh and Nagaland

Species/ Characters	***Lepidotrigona arcifera***	***Tertragonula* sp I (white)**	***Tertragonula* sp I (black)**	***Tetragonula* sp II**
Head and thorax	Dense tessellation present. Densely plumose hair at the margin of mesoscutum	Dark head and thorax. Mesoscutum with glabrous interspace. No setose around and ocelli and mesoscutum.	Dark head and thorax. Mesoscutum with glabrous interspace. No setose around and ocelli and mesoscutum	Dark head and mesoscutum

Plate 7a: Head and thorax

Plate 7b : Antennae

Plate 7c: Abdomen

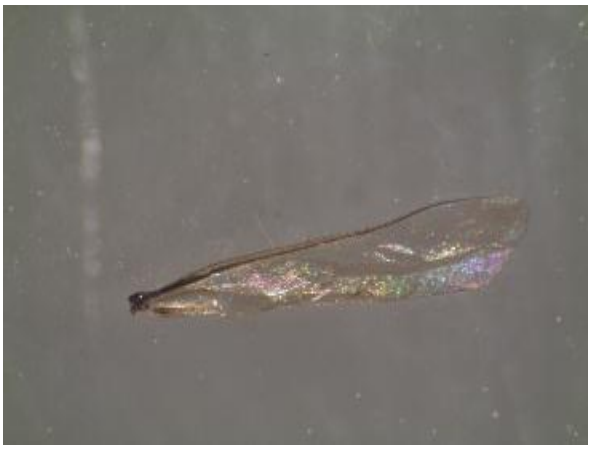


Plate 7d: Hind wing

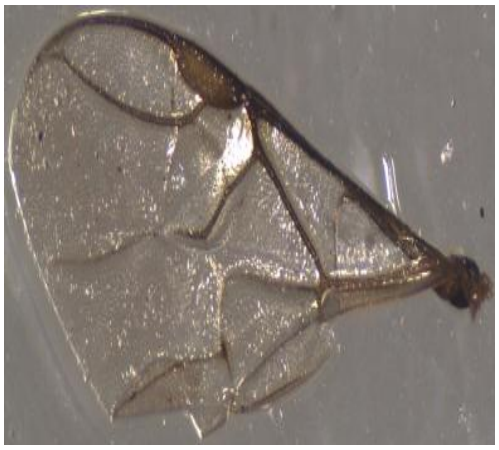


Plate 7e: Fore wing

Plate 7f: Over view

Plate 7 : *Lepidotrigona arcifera* (Cockrell 1929); Stingless bee of Arunachal Pradesh

Antennae	Black ventrally	Reddish brown ventrally	Reddish brown ventrally	Dark brown ventrally
Abdomen	Dark band present. Yellow coloration present at propodeum.	Light brown, three number of band present	Uniform light brown	Dark brown, three lines with illuminated small dots present.
No. of hamuli	6	6	5	6
Colour of entrance funnel	White/ light brown	White/ light brown	Black Stout funnel	Black Stout funnel

Colonies collected from Arunachal Pradesh are identified as *Lepidotrigona arcifera* (Cockrell 1929). It is holotype and the voucher specimens for all these colonies of *Lepidotrigona arcifera* have been deposited and available with Dr. Shashidhar Viraktamath, Emeritus Scientist, Department of Entomology, University of Agricultural Sciences, GKVK, Bengaluru-560065, Karnataka. With respect to molecular analysis of all these colonies, the nucleotide length of final good quality DNA sequence of partial COI gene was 575 bp which encoded 191 amino acids. The barcode for all these colonies of *Lepidotrigona arcifera* collected from Arunachal Pradesh have been developed and deposited to the international GenBank NCBI (National Centre for Biotechnology Information) vide Accession Numbers: MH347232 to MH347236 (Table 4.8). Among five colonies, the DNA sequences of three colonies (NH2, NH5 and LAH6) were 100% identical. However, colony NH4 has three mutation (Nucleotide polymorphism) and colony LAH5 has two mutations similar except Colony NH4 has three mutation. Even with these three and two mutations within in sequences of

colonies collected from Arunachal Pradesh, the overall identity at taxonomic and molecular level did not changed and all these colonies were belonged to *Lepidotrigona arcifera* because all these mutations were silent mutations. It is worth to note that, the present study has generated a molecular data for the first time for *Lepidotrigona arcifera*. The molecular data/DNA barcodes generated from this study would be certainly useful to the other researchers working on *Lepidotrigona arcifera* across the globe. The DNA data generated from present study has been deposited in the international GenBank which can be used by other researchers for confirmation of molecular identity of their specimens (Plate 8). Nucleotide sequence of the specimens has been submitted to NCBI, USA and acquired their accession Numbers.

This result is conformity with the findings reported by Rasmussen (2013) who reported that *Lepidotrigona arcifera* is holotype specimen and is identified with diagnostic dense tessellation on head and thorax and densely plumose hairs on the margin of mesoscutum.

Similar finding was reported by Vijayakumar (2014) that worker bee of *Lepidotrigona arcifera* had usually the mesonotum usually enclosed by a border of short thick scale like or tomentose yellowish to whitish hairs.

Table 4.8: Molecular characterization of stingless bee of Arunachal Pradesh

Sl. No.	Taxonomic Identification	Collection Code	Nucleotide Length (bp)	Protein Length	NCBI GenBank Accession Numbers	Molecular results
1	*Lepidotrigona arcifera*	NH 2 1655/GTB204	575bp	191	MH347232	No Mutation
2	*Lepidotrigona arcifera*	NH 4 1656/GTB208	575bp	191	MH347233	3 mutations
3	*Lepidotrigona arcifera*	NH 5 1657/GTB217	575bp	191	MH347234	No Mutation
4	*Lepidotrigona arcifera*	LAH 5 1661/GTB228	575bp	191	MH347235	2 mutations
5	*Lepidotrigona arcifera*	LAH 6 1662/GTB229	575bp	191	MH347236	-

4.4.2 Specimens from Nagaland

4.4.2.1 Morphological characters of stingless bees

Three colonies were collected from Nagaland, *viz*. Black SASRD, white SASRD and underground SASRD. In this study a distinct variations in number of hamuli present in hind wings in different specimens were detected. Abdominal variation also observed in white SARSD, black SASRD, and underground SASRD. Difference in structure and color of the entrance funnel was observed in three colonies prominently.

White SASRD and black SASRD stingless bee colonies were identified as *Tetragonula* sp.I (Table 4.7 and Plate 9-10) based on the morphological characters. In present investigations it was revealed that all the specimens collected from Nagaland were having head and thorax with dark coloration. Antennae are ventrally reddish brown in all the specimens. Their mesoscutum were having broad glabrous interspaces. However, underground SASRD stingless bee specimen is identified as *Tetragonula* sp.II (Table 4.7 and Plate11) due to distinct variations in number of hamuli present in hind wings in different specimens, abdominal variation in infuscation, body colour and difference in structure and colour of the entrance funnel (Plate 12). This distinctness was further supported by molecular analysed result. Sequences of white SASRD and black SASRD are 100% identical and hence same species (*Tetragonula* sp. I).

The good quality DNA sequences were successfully obtained for the representative specimens of these three colonies. The nucleotide sequence length for colony; White SASRD and Balck SASRD were 666 bp (221 amino acids) and 677 bp (225 amino acids), respectively (Table 4.9). In absence of matching molecular data in international GenBank, the molecular identity for White SASRD and Black SASRD could not be established. The identity of

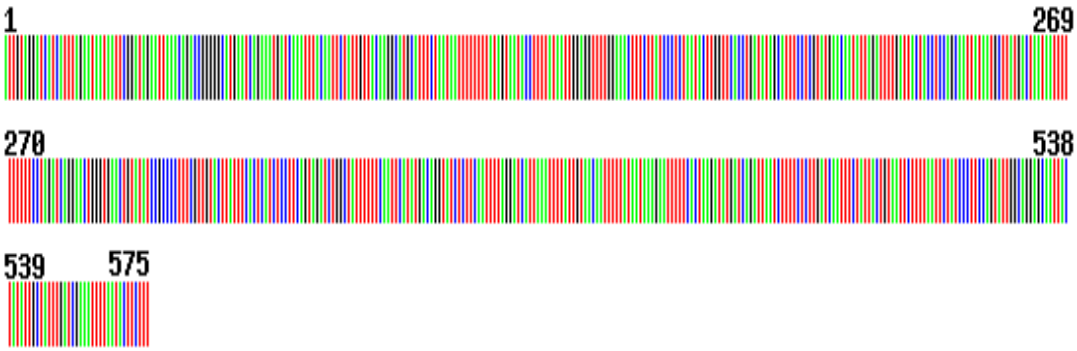


Plate 8a: Barcode of *Lepidotrigona arcifera*

>ATTGTAGGATCATCATTTAGAATAATAATTCGGATAGAATTAAAC
AGACCGGGGGCATGAATCAGAAATGATCAAATTTACAATTCTATT
GTTACAAGGCATGCATTTCTAATAATTTTTTTTATAGTAATACCTTT
TATAATTGGAGGTTTTGGAAACTTTCTTATCCCTCTAATACTTGGT
TCACCTGATATAGCATTTCCTCGTATGAACAATATTAGATTTTGAT
TACTACCTCCAGCAATTATAATGCTTATGACTAATAATTTTTTTTTT
CCTAGATCAGGAACTGGGTGAACTGTATATCCGCCCCTTTCGTTG
TACTTATTTCACTCATCTCCTTCAGTAGATCTGGCTATTTTTTCAA
TTCATATGACAGGTATCTCTTCAATTTTAGGATCATTAAATTTTATT
GTAACAATTTTTATAATAAAGAATTTTTCACTAAGATATGATCAGA
TTAATCTTTTCTCTTGATCAATTTCTATTACTGTAATTCTTTTAATT
CTATCCCTTCCAGTATTGGCAGGAGCAATTACTATATTGCTATTTG
ATCGAAATTTTAATACTTCTTT

Plate 8b: Nucleotide sequence of *Lepidotrigona arcifera*

IVGSSFSMMIRMELNSPGAWISNDQIYNSIVTSHAFLMIFFMVMPFMIG
GFGNFLIPLMLGSPDMAFPRMNNISFWLLPPAIMMLMTNNFFFPSSGT
GWTVYPPLSLYLFHSSPSVDLAIFSIHMTGISSILGSLNFIVTIFMMKNFS
LSYDQINLFSWSISITVILLILSLPVLAGAITMLLFDRNFNTS

Plate 8c: Protein sequence (nucleic acid) of *Lepidotrigona arcifera Smith*

Plate 8: Nucleotide sequence and barcode of *Lepidotrigona arcifera*

Plate 9a: Mouth Part

Plate 9b: Head & thorax

Plate 9c: Abdomen

Plate 9d: Over view

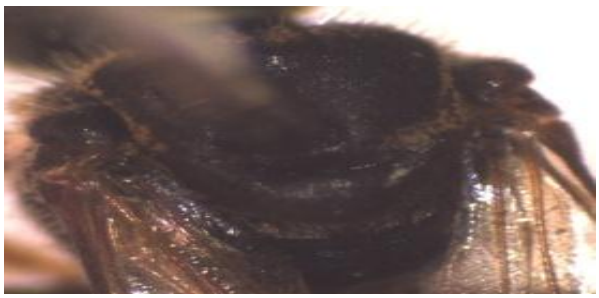


Plate 9e: Thorax

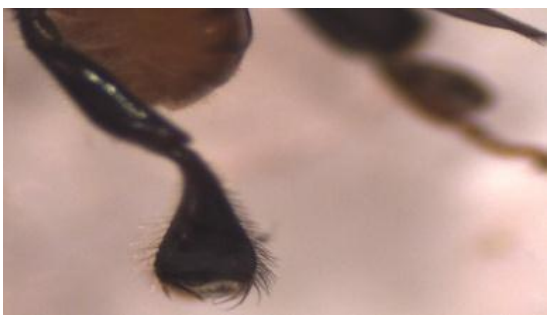


Plate 9f: Hind leg

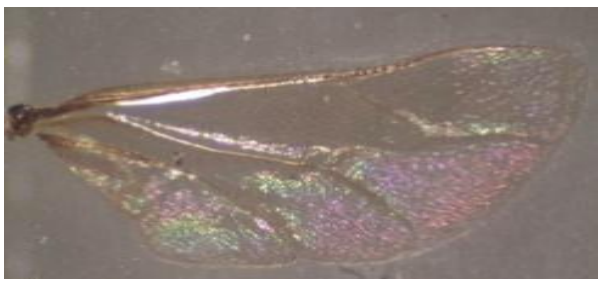


Plate 9g: Hind wing

Plate 9h: Fore wing

Plate 9: White SASRD, (*Tetragonula* sp. I) Stingless bee of SASRD, Nagaland

Plate 10a: Antennae

Plate 10b: Head and thorax

Plate 10c: Abdomen

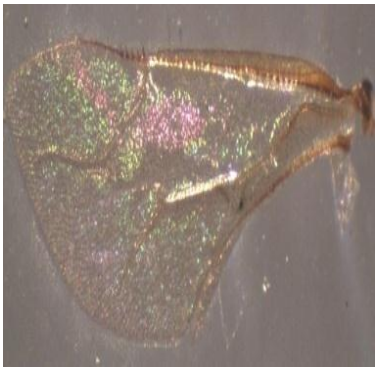


Plate 10d: Hind wing

Plate 10e: Fore wing

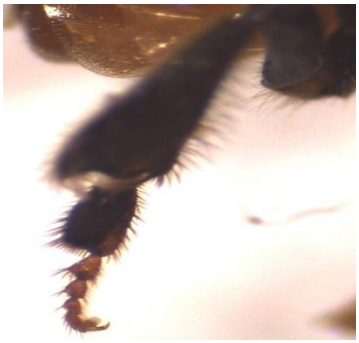


Plate 10f: Hind leg

Plate 10g: Overview

Plate 10: Black SASRD, (*Tetragonula* sp. I) stingless bee of SASRD, Nagaland

Plate 11a: Over view

Plate 11b: Thorax

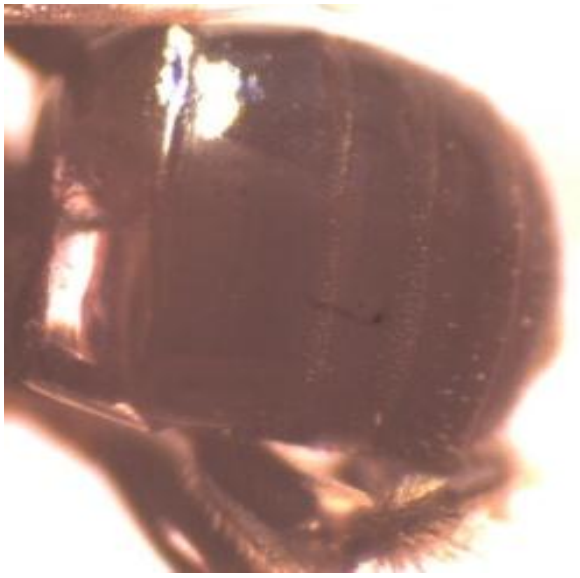


Plate 11c: Abdomen

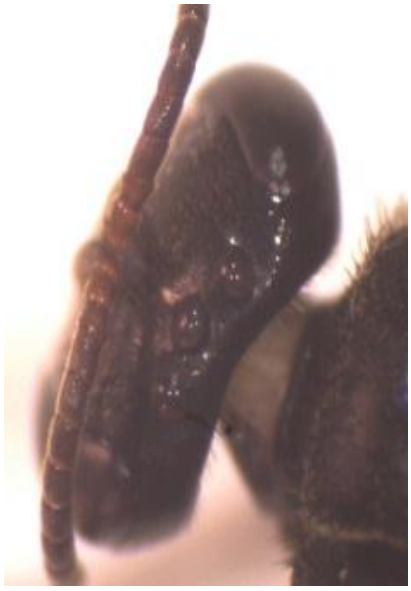


Plate 11d: Head

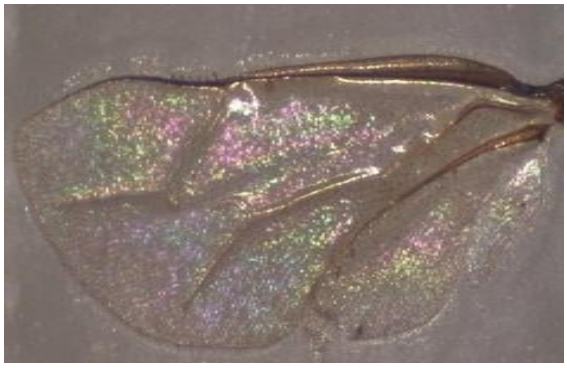


Plate 11e: Hind wing

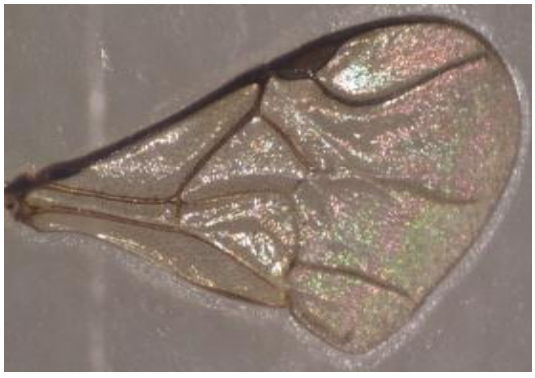


Plate 11f: Fore wing

Plate 11: Underground bee of SASRD, *(Tetragonula* sp. II) stingless bee of SASRD, Nagaland

Plate 12a: Entrance funnel of Black SASRD

Plate 12b: Entrance funnel of underground SASRD

Plate 12: Entrance funnel of stingless bee of Nagaland

these two colonies was established as *Tetragonula* sp. I. The DNA sequences of both the colonies were 100% identical. In case of colony referred as Underground SASRD collected from Nagaland, the nucleotide length of this colony was 677 bp which encoded into 225 amino acids. The multiple sequences alignment between White SASRD, Black SASRD and Underground SARSRD revealed that, there was 10% variation at molecular level in case of Underground SASRD. That amount of genetic variation always exists in between species. Taxonomically, the specimens of Underground SASRD colony were unambiguously identified as *Tetragonula* sp.II. At morphological and molecular level, Underground SASRD was significantly different from the White and Black SASRD colonies. The DNA barcodes of White SASRD, Black SASRD and Underground SASRD have been successfully submitted to the GenBank (NCBI) vide accession Numbers; MH347237, MH347237 and MH347237, respectively (Table 4.9 and Plate 14). Again it is interesting to note that, the DNA barcodes (Plate 13) for *Tetragonula* sp.I and *Tetragonula* sp.II have been generated for the first time in present study which would certainly be helpful to the other researchers working on these species for establishment of molecular identity of these species.

The present investigation revealed that all the specimens collected from Nagaland were having head and thorax with dark coloration but underground SASRD specimen has body black in colour while other two are comparatively lighter in body colour. It has also been noticed that white SASRD and underground SASRD have 6 numbers of hamuli in their hind wings. Whereas black SASRD has 5 numbers of Hamuli. Abdomen of white SASRD brownish in color with three distinct bands and black SASRD specimens having light brown colour uniformly. Whereas underground SASRD has dark brown with three distinct bands. In black SASRD, entrance gate bounded by projecting short and stout funnel in dark brown to black in colour (Plate13). White SASRD colony with white to light brown funnel entrance gate of 3-4 cm in

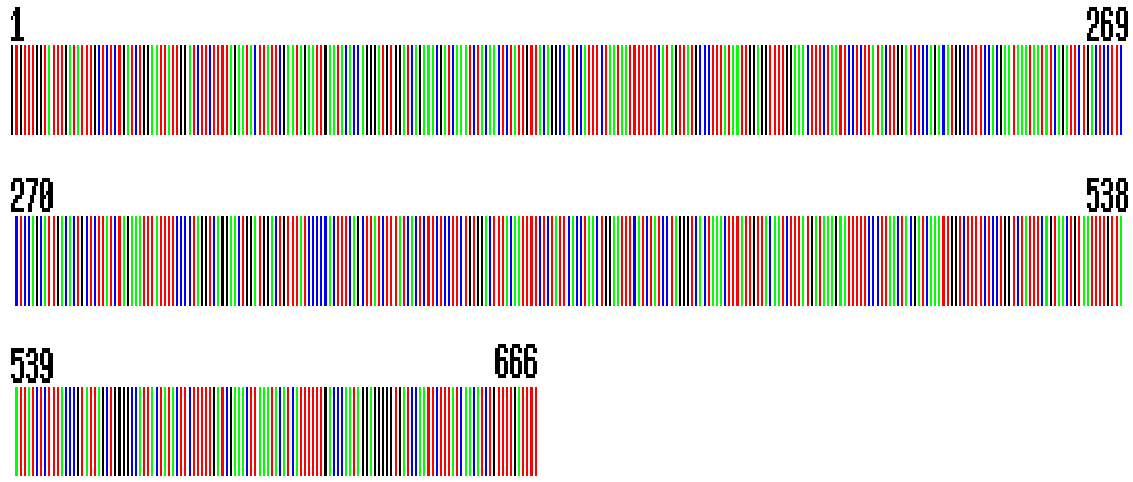


Plate 13a: Barcode of underground stingless bee, *Tetragonula* spp.

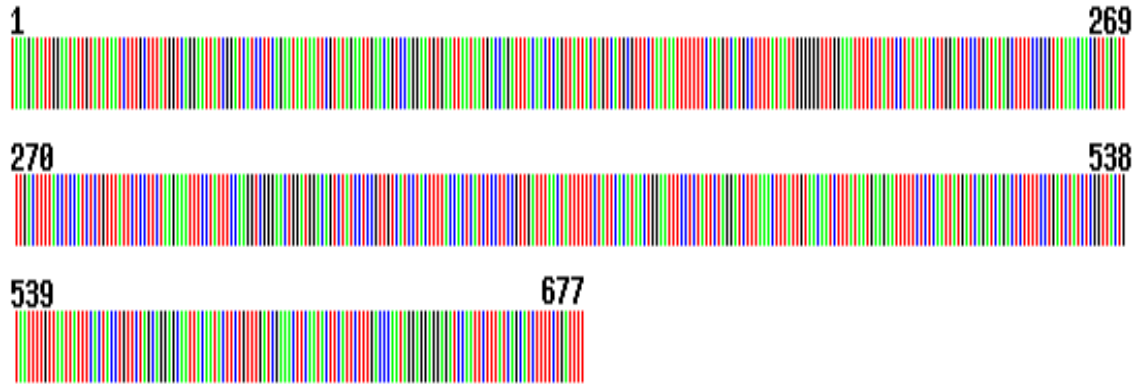


Plate 13b: Barcode of black stingless bee, *Tetragonula* spp.

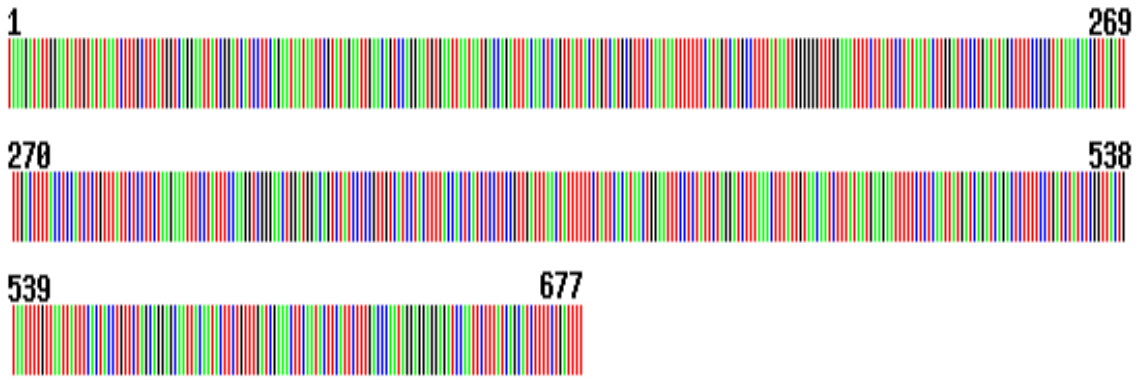


Plate 13c: Barcode of white stingless bee, *Tetragonula* spp.

Plate 13: Nucleotide barcode of stingless bees from Nagaland

KDIGMLYMIFALWSGIIGSSFSMMIRMELNSPGMWINND
QIYNSVITSHAFLMIFFMVMPFMIGGFGNFLIPMMLGSP
DMAFPRMNNVSFWLLPPSLFILLLSNFLFPSSGTGWTVY
PPLSSYFYHSSPSVDLTIFSIHMTGISSILGSLNFIVTI
FMMKNFSLNYDQISLFSWSISVTVILLIISLPVLAGAIT
MLLFDRNFNTSFFDPMGGGDPILYQHLFWF

Plate 14a: *Tetragonula* sp. I-White

KDIGMLYMIFALWSGIIGSSFSMMIRMELNSPGMWINNDQIYNSVITSHA
FLMIFFMVMPFMIGGFGNFLIPMMLGSPDMAFPRMNNVSFWLLPPSLFI
LLLSNFLFPSSGTGWTVYPPLSSYFYHSSPSVDLTIFSIHMTGISSILGSLN
FIVTIFMMKNFSLNYDQISLFSWSISVTVILLIISLPVLAGAITMLLFDRNF
NTSFFDPMGGGDPILYQHLFWF
MKNFSLNYDQIGLFSWSISVTVILLIISLPVLAGAITMLLFDRNLNTSFFD
PMGGGDPILYQHLFWF

Plate 14b: *Tetragonula* sp. I-Black

VWYLMFALWSGIIGSSFSMLIRMELNTPGMWISNDQIYN
SIVTGHAFLMIFFMVMPFMIGGFGNFLIPLMLGSPDMAF
PRMNNISFWLLPPALTLLLSSNLFSPSSGTGWTVYPPLS
AYLYHSSPSVDFTIFSIHLTGISSILGSLNFIVTIFM

Plate 14c: Underground stingless bee

Plate 14 : Protein sequence of stingless bees from Nagaland

length. Whereas underground SASRD had blackish entrance gate without long funnel entrance tube. As the fact of multiple morphological variations among the genus, at present study, specimens could not be identified at species level. Identification of the specimens was confirmed by Dr. Shashidhar Viraktamath, Emeritus Scientist, Department of Entomology, University of Agricultural Sciences, GKVK, Bengaluru-560065, Karnataka.

The present findings is in conformity with the observations made by Rusmussen (2013), who reported that antennae of *Tetragonula* spp. orient ventrally mostly testaceous to ferruginous and *Tetragonula* species of the "*iridipennis*" species group are characterized by having a dark mesoscutum with four distinct hair bands separated by broad glabrous interspaces. Similarly, Rahman *et al.* (2015) reported that *Tetragonula* spp. have black head and mesosoma and brownish antennae and their head is devoid of pubescence.

Table 4.9: Molecular characterization of stingless bee of Nagaland

Sl. No.	Taxonomic Identification	Collection Code	Nucleotide Length (bp)	Protein Length	NCBI GenBank Accession Numbers	Molecular results
1	*Tetragonula* sp.I	White SASRD 1851/ GTB261	666 bp	221	MH347237	100% similarity
2	*Tetragonula* sp.I	Black SASRD 1850 GTB256	666 bp	221	MH347239	100% similarity
3	*Tetragonula* sp.II	Underground SASRD 1853/GTB266	677 bp	225	MH347238	More than 1% difference at molecular level.

4.5 Comparative Study of architectural Design of stingless bee hives

Among three different hive types designed, wooden hive (LAH) were the best followed by Natural hives (NH). Bamboo hives were worst in term of following parameters:

4.5.1. Establishment and growth of colony (days)

Data on establishment and growth of stingless bee, *Lepidotrigona arcifera* colonies revealed that the establishment were significantly different among all three hives designed (Table 4.10 and Figure 4.1).

Table 4.10: Establishment and growth of colony (days) in different hives

Hive types and height	Days (2016-17)	Days (2017-18)	Pooled	Honey and pollen (cm^3)- (2016-17)	Honey and pollen (cm^3)- (2017-18)	Pooled
MoHo	234.333	214.666	224.499	111.666	225.000	168.333
M_1 Ho	70.000	39.333	54.666	70.000	60.000	65.000
M_2 Ho	325.000	366.333	345.666	298.333	441.666	739.999
MoH_1	366.000	366.000	366.000	207.000	215.000	211.000
M_1 H_1	192.333	40.666	116.498	115.833	64.333	90.083
M_2 H_1	183.000	366.000	274.500	228.333	473.333	350.833
SEm±	49.569	31.024		87.378	42.251	
CD = 0.05	179.459	112.322		NS	152.968	

All the hives placed above 5 feet (H1) from the ground performed better in comparison to 2 feet above the ground (Ho) (Table 4.10) but were Non Significant (NS) statistically.

Interaction effect on hive height placement and hive types were significantly different (Table 4.10 and Figure 4.1). Colonies in Mo hives placed at H1 performed better with 366 days in comparison to Ho with 224.4 days. Colonies stayed longer at BH placed at H1 (116.4 days) than the Ho (54.6 days). Performance of colonies in Mo are at par at Ho and H1.

It is observed that *L. arcifera* stayed longer period in M2 (LAH) 310.083 days in comparison to Mo (NH) with 295.25 days. Shortest duration recorded in M1 (Bamboo hive) with 85.58 days (Table 4.11).

Table: 4.11: Establishment (days) of stingless bee, *Lepidotrigona arcifera* colonies in different hives

Hive types	Days (2017)	Days (2018)	Pooled	Size of chamber (cm^3)-2017	Size chamber (cm^3)- 2018
Mo	300.167	290.333	295.25	159.333	220.000
M_1	131.167	40.000	85.583	92.917	62.167
M_2	254.000	366.167	310.083	263.333	457.500
SEm±	35.050	0.982		61.785	29.876
CD = 0.05	126.897	3.554		NS	108.16

NS= Non significant

Present study concluded that 15 colonies established as perennial colony out of 36 colonies introduced in different hives. M2 hive recorded best with 8 (66.66%) colonies out of 12 colonies introduced, followed by 7 (58.33%) in Mo hives. BH recorded with nil colony establishment rate. Overall establishment rate recorded as 41.66% (Table 4.12a).

Table 4.12a: Per cent establishment of stingless bee, *Lepidotrigona arcifera* colonies established in different hives

Hive types	No. of colony established during 2016-17	No. of colony established during 2017-18	Total no. of colony established	Establishment (%)
Mo	03/6	04/6	07/12	58.33
M1	00/6	00/6	00/12	-
M2	02/6	06/6	08/12	66.67
Total	**05/18**	**10/18**	**15/36***	

Martins *et al.* (2004) have also reported that in North-eastern Brazil, mostly tree trunks were used to domesticate the stingless bee by Meliponinae

beekeepers. In the present study, the colonies placed at higher elevation comparatively performed better though they are not statistically significant. The result of the present finding is in accordance with the report of Nayak *et al*. (2012) who studied the nesting heights of Trigona. They reported that the nesting elevations offered by Trigona above ground level showed very distinct preference of 47% between an elevation range of 11–15 ft from the ground point , while between 0–5 ft and 6–10 ft of ranges only 28% of nests were found.

4.5.2 Honey and pollen chamber distribution and dimensions

Internal nest structure studies during honey harvest revealed honey and pollen pots of *Lepidotrigona arcifera* were located on the periphery of the brood chamber (Plate 15). The dimensions in terms of size of honey and pollen chambers in different hives were statistically non significant (Table 4.10). Statistically directly proportional relationship was observed in duration of stay and honey production in the hive (Table 4.10).

The interaction effect in between different hive types and hive height placement in relation to the size of honey and pollen chambers are not significant statistically (Table 4.10 and Figure 4.2).

At the time of edition of this thesis, literature was not available in relation to the description about the size of honey and pollen chamber in new hives with especial reference to *Lepidotrigona arcifera* however, in Australia for honey production (1 kg/ hive/year), the beekeepers used different types of hives designed box for *Austroplebeia australis* so that the honey stores can be reached without damaging the rest of the nest structure. Box designs for honey production provide a separate compartment for the honey stores so that honey pots can be removed without spilling honey into other areas of the nest (https://www.aussiebee.com.au/honeyproduction.html).

Plate 15a: Hive ready to be opened

Plate 15b: Opened hive for honey extraction

Plate 15c: Honey and pollen chamber

Plate 15d: Opened hive for honey extraction

Plate 15e: Brood Chamber

Plate 15: Internal structure of *Lepidotrigona arcifera* colony in Arunachal Pradesh

4.5.3 Honey production (ml) /year

Data presented in Table 4.12b disclosed that mean honey production in wooden hives (M2) was much higher (2445.8 ml) than natural hives (Mo) which was 1766.5 ml but in bamboo hives (M1), the honey production was negligible (1.75 ml) during 2016-17 and 2017-18. However, in 2017 the honey production in M2 was found less due to use of split type M2 (LAH) in which rate of absconds was high. Total honey production in different hives was recorded with 4891.7 ml (58.03%) in M2, 3533.1 ml (42%) in Mo and 3.5 ml (0.04%) in M1 over two years (Table 4.12b).

Table 4.12b: Honey production from different hives during 2017 and 2018

Sl. No.	Hives	Honey production (ml)		Mean	Per cent production
		2017-18	2016-17		
01	Mo	1522.00	2011.00	1766.55	42.22
02	M1	2.20	1.30	1.75	0.04
03	M2	1201.70	3690.00	2445.85	58.03
Mean	12	908.60	1900.7	1404.71	

*Each hive having 12 numbers

Data on the performance of different hives individually during the years 2017 and 2018, the wooden hive (LAH 5) was found best with average yield of 665 ml over two years. Natural hives (NH5), (NH4) and (NH1) were recorded with a mean yield of 550 ml, 540 ml and 500 ml respectively (Table 4.13).

Table 4.13: Honey production against hive types and dimension (2016-17 and 2017-18)

Hives	Hives (2017-18)			Hives (2016-17)		Mean honey production ml/hive
	Dimension of hive (Lx B x H)	Honey production /hive (ml)	Name of hive	Dimension of hive (L x B x H)	Honey production /hive (ml)	
NH3	17 x 7 x 5.04	0.7	NH3	17 x 7 x 5.04	0.60	0.65
NH6	20 x10 x 5.07	0.4	NH6	20 x10 x 5.07	0.40	0.40
NH2	18 x 6 x 5.09	1.0	NH2	18 x 6 x 5.09	350.00	175.5
BH1	80 x 7.5	0.1	BH1	80 x 7.5	0.30	0.2

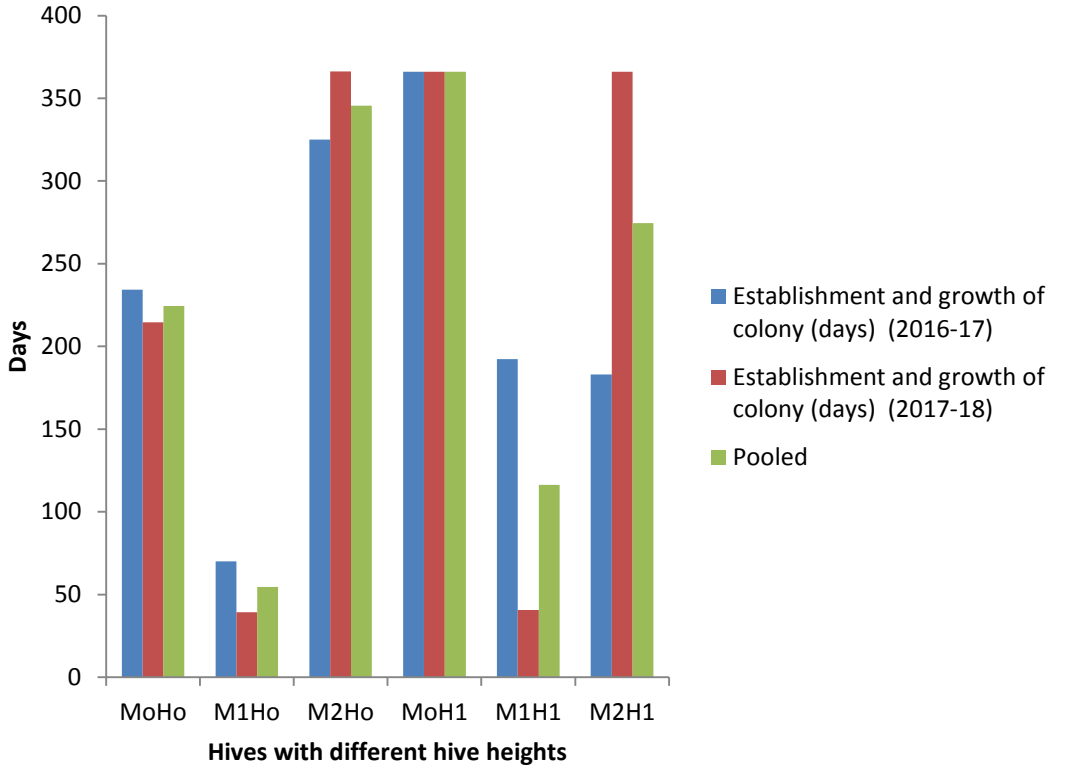


Fig 4.1: Interaction effect on establishment and growth in hives on different heights

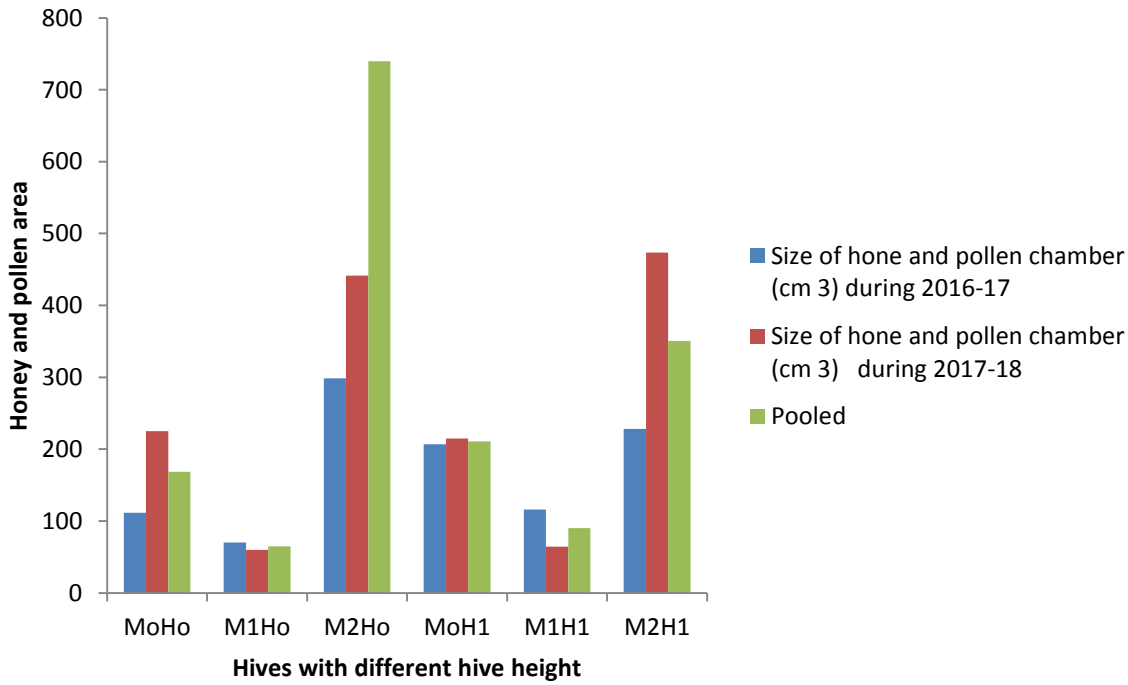


Fig 4.2: Interaction effect on honey and pollen size in hives at different heights

BH4	82 x 8	0.5	BH6	82 x 8	0.20	0.35
BH2	45 x 6.5	0.6	BH2	45 x 6.5	0.10	0.35
LAH3	100 x 10 x 5	0.7	LAH3	60 x 16 x 5	540.00	270.30
LAH6	40 x 18 x 8	550	LAH6	40 x 18 x 8	670.00	610.00
LAH4	45 x 7 x 5	0.7	LAH4	98.8 x 10.8 x 6	500.00	250.30
NH4	18 x 8.5 x 6.04	480	NH4	18 x 8.5 x 6.04	600.00	540.00
NH1	30 x 7 x 4	500	NH1	30 x 7 x 4	500.00	500.00
NH5	24 x 10 x 7	540	NH5	24 x 10 x 7	560.00	550.00
BH5	82 x 8	0.5	BH5	82 x 8	0.20	0.35
BH6	45 x 6.5	0.2	BH4	45 x 6.5	0.40	0.30
BH3	80 x 7.5	0.3	BH3	80 x 7.5	0.10	0.20
LAH1	45 x 7 x 5	0.1	LAH1	99 x 11 x 5.87	660.00	330.05
LAH5	40 x 18 x 8	650	LAH5	40 x 18 x 8	680.00	665.00
LAH2	60 x 18 x 7	0.2	LAH2	60 x 16 x 5	640.00	320.10

The interaction effect in between different hive types and hive height placement in relation to the honey production are not significant statistically (Figure 4.3).

In the present study the average honey production of *Lepidotrigona arcifera* was recorded to be 1404 ml while per hive production is 117.02 ml/hive per season (Table 4.12b), which is conformity with the finding of Rahman *et al.* (2015) in which they reported that stingless bees produce honey around 200-500 g per season.

Similarly, Kumar *et al*. (2012) reported that 600-700 g/year honey was extracted from the domestication of *Tetragonula iridipennis* in log cylindrical hives and he also reported about the successful multiplication and seasonal management of colony.

4.5.4. Durability and portability of hives

The durability and portability of different hives in terms of non cracking/ split into aperture, enable easy handling and required less maintenance and lasts longer without trivial maintenance and care off, M2 (10.81 months) hives were best followed by Mo (10.13 months) expressed in term of months While bamboo hives M1 (5.71 months) required frequent maintenance in sealing the cracks. All the treatments were significantly different (Table 4.14 and Figure 4.4).

Table 4.14: Durability and portability (days) of hives

Hive types	Durability (months) (2016-17)	Durability (months) 2017-18)	Pooled (2017 & 2018)
Mo	9.883	10.383	10.133
M_1	5.433	6.000	5.716
M_2	9.517	12.117	10.817
SEm±	0.982	0.524	
CD = 0.05	3.554	1.896	

Durability of the different hives was affected to some extend with the hive height placement. M1 (bamboo hives) placed at 5 feet (H1) above the ground lasts longer than those placed at Ho. However, all the treatments were not statistically significant. The interaction effect in between different hive types and hive height placement in relation to the durability are significant statistically (Table 4.15). An average durability of Mo hives at H1 last longer (12.10 months) than those (Mo) placed at Ho (8.1months). Similarly, M2 hives placed at Ho is at par with that of H1. Nayak *et al*. (2012) also studied the nesting heights of Trigona. They reported that the nesting elevations offered by Trigona above ground level showed very distinct preference of 47% between an elevation range of 11–15 ft from the ground point , while between 0–5 ft and 6–10 ft of ranges only 28% of nests were found which is in conformity with the present studies.

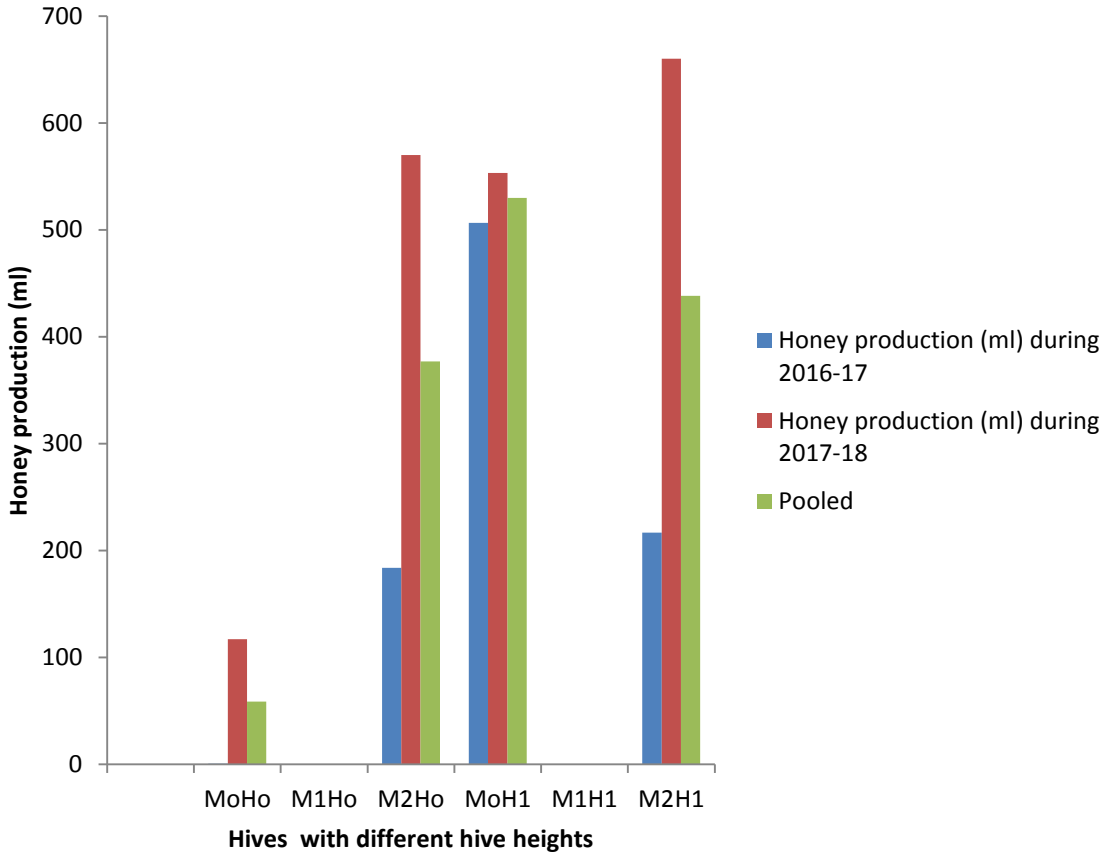


Fig 4.3: Effect of different heights on honey production

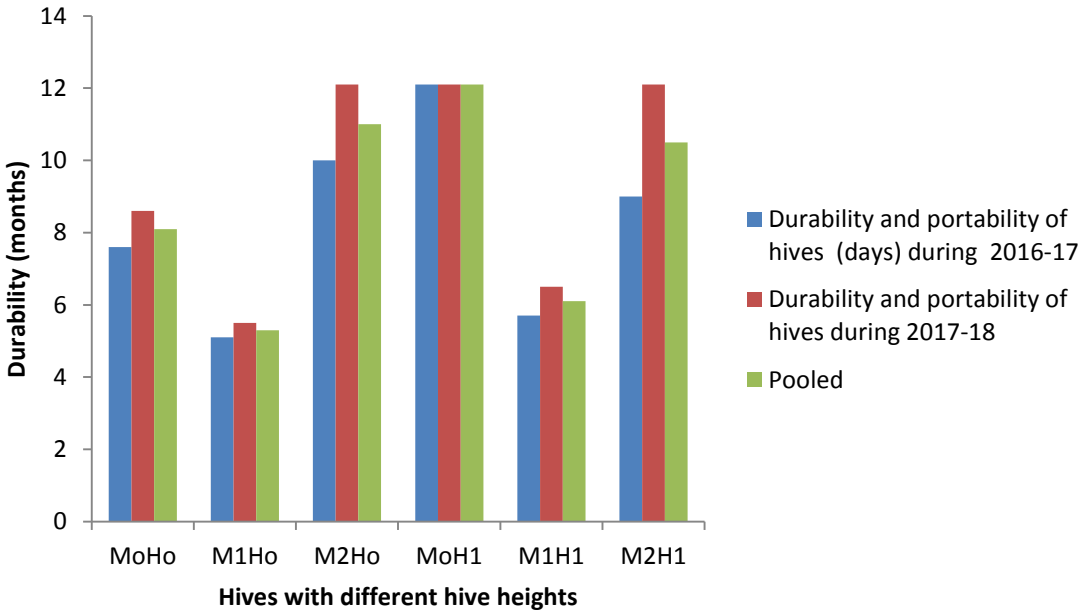


Fig 4.4: Effect of different heights durability of hives

Table 4.15: Effect of height on durability and portability (days) of hives

Hive and height types	Durability (days, 2016-17)	Durability (days, 2017-18)	Pooled
MoHo	7.66	8.66	8.16
M_1 Ho	5.16	5.50	5.33
M_2 Ho	10.03	12.13	11.08
MoH_1	12.10	12.10	12.10
M_1 H_1	5.70	6.50	6.10
M_2 H_1	9.00	12.10	10.55
SEm±	1.38	0.74	
CD = 0.05	NS	2.68	

During the studies it was observed that on exposures to direct sunlight and rain during summer and rainy seasons the hives were less durable. Similar findings have been reported by Kwapong *et al*. (2010) in which they reported about the possible site for hive placement in which they recommended that stingless bee may be kept under sheltered areas such as near homes – at the backyard, on corridors or verandas of houses and on the farm – at fruit orchards and tree shaded area having protection from rain and direct sun.

4.5.5 Absconded colonies

Out of 36 colonies in Arunachal Pradesh, fifteen colonies established in new hives during 2016-17 and 2017-18 and rest colonies absconded. Dates of absconds were not same. The known reasons of absconds are attributed to:

1) Frequent opening of hive while monitoring the internal colony development.
2) Bamboo hives bent to split/ crack at hot summer months (Plate 16).
3) On exposures to direct contact of hives to rain during rainy season.
4) Freshly designed log hive slacken off the tied rope and shrink to develop fissures/crevices.
5) Insect pest such as fire ants and orange gaster *Oecopylla smaragdina*.

Plate 16a: Internal view of absconded colony

Plate 16b: External view of absconded colony

Plate 16c: *Lepidotrigona arcifera* colonies before absconding in greenhouse

Plate 16d: *Lepidotrigona arcifera* colonies after absconding in greenhouse

Plate 16: Absconded stingless bee colonies

6) Cause of absconding could be due to diseases, yet to investigate for disease diagnosis.

In the present study, the causes of colony absconds observed are in accordance with the repors of Kwapong *et al*. (2010) in which they reported that when the colony transfer operation is over the beekeeper should make sure that the site of the new hive is free of pest especially ants. Hive stands and hanging ropes should be effectively protected from ants and other pests. Dirty engine oil in canes and grease on ropes can be used to stop the invasion of the new nest by ants and other crawling insects. Placement of hives should be done in such a way to prevent pests such as ants, lizards, spiders and other intruders having access to nests.

4.6. Effect of stingless bee *Lepidotrigona arcifera* pollination on tomato under protected conditions

The present studies were conducted to know the pollination potential of stingless bee, *Lepidotrigona arcifera* on off season tomato crop under protected cultivation. The different parameters are discussed under the following heads:

4.6.1 Foraging activity

4.6.1.1 Foraging rate of the *Lepidotrigona arcifera* on flowering crop of tomato during 6th January – 31st January, 2017 and 2018

During the studies stingless bee colonies were introduced inside the polyhouse and foraging rate was observed from early morning till evening hours (Plate 17). The bees started foraging at 0700 h (6.9 flowers/minute) and cessation at 1700 h (7.1 flowers/minute). The maximum number of flowers visited by the bees were recorded at 1200 h (8.4 flowers/minute) while the minimum foraging rate was found at 1500 h (6.6 flowers/minute) (Table 4.16). Rokozeno (2015) studied the foraging activity of stingless bees on cucumber. She reported that stingless bees visited 1.87 flowers/minute. Similarly, Singh

Plate 17a: *Lepidotrigona arcifera* hive placed in greenhouse

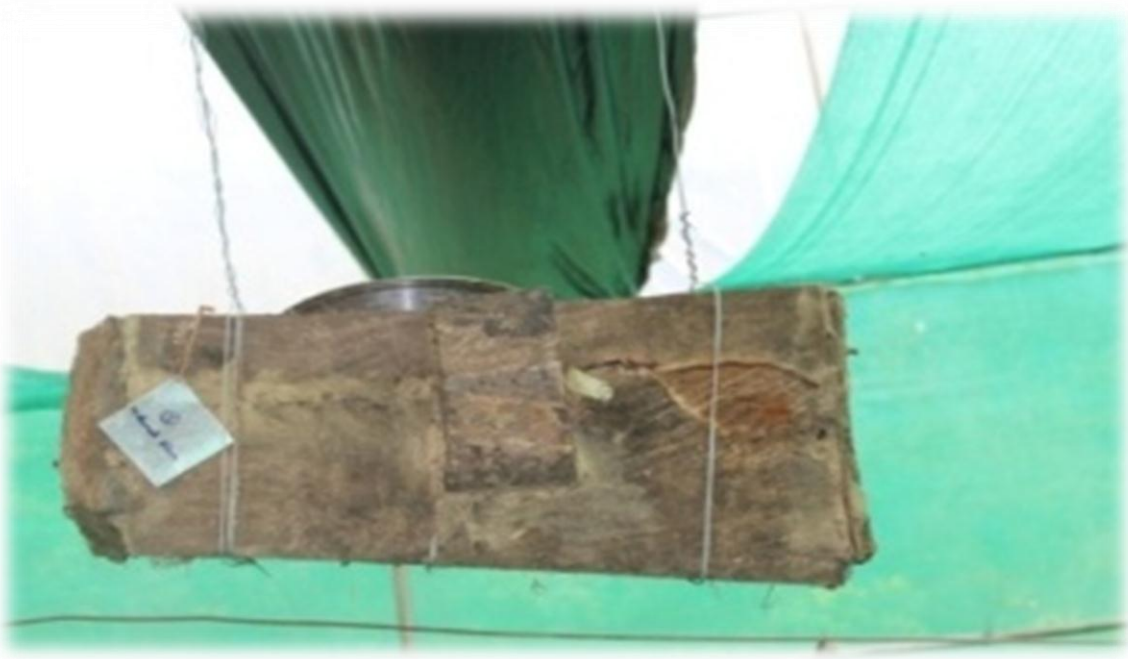


Plate 17b: Artificial feeds on tray being placed on the top of hives in greenhouse

Plate 17: Stingless bee hives placed in tomato greenhouse

and Chauhan (2018) described the foraging activity of *Tetragonula iridipennis* on ash gourd. They reported 7.60 flowers/minute were visited by the stingless bees which are in conformity with the present investigations. Present findings was found in similarity with the finding of Ramalho *et al*. (1994) who reported that stingless bees are floral constancy, workers bee visit only one plants species on a single trip (Table 4.18). Similarly, Rajashri *et al*. (2012) reported that *Trigona iridipennis* made 53.8 visits on a capitalum of sun flower in five minutes.

Table 4.16: Mean foraging rate (Number of flowers/ minute) of *Lepidotrigona arcifera* on tomato flowers

Time (h)	R1	R2	R3	R4	R5	R6	R7	Mean
0700	9.2	6.6	5.6	5.5	9.2	5.5	6.7	6.9
0800	6.6	6.4	9.2	5.6	6.7	7.4	9.2	7.3
0900	7.5	5.4	10.0	9.5	9.0	8.5	8.5	8.3
1000	7.5	7.0	9.2	7.5	8.5	7.5	7.8	7.8
1100	8.5	9.0	5.4	10	8.5	7.5	9.2	8.3
1200	8.5	5.4	10.5	9.5	9.5	8.5	7.5	8.4
1300	9.2	7.6	6.6	10	9.2	7.5	7.5	8.2
1400	5.4	10	10.5	5.4	7.5	8.4	9.2	8.0
1500	9.2	7.5	8.5	6.6	9.2	5.6	5.8	7.4
1600	5.5	5.5	5.5	9.2	9.2	6.4	5.5	6.6
1700	8.0	6.6	6.4	9.2	5.6	6.7	7.4	7.1
Mean	7.7	7.0	7.9	8.0	8.3	7.2	7.6	7.7
$CD_{0.05}$								0.21

4.6.1.2 Foraging speed of the *Lepidotrigona arcifera* on tomato

Data on mean foraging speed of the *Lepidotrigona arcifera* on flowering crop of tomato during 6th January – 31st Jan 2017and 2018 revealed that maximum time spent per flower was recorded at 700 h (9.7 sec) followed by 1600 h (9.4 sec). The minimum time spent per flower was recorded at 900 h. Similar results were observed by Singh and Chauhan (2018) in ash gourd. They found that *Tetragonula iridipennis* workers spend 4.33 seconds on 4.15 seconds on flowers. However in cucmber it was 3.83 seconds. Soliman *et al.*

(2013) who reported that average time spent per sesame flower by small carpenter bee was relatively longer with 32.10 seconds/ flower in comparison to that of honey bee. However, Rokozeno (2015) reported that mean foraging speed, second (s)/ flower by *Tetragonula* sp. on cucumber was 30.26 seconds.

Table: 4.17: Mean foraging speed (time spent/flower) of *Lepidotrigona arcifera* on tomato flowers

Time (h)	R1	R2	R3	R4	R5	R6	R7	Mean
0700	9.2	9.0	10.7	10.9	9.2	10.5	8.9	9.7
0800	9.0	9.3	9.2	10.7	8.9	8.1	9.2	9.2
0900	8.0	5.4	5.7	6.0	6.3	6.6	7.0	6.4
1000	8.0	8.5	9.2	8.0	7.0	8.0	7.6	8.0
1100	7.0	6.6	5.4	6.0	7.0	8.0	9.2	7.0
1200	7.0	5.4	5.7	6.3	6.3	7.0	8.0	6.5
1300	9.2	7.8	9.0	6.0	9.2	7.1	8.0	8.0
1400	5.4	6.0	5.7	5.4	8.0	7.1	9.2	6.6
1500	9.2	8.0	7.0	9.0	9.2	10.71	10.3	9.0
1600	10.9	10.9	10.9	9.2	9.2	9.3	5.5	9.4
1700	7.5	9.0	9.3	9.2	10.7	8.9	8.1	8.9
Mean	8.2	7.8	7.9	7.8	8.2	8.3	8.2	
$CD_{0.05}$								**0.51**

4.6.1.3 Diurnal abundance of *Lepidotrigona arcifera*

Diurnal abundance of stingless bee *Lepidotrigona arcifera* was observed during the month of January 2017 and 2018. The maximum activity of bees was recorded at 800-900 h, 1000-1200h (1.20 bees / 10 minutes). The activity of the bees started decreasing at 1300 h upto1700 h which is in conformity with the results of Singh and Chauhan (2018) who observed the relative abundance of stingless bees on ash gourd and concluded that the activity of the bees started in the morning hours and maximum during early noon hours followed by decrease in the activity in early evening hours. Similarly, Anonymous (2018) reported diurnal abundance of stingless bees in litchi, cucumber and guava was maximum in the morning hours and minimum

in the evening hours of the day which is in conformity with the present investigations.

Table 4.18: Diurnal abundance of *Lepidotrigona arcifera* on tomato flowers

Time (h)	R1	R2	R3	R4	R5	Mean
0600-700	1.00	1.00	1.00	1.00	1.00	1.00
0700-800	1.00	1.00	1.00	1.00	1.00	1.00
0800-900	1.00	2.00	1.00	1.00	1.00	1.20
900-1000	1.00	1.00	1.00	1.00	1.00	1.00
1000-1100	1.00	1.00	1.00	2.00	1.00	1.20
1100-1200	1.00	1.00	2.00	1.00	1.00	1.20
1200-1300	1.00	1.00	2.00	1.00	1.00	1.20
1300-1400	1.00	1.00	1.00	1.00	1.00	1.00
1400-1500	1.00	1.00	1.00	1.00	1.00	1.00
1500-1600	1.00	1.00	1.00	1.00	1.00	1.00
1600-1700	1.00	1.00	1.00	1.00	1.00	1.00
Mean	1.00	1.20	1.30	1.20	1.00	
$CD_{0.05}$						0.11

The result of the present findings is not in accordance with the findings of Ciar *et al.* (2013) who reported that *Tetragonula* sp prefer the feeder (food source) located 1m above the ground, which are directly in front of the hive opening. Ciar also reported that nearest or 1m distance is the most preferred distance for food source. Whereas, in the greenhouse *Lepidotrigona arcifera* two hives were hang at 1m above the ground during the flowering of tomato crop. Despite of having these, the size of foraging population were recorded scantly (Table 4.18). Similarly, Bartelli *et al*. (2014) reported that *Melipona quadrifasciata* landed on anther of tomato flower 6 months from the date of introduced in the greenhouse. Whereas, Heard (1999) reported that stingless bees foraged for a mean period of 7 h per day as compared with 10 h for honey bees.

4.6.1.4. Initiation and cessation time of the *Lepidotrigona arcifera*

Data on initiation and cessation time of the *Lepidotrigona arcifera* in greenhouse during winter months was recorded which revealed that the bees started foraging at 0645 h and the activity ceased at 1651 h respectively (Table 4.19). Similar results were obtained by Anonymous (2018) on cucumber and litchi. The bees started their activity in the morning hours and ceased their activity in late evening hours. The present results are also in proximity with the findings of Rokozeno (2015), who reported the initiation (0626 h) and cessation (1647 h) time of the stingless bee, *Tetragonula iridipennis* under open conditions.

Table 4.19: Initiation and cessation activity of *Lepidotrigona arcifera* in greenhouse

S.No	Pollinator	Initiation (h)	Cessation (h)
01	*Lepidotrigona arcifera*	0632	1622
02		0700	1655
03		0635	1705
04		0645	1625
05		0615	1649
Mean		0645	1651

4.6.1.5 Loose pollen grains

Data on loose pollen grains were observed on the body of the foragers which revealed that *L.arcifera* carries 2380 numbers of loose pollen grains on their body. Similar investigations were made by Singh and Chauhan (2018) who reported 1290 pollen grains in cucumber and ash gourd.

4.6.1.6 Pollination efficiency

Pollination efficiency of *L. arcifera* was calculated on tomato crop grown under greenhouse conditions which was found to be 3. Singh and Chauhan (2018); Anonymous (2018) calculated the pollination efficiency of stingless bee on ash gourd, cucumber and litchi. The pollination index was calculated to be 24 which is higher than the pollination index in tomato.

4.6.1.7 Flowers monitoring for bee activity

The bee activity in terms of flower bruishing was recorded. The tomato flowers where bees visited on tagged plants were observed visually to find out visible bite bruises on the flower cone. No signs of bite bruises were observed on the tomato flower cones of tagged plants that were visited by the bees. Similar results were obtained by Amano (2004). He studied crop pollination of different crops under greenhouse conditions. He found that the stingless bees visited about 82% of the flowers of tomato.

4.7 Tomato berry harvest and yield

The average yield of tomato plant in greenhouse with stingless bee (1347.63 g) is higher than open condition (1050.11 g) during 2017 and 2018. The average yield of T1 (1347.63 g) is at par with T2 (1330.71 g) during 2017 and 2018. Yield per plant under greenhouse was higher than that of open condition due to growing and fruiting longevity of the plants in the greenhouse (Table 4.20 and Figure 4.5 and 4.6). The results of the present investigations are in proximity with the results of Amano (2004) who laid experiments in Japan for the pollination of tomato under greenhouse conditions. He observed that stingless bees visited large numbers of tomato flowers but the fruit set is only 8% of total pollinated plants. However, Santos *et al*. (2004a) compared pollination effectiveness *of Melipona. quadrifasciata* and *Apis mellifera* (each species in a 86 m^2 greenhouse), and found that tomatoes were bigger, heavier

Table 4.20: Effect of stingless bee, *Lepidotrigona arcifera* pollination on tomato yield in greenhouse

Plant No.	2017			2018		
	Control	Stingless bee pollination	Open pollination condition	Control	Stingless bee pollination	Open pollination condition
1st plant	1540	1300	1100	1140	1350	1210
2nd plant	1270	1570	1250	1450	1400	700
3rd plant	1020	1320	900	1560	1300	1100
4th plant	1400	1200	1150	1650	1140	1050
5th plant	1730	1550	750	1080	1550	1150
6th plant	1210	1430	1150	1160	1350	1040
7th plant	1230	1300	1110	1190	1100	1050
Mean	1342.85	1381.42	1058.57	1318.571	1312.857	1041.667
CV	14.793			15.130		
SEm±	70.503			70.043		
CD = 0.05	243.784			242.194		

and had more seeds following pollination by *M. quadrifasciata* compared to *A. mellifera*. The present study observed that the yield per plant was higher in case of greenhouse conditions. Present finding is in agreement with the findings of Gualberto *et al*. (2007) who reported that the performance of tomato was statistically superior in polyhouse cultivation compared to open condition. Similar results were obtained by Singh and Asrey (2005) as they found excellent tomato crops in poly houses compared to open condition.

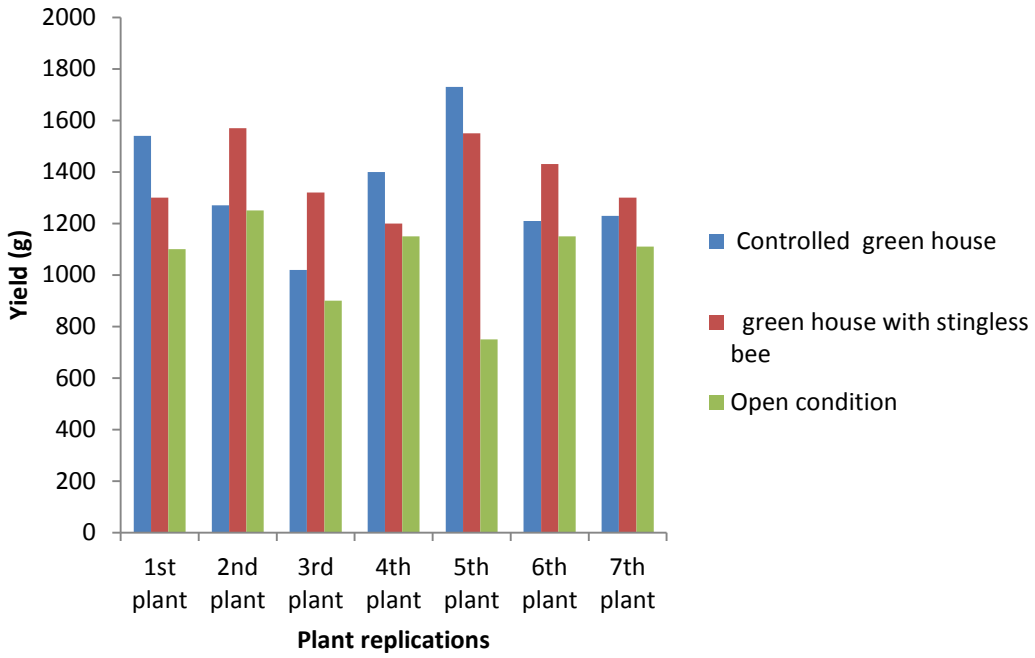


Fig 4.5: Effect of pollination on tomato yield under greenhouse conditions 2017

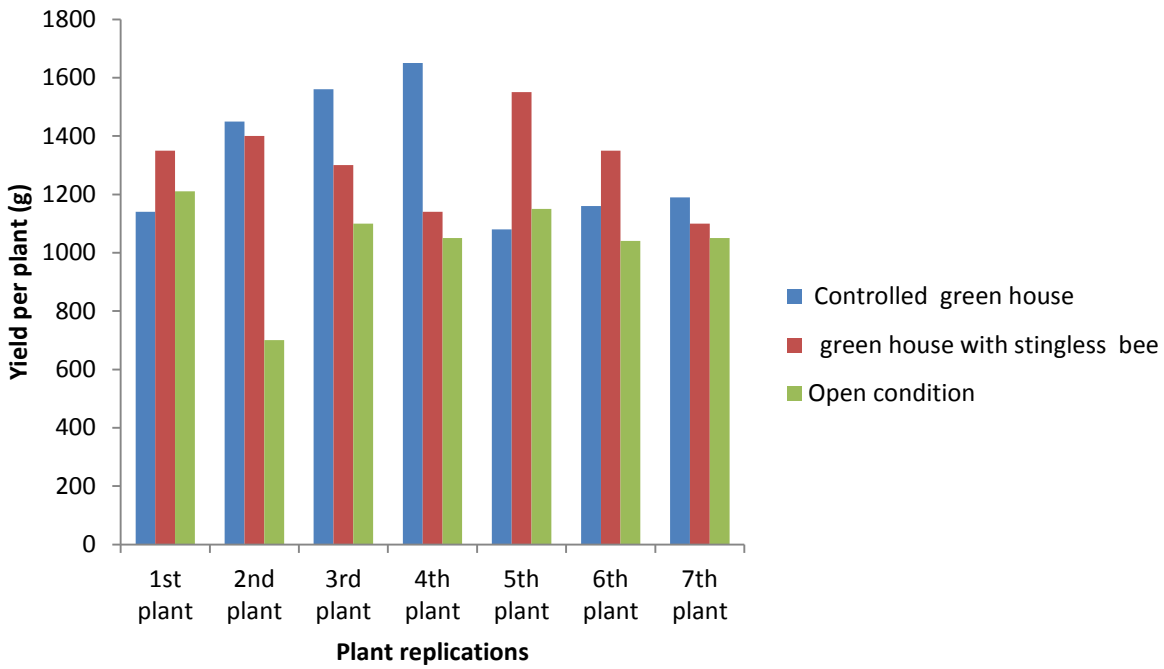


Fig 4.6: Effect of pollination on tomato yield under greenhouse conditions 2018

4.7.1 Total yield

Average tomato yield in greenhouse with stingless bee (T1) was 24.2 kg/plot. Greenhouse without stingless bees (T2) 23.9 kg/plot and open pollinated plant (T3) yielded 18.9 kg/plot. Average tomato yield in green house with stingless bee (T1) was 107.7 ton/ha. Greenhouse without stingless bees (T2) 106.4 ton/ha. and open pollinated plant (T3) yielded 84 ton/ha.

CHAPTER - V

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

The present investigation entitled, "Studies on stingless bees and their utilization in tomato (*Lycopersicon esculentum*) Bailey production" were carried out during 2016-2018 in the laboratory at ICAR, Umiam, Meghalaya and field in Arunachal Pradesh and School of Agricultural Sciences and Rural Development, Nagaland University Campus Medziphema, India.

The stingless bee colonies from different altitudes of Nagaland and Upper Subansiri district of Arunachal Pradesh were collected and their identification by traditional and molecular taxonomy was conducted. Standardization of new hives was tested for their domestication. The reared colonies of *Lepidotrigona arcifera* were used in pollination of tomato crop grown under greenhouse conditions. The findings of the objectives are summarised below:

1) Total thirty four colonies (thirty one colonies from Arunachal Pradesh and three colonies from Nagaland) of stingless bees were collected from the forest and re-established in the new designed hives *viz*. natural hives, bamboo hives and wooden hives having different dimensions.

2) The stingless bee colonies collected from Arunachal Pradesh were identified as *Lepidotrigona arcifera* Cockrell through traditional and molecular analysis having 575 bp nucleotide length and 191 protein length.

3) White SASRD and Black SASRD colonies collected from Nagaland were confirmed as *Tetragonula* sp.I and underground SASRD as *Tetragonula* sp.II. *Tetragonula* sp.I with nucleotide sequence resulted to 666 bp and having 221 protein lengths. The DNA sequence of underground SASRD with 677 bp nucleotide length and having 225 protein lengths.

4) Two type of hive height placement (2 feet and 5 feet) were tested in the shed houses. Hives placed at five feet height performed better (366 days) in term of colony establishment in hives but were statistically non-significant from those placed at two feet height above the ground.

5) New hives were standardized on the basis of establishment and growth of colony, size of honey and pollen chamber, honey production and durability and portability of hives. It was observed that wooden hives (M2) were found better with honey production of 615 ml and colony stayed for 254 days as compared to natural hives (335 g and 253 days) and bamboo hives (217 g and 40 days). The durability and portability of the hives were directly depending on the exposure of sun and rain during summer and rainy seasons. The hives at five feet high were better than the hives placed at two feet in terms of durability in months.

6) The foraging activity of *Lepidotrigona arcifera* was observed in tomato crop in greenhouse during 2017 and 2018. The initiation and cessation time of the *Lepidotrigona arcifera* in greenhouse during winter months was recorded which revealed that the bees started foraging at 0645 h and the activity ceased at 1651 h, respectively. Mean foraging rate was found to be 7.7 flowers while foraging speed was 8.9 seconds. Diurnal abundance was found maximum during morning hours (1.2 bees) and minimum in late evening hours (1700 h) with 1.00 bees.

7) The yield of tomatoes under different conditions *i.e*. greenhouse with stingless bee pollination (T1), greenhouse without bees (T2) and T3 open condition were significantly different. The present results revealed that the yield of T1 (1347.1 gm/plant) was at par with that of T2 (1330.7 gm/plant) and open pollinated plant (T3) yielded 1050.1 g/plant. The yield of tomatoes in greenhouse was higher than that of open condition (T3). The cause of

increased in yield of tomatoes in greenhouse were due to longer longevity of the tomatoes crop and their healthier plant growth in the greenhouse.

CONCLUSION

The present investigations disclosed that feral stingless bee fauna of Arunachal Pradesh and Nagaland can be domesticated in new hives for both honey production and pollination of tomato crop grown under protected conditions which can augment the income of farmers. *Lepidotrigona arcifera* is the stingless bee which is available dominantly in Arunachal Pradesh. In the present studies it has been observed that *Lepidotrigona arcifera* can be domesticated in log artificial hives (wooden hives), having different dimensions for better honey yield and pollination of crops. Wooden hive (LAH) yielded mean honey production of 665 ml/hive/season with damage to the brood. Therefore, further research should be conducted to standardise the hives and the honey extraction method without damaging the brood chamber of the colony systematically in the days to come. In the present line of work, an efficient and tangible rearing and their nucleus colony multiplication technology for sustainable farming in North East India is need of hour. The hives placed at height five feet above from the ground are less susceptible to abscond due to less exposure to weather conditions and pests. Stingless bees of Arunachal Pradesh and Nagaland can be identified through morphology and molecular analysis. Stingless bees can be utilized for the pollination of tomato crop as the bees were foraging on tomato flowers from morning till evening. The yield of tomato was higher under greenhouse conditions but at par with the stingless bee pollination. Quality parameters were not the objectives of the study so it is important to study the effect of stingless bee pollination keeping in view the different quality parameters to ascertain their specific role in pollination of tomato crop under protected conditions.

REFERENCES

REFERENCE

Al-Attal, Y. Z., Kasrawi, M. A. and Nazer, I. K. 2003. Influence of pollination technique on greenhouse tomato production. *Agric. Mar. Sci.* **8**: 21-26.

Alves, D. J. 2006. Uso do marcador molecular RAPD para estudo de polimorfismos em populacoes de *Tetragonisca angustula* L. (Apidae: Meliponinae). Dissertacao de Mestrado em Genetica e Melhoramento. Universidade Estadual de Maringa, Maringa, PR. 45.

Amano 2004. Attempts to introduce stingless bees for the pollination of crops under greenhouse conditions in Japan. Food & Fertilizer Technology Center. http://www.fftc.agnet.org./library/article/tb167.html.

Annual Report 2016-17 of DAC & FW, Department of Agriculture Cooperation and Farmers welfare Ministry of Agriculture & Farmers welfare Government of India, Krishi Bhawan, New Delhi-110001.

Anonymous. 2018. AICRP-Honey & Pollinators. Biennial Report 2017-18, Medziphema Nagaland.

Antunes, O.T., Calvete, E. O., Rocha, H. C., Nienow, A. A., Cecchetti, D., Riva, E. and Maran R. E. 2007. Produção de cultivares de morangueiro polinizadas pela abelha jataí em ambiente protegido. *Horticultura Brasileir*a. **25**: 94-99.

Asada, S. and Ono M. 1997. Tomato pollination with Japanese native bumble bees (*Bombus* spp.). *Acta Hotic.* **437**: 289-292.

Asokan, R., Rebijith, K. B., Singh, S. K., Sidhu, A. S., Siddharthan, S., Karanth, P. K., Ellango, R., and Ramamurthy, V. V. 2011. Molecular identification and phylogeny of *Bactrocera* species (Diptera: Tephritidae). *Fla. Entomol.* **94** (4): 1026-1035.

Banda, H. J. and Paxton, R. J. 1991. Pollination of greenhouse tomatoes by bees. *Acta Hortic.* **288**: 194-198.

Bartelli, B. F., Santos, A. O. R. and Nogueira, F. H -Ferreira. 2014. Colony performance of *Melipona quadrifasciata* (Hymenoptera, Meliponina) in a Greenhouse of *Lycopersicon esculentum* (Solanaceae). *Sociology*. **60** (1): 50-67.

Batista, M. A., Ramalho, M. and Soares, A. E. E. 2003. Nesting sites and abundance of Meliponini (Hymenoptera: Apidae) in heterogeneous habitats of the Atlantic rain forest, Bahia, Brazil. *Lundiana.* **4**: 19-23.

Behere, G. T., Tay, W. T., Russell, D. A., Heckel, D. G., Appleton, B. R., Kranthi, K. R. and Batterham, P. 2007. Mitochondrial DNA analysis of field populations of *Helicoverpa armigera* (Lepidoptera: Noctuidae) and of its relationship to *H. zea.* **In:** BMC Evolutionary Biology. **7**: 117.

Benton, J. J. Jr. 1998.Tomato plant culture in the field, greenhouse and home garden. **In**: CRC Press, Boca Raton, FL. Tomato Plant Culture: 635-642.

Bhambura.1958. Further studies on the importance of honey bees in pollination of cucurbitaceae. *Indian Bee Journal*. **20**: 189-191.

Biesmeijer, J. C. and Slaa, E. J. 2004. Information flow and organization of stingless bee foraging, *Apidologie*. **35**: 143-157.

Biesmeijer, J. C., Giurfa, M., Koedam, D., Potts, S. G., Joel, D. M., and Dafni, A. 2005. Convergent evolution: Floral guides, stingless bee nest entrances, and insectivorous pitchers. *Naturwissenschaften.* **92**: 444-450.

Biesmeijer, J. C., Roberts, S. P. M. and Reemer, M. 2006. Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science*. **313**: 351-354.

Bingham, C. T. 1897. "Hymenoptera, wasp and bees". **In**: W.T. Blandford (Ed), "The fauna of British India including Ceylon and Burma". Taylor and Francis, London, pp 579-1897.

Bohart, G. E. and Nye, W. P. 1960. Insect pollinators of carrots in Utah. *Utah Agr. Exp. Sta. Bull*. **419**: 16.

Boogert, N. J, Hofstede, F. E. and Monge, I. A. 2006. The use of food source scent marks by the stingless bee *Trigona corvina* (Hymenoptera: Apidae): the importance of the depositor's identity. *Apidologie*. **37** (3): 366-375.

Boongird, S. 2011**.** Aspects of culturing, reproductive behavior, and colony formation in the stingless bee *Tetragonula fuscobalteata* (Hymenoptera: Apidae: Meliponini). *Journal of The Kansas Entomological Society.* **84** (3): 190–196.

Buchmann, S. L. 1983. Buzz Pollination in Angiosperms. **In**: Handbook of Experimental Pollination Biology (Jones CE and Little RJ, eds.). Van Nostrand Reinhold, New Rome: Food Agric. Org. York. pp 73-113.

Buchmann, S. L. 1995. Pollen, anthers and dehiscence. **In** : Pollination of Cultivated Plants in the Tropics, ed. DW Roubik. pp 121-123.

Buchmann, S. L. and Nabhan, G. P. 1996. The forgotten pollinators, Island Press, Washington, D C.

Camargo, J. M. F. 1970. Ninhos e biologia de algumas espécies de Meliponideos (Hymenoptera: Apidae) da região de Porto Velho, Território de Rondônia, Brasil. *Rev. Biol. Trop*. **16**: 207-239.

Camargo, J. M. F. and Pedro, S. R. M. 2003. Meliponini neotropicais: o gênero *Partamona* Schwarz, 1939 (Hymenoptera, Apidae, Apinae) - bionomia e biogeografia. *Revista Brasileira de Entomologia*. **47**: 311-372. http://dx.doi.org/.

Camargo, J. M. F. and Pedro, S. R. M. 2004. Meliponini neotropicais: o gênero *Ptilotrigona* Moure (Hymenoptera, Apidae, Apinae). *Revista Brasileira de Entomologia*. **48**: 353-377.

Cameron, P. 1897 Hymenoptera Orientalia, or contributions to knowledge of the Hymenoptera of the Oriental zoological region. Part V. *Memoirs and Proceedings of the Manchester Literary and Philosophical Society*. **41** (4): 1-144.

Campos, L. A. O. 1987. Abelhas indígenas sem ferrão: o que são? *Info. Agrop*. **13** (147).

Cane, J. H. and Tepedino, V. J. 2001. Causes and extent of declines among native North American invertebrate pollinators: detection, evidence, and consequences. *Conserv. Ecol*: **5** (1). http://www.consecol.org/vol5/iss1/art1. Accessed on 6 March 20018.

Carvalho, A. T., Koedam, D., Imperatriz-Fonseca, V. L. 2014. Register of a New Nidification Substrate for *Melipona subnitida* Ducke (Hymenoptera: Apidae: Meliponini): The Arboreal Nest of the Termite *Constrictotermes cyphergaster* Silvestri (Isoptera: Termitidae: Nasutitermitinae). *Sociobiology*. **61** (4): 428-434.

Cauich, O., Quezada-Eußn, J. J. G., Macias-Macias, J. O., Reyes-Oregel, V., Medina-Peralta, S. and Parra-Tabla, V. 2004. Behavior and pollination efficiency of *Nannotrigona perilampoides* (Hymenoptera: Meliponini) on greenhouse tomatoes *(Lycopersicum esculentum)* in Subtropical Mexico, *Hortic. Entomol.* **97**: 475-481.

Chahuan, A. and Thakur, R. K. 2014. Studies on nest architecture and pollination potential of bumble bee *Bumbus haemorrhoidalis*. *Indian Journal of Ecology*. **41** (1): 158-164

Chauhan, A., Rana, B. S., Thakur, M and Katna, S. 2017. RAPD-PCR studies on bumble bees (*Bombus haemorrhoidalis* and *Bombus rufofasciatus*) and their important pests (*Physocephala tibialis* and *Aphomia sociella).* **In**: Ecology, Environment and Conservation Paper. **23** (4): 2280-2284.

Ciar, R. R., Bonto, L. S., Bayer, M. H., Rabajante, J. F., Lubag, S. P., Fajardo, A. C. and Cervancia, C. R. 2013. Foraging behaviour of stingless bee (*Tetragonula* sp: Distance, Direction and height of preferred food source. https://www.researchgate.net/publication/. Accessed on 20th June 2016.

Cortopassi-Laurino, M., Knoll, F. R. N., Ribeiro, M. F., Heemert, C., Ruijter, A. 1991. Food plant preferences of *Friesella schrottkyi*. *Acta Hort.* **288**: 382-385

Costanza, R., Arge, Rd'. de Groot, R., Farber, S,Grosso, M., Hannon, B., Limburg, K. and Naeem, S. 1997. The value of the world's ecosystem services and natural capital. *Nature*. **387**: 253-260.

Cruz, D de O., Freitas, B. M., Silva, L. A., da, Silva, S.E. M. D. and Bomfim, I. G. A. 2005. Pollination efficiency of the stingless bee *Melipona subnitida* on greenhouse sweet pepper, Pesq. Agropec. *Bras., Brasilia.* **40**: 1197-1201.

Danaraddi, C. S., Viraktamath, S. 2009. Morphometrical studies on the stingless bee, *Trigona iridipennis* Smith. *Karnataka Journal of Agricultural Sciences*. **22**: 796-797.

Delfinado-Baker, M., Baker, E. W., Phoon ,A. C. G. 1989. Mites (Acari) associated with bees (Apidae) in Asia, with description of a new species. *Am. Bee J.* **129**: 609-13.

Dogterom, M. H., Matteoni, J. A. and Plowright, R. C. 1998. Pollination of greenhouse tomatoes by the North American *Bombus vosnesenskii* (Hymenoptera: Apidae). *J. Econ. Entomol.* **91**: 71-75.

Dollin, A. E., Dollin, L. J. and Sakagami, S. F. 1997. Australian stingless bees of the genus *Trigona* (Hymenoptera: Apidae). *Invertebrate Taxonomy*. **11**: 861-896.

Eardley, C. D. 2004. Taxonomic revision of the African stingless bees (Apoidea: Apidae: Apinae: Meliponini). *African Plant Protection.* **10** (2): 63-96.

Eltz, T., Brühl, C. A., Imiyabir, Z. and Linsenmair, K. E. 2003. Nesting and nest trees of stingless bees (Apidae: Meliponini) in lowland dipterocarp forests of Sabah, Malaysia, with implications for forest management. *Forest Ecol. Manag.* **172**: 301-313.

Erniawati. 2013. Kajian Biologi Lebah Tak Bersengat (Trigona) di Indonesia, *Fauna Indonesia Journal*. **12**: 37-38

Estay, P., Wagner, A. and Escaff, M. 2001. Evaluación de *Bombus dahlbomii* (Guér.) como agente de flores de tomate (*Lycopersicon esculentum* (Mill), bajo condiciones de invernadero. [Evaluation of *Bombus dahlbomii* (Guér.) as a pollinating agent for tomato (*Lycopersicon esculentum* (Mill.)) flowers under greenhouse conditions]. *Agric. Tec.* **61**: 113-119.

Eswarappa, G. 2001. Pollination potentiality of different species of honey bees in increasing the productivity of chow-chow (*Sechium edule* (Jacq) S. W.). M.Sc. (Agri.) Thesis, University of Agricultural Sciences, Bangalore.

Folmer, O., Black, M., Hoeh, W., Lutz, R. and Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial cytochrome Coxidase subunit I from diverse metazoan invertebrate. *Mol. Mar. Biol. Biotechnol.* **3** (5): 294-299.

Free, J. B. 1993. Insect pollination of crops. 2nd edition London Academic. pp 684.

Ghazoul, J. 2005a. Buzziness as usual? Questioning the global pollination crisis, *Trends Ecol. Evol*. **20**: 367-373.

Ghazoul, J. 2005b. Response to Steffan-Dewenter *et al*.: Questioning the global pollination crisis, *Trends Ecol. Evol*. **20**: 652-653.

Giannini, T. C., Boff, S., Cordeiro, G. D., Cartolano, Jr. E. A., Veiga, A. K., Imperatriz-Fonseca, V. L. and Saraiva, A. M. 2015b. Crop pollinators in Brazil, a review of reported interactions. *Apidologie*. **46**: 209-223.

Gibbs, J. 2009. Intergative taxonomy indentifies new (and old) species in the *Lasioglossum (Dialictus) tegulare* (Robertson) (Hymenoptera, Halictidae) species group. *Zootaxa.* **2032**: 1-38.

Goulson, D. Lye G. C. and Darvill, B. 2008. Decline and Conservation of Bumble Bees. *Annu Rev Entomol.* **53**: 191.

Gualberto, R., Resende, F. V., De Guimaraes, A. M. and Ambrosio, C. P. 2007 - Performance of long-life salad tomato cultivars grown in a protected environment and under field conditions. - *UNIMAR Ciencias*. **7** (2): 133-138.

Heard, T. A. 1988. Propagation of hives of *Trigona carbonaria* Smith (Hymenoptera: Apidae). *J. Aust. Entomol. Soc.* **27**: 303-304.

Heard, T. A. 1999. The role of stingless bees in crop pollination. *Annual Review of Entomology*. **44**: 183-206.

Heard, T. A.and Hendrikz. J. K. 1993. Factors influencing flight activity of colonies of the stingless bee *Trigona carbonaria* (Hymenoptera: Apidae). *Aust. J. Zool.* **41**: 343-353

Heard. T. A. and Dollin, A. 2000. Stingless beekeeping in Australia, snapshot of an infant industry, *Bee World.* **82**: 116-125.

Hebert, P. D. N., Penton, E. H., Burns, J. M., Janzen, D. H. and Hallwachs, W. 2004. Ten species in one: DNA barcoding reveals cryptic species in the neotropical butterfly *Astraptes fulgerator*. *Proc Natl Acad Sci USA.* **101:** 14813-14817.

Hebert, P. D. N., Ratnasingham, S., and de Waard, J. R. 2003. Barcoding animal life: cytochrome *c* oxidase subunit 1 divergences among closely related species. *Proc. R. Soc. Lond. B. Biol. Sci.,* **270** (1): 96-99.

Heithaus, E. R. 1979a. Flower-feeding specialization in wild bee and wasp communities in seasonal neotropical habitats*, Oecologia.* **42**: 179-194.

Heithaus, E. R. 1979b. Flower visitation records and resource overlap of bees and wasps in northwest Costa Rica, *Brenesia.* **16**: 9-52.

Hilario, S. D. and Imperatriz, F. 1999. Flight activity and colony strength in stingless bee *Melipona bicolor bicolour* (Apidae, Meliponinae). *Rev. Brasil. Biol*. **60** (2): 299-306.

Hubbell, S. P. and Johnson, L. K. 1977. Competition and nest spacing in a tropical stingless bee community. *Ecology.* **58:** 949-963.

Inoue, T., Sakagami, S. F., Salmah, S., Nukmal, N. 1984. Discovery of successful absconding in the stingless bee *Trigona* (*Tetragonula*) *laeviceps*. *J. Apic. Res.* **23**: 136-42.

Inoue, T., Salmah, S., Abbas, I., Yusuf, E. 1985. Foraging behavior of individual workers and foraging dynamics of colonies of three Sumatran stingless bees. *Res. Popul. Ecol.* **27**: 373-392.

Jaenike, J. J. and Holt, R. D. 1991**.** Genetic variation for habitat preference: Evidence and explanations, *American Naturalist*. **137**: 67-90.

Jianhuan, Z., Xiaolin, L., Fusou, Z. and Junliang, L. 2004. Response of green house tomatoes and pepper yield and nitrogen dynamics to applied compared fertilizer. *Pedosphere.* **14**: 213-222.

Kakutani, T., Inoue, T., Tezuka, T. and Maeta, Y. 1993. Pollination of strawberry by the stingless bee, *Trigona minangkabau*, and the honey bee, *Apis mellifera*: an experimental study of fertilization efficiency, Res*. Popul. Ecol*ogy. **35**: 95-111.

Katayama, E. 1987. Utilization of honeybees as pollinators for strawberries in plastic greenhouses. *Honeybee Science.* **8**: 147-150.

Kearns, C. A. and Inouye, D. W. 1993. Techniques for pollination biologists, University Press of Colorado, Colorado. USA *Bio Science.* **47**: 297-307.

Kearns, C. A., Inouye, D. W. 1997. Pollinators, flowering plants, and conservation biology. *Annual of Botany.* **102**: 23-30.

Kelly, N., Farisya, M. S. N., Kumara T. K. and Marcela, P. 2014. Species diversity and external nest characteristics of stingless bees in Meliponiculture. *Pertanika Journal Tropical Agricultural Science.* **37** (3): 293-298.

Kerr, W. E., Carvalho, G. A. and Nascimento, V. A. 1996. Abelha uruçu: biologia, manejo e conservação. Fundação Acangaú.Belo Horizonte. pp. 31-35.

Kevan, P. G., Straver, W. A., Offer, M. and Laverty, M. 1991. Pollination of greenhouse tomatoes by bumble bees in Ontario. *Proc. Entomol. Soc. Ont.* **122**: 15-19.

Kiatoko, N., Kumar, R. S. and Langevelde, F. 2016. A vertical compartmented hive design for reducing post-harvest colony losses in three afro tropical stingless bee species (APIDAE: MELIPONINAE). *International Journal of Development Research*. **6** (8): 9026-9034.

Kiatoko, N., Raina, S. K. S., Muli, E., Mueke, J., Nkoba, K., Raina, S. K. S. and Mueke, J. 2014. Enhancement of fruit quality in *Capsicum annum* through pollination by *Hypotrigona gribodoi* in Kakamega, Western Kenya. *Entomological Science*. **17** (28): 106-110.

Kishan, T. M., Srinivasan, M. R., Rajashree, V. and Thakur, R. K. 2017. Stingless bee *Tetragonula iridipennis* Smith for pollination of green house cucumber. *Journal of Entomology and Zoology Studies*. **5** (4): 1729-1733.

Klein, A. M., Vaissière, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A. and Kremen, C. 2007. Importance of pollinators in changing landscapes for world crops. Proceedings of the Royal Society B: *Biological Sciences.* **274**: 303-313.

Koch, H. 2010. Combining Morphology and DNA Barcoding Resolves the Taxonomy of Western Malagasy *Liotrigona* Moure, 1961. (Hymenoptera: Apidae: Meliponini). http://www.bioone.org/. Accessed on 23rd April 2015.

Kumar, M. S., Singh, A. J. A. R. and Ahugunuth, G. 2012. Traditional Beekeeping of stingless bee (*Trigona* sp.) by Kani Tribes of Western Ghats, Tamil Nadu,India. *Indian Journal of Traditional Knowledge.* **11** (2): 342-345.

Kuotsu, K. 2016. Development of DNA Barcodes of Insect Pests and Natural Enemies of Major Cereal Crops in Mid Hills of Meghalaya.M.Sc. (Agri) Thesis, School Of Crop Protection College Of Post Graduate Studies (Central Agricultural University, Imphal) Umiam- 793103, Meghalaya, India.

Kwapong, P., Aidoo, K., Combey, R. and Karikari, A. 2010. Stingless bees: Importance, management and utilisation; A Training Manual for Stingless Beekeeping. pp 52-59.

Lee, S.Y., Park, H., Boo, K. S., Park, K. T., and Cho, S. 2005. Molecular identification of *Adoxophyes honmai* (Yasuda) (Lepidoptera: Tortricidae) based on mitochondrial COI gene sequences. *Mol. Cells*. **19** (3): 391-397.

Lindauer, M. and Kerr, W. E. 1960. Communication between the workers of stingless bees. *Bee World.* **41**: 65-71.

Liua, S., Leea, I. M., Ajania, U., Colea, S. R., Buring, J. E. and Mansona, J. A. 2001. Intake of vegetables rich in carotenoids and risk of coronary heart disease in men: the physicians' health study. *Intl. J. Epidemiol*. **30**:130-135.

Maeta, Y., Tezuka, T., Nadano, H. and Suzuki, K. 1992. Utilization of the Brazilian stingless bee, *Nannotrigona testaceicornis* as a pollinator of strawberries. *Honeybee Science*. **13**: 71-78.

Manger, A. 2015. Molecular characterization of fruit fly species of the genus Bactrocera in mid altitudes of Meghalaya. M. Sc. (Agri.) Thesis, School of Crop Protection College of Post Graduate Studies Central Agricultural University Umiam-793103, Meghalaya, India.

Martins, C. F., Cortopassi-Laurino, M., Koedam, D. & Imperatriz-Fonseca, V. L. 2004. Tree species used for nidification by stingless bees in the Brazilian Caatinga (Seridó, Pb; João Câmara, Rn*). Biota Neotropica*. **4** (2): 1-8.

Mc Gregor, S. E. 1976. Insect pollination of cultivated crop plants. Washington, DC: USDA (USDA agriculture handbook): pp 411-496.

Michener, C. D. 1990. Classification of the Apidae (Hymenoptera). Appendix: *Trigona genalis* Friese, a hitherto unplaced New Guinea species. University of Kansas Science Bulletin. **54**: pp 75-163.

Michener, C. D. 2000. The bees of the world. Johns Hop-kins University Press, Baltimore, **14** (1): 913.

Michener, C. D. 2007. The bees of the world. Second edition. Johns Hopkins University Press, Baltimore. **16**: 953.

Michener, C. D. 2013. The Meliponini. **In**: Vit, P., Pedro, S. R. M. and Roubik, D.W. (Eds.), Pot-Honey: A legacy of stingless bees. Springer, New York, pp 3-17.

Momose, K., Yumoto, T., Nagamitsu, T., Kato, M., Nagamasu, H., Sakai, S., Harrison, R. D., Itioka, T., Hamid, A. A. and Inoue, T. 1998. Pollination biology in a lowland dipterocarp forest in Sarawak, Malaysia. I. characteristics of the plant-pollinator community in a lowland dipterocarp forest. *American Journal of Botany*. **85** (10): 1477-1501.

Moure, J. S. 1961. A preliminary supra-specific classification of the Old World Meliponinae bees (Hymenoptera-Apoidea). *Studia Ent. Rio de J*. **4**: 181-242.

Murthy, K. S., Rajeshwari, R., Venkatesan, T. and Baby, N.L. 2011. Detection and characterization of *Wolbachia* in *Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae), a parasitoid of the diamond back moth *Plutella xylostella* (Linn.). *Journal of Biological Control*. **25** (3): 213-216.

Muthuraman, M. and Saravanan, P. A. 2004. Utilization of stingless bees for crop pollination. *Indian Bee Journal*. **66**: 58-64.

Nabhan, G. P., Allen-Wardell, G., Bernhardt, P. And Bitner, R. 1998. The potential consequences of pollinator declines on the conservation of biodiversity and stability of food crop yields, *Conserv. Biol*. **12**: 8-17.

Nayak, P. P. and Prakash, J. 2017. Molecular Characterization of the Indigenous Stingless Bees(*Tetragonula* spp. Complex) Using ISSR Marker from Southern Peninsular India. Sociedade Entomológica do Brasil 2017. https://www. researchgate. net/publication/318548508.

Nayak, P. P., Reddy, S. M. and Jayaprakash. 2012. Nesting Pattern Preferences of Stingless Bee, *Trigona iridipennis* Smith (Hymenoptera: Apidae) in Jnanabharathi Campus, Karnataka, India. *International Research Journal of Biological Sciences.* **2** (2): 44-50

Ndungu, N. N., Kiatoko, N., Ciosi, M., Salifu, D., Nyansera, D., Masiga, D. and Raina, S. K. 2017. Identification of stingless bees (Hymenoptera: Apidae) in Kenya using Morphometrics and DNA barcoding. *Journal of Apicultural Research*. **56** (4): 341-353.

Nei, M. 1978. Estimation of average heterozigosity and genetic distance from a small number of individuals. *Genetics*. **89**: 583-590.

NHB. 1998 and 2013.www.agriinfo.in

Nieh, J. C. 2004. Recruitment and communication in stingless bees (Hymenoptera, Apidae, Meliponini). *Apidologie*. **35**: 159-182.

Nkoba, K., Raina, S. K., Muli, E., Mithöfer, K.and Mueke, J. 2012. Species richness and nest dispersion of some tropical meliponine bees (Apidae: *Meliponinae*) in six habitat types in Kakamega forest, western Kenya*. International Journal of Tropical Insect Science.* **32**: 194-202.

Nogueira-Neto, P. 1997. Vida e Criação de Abelha sem Ferrão.. Editora Nogueirapis, São Paulo. pp 447.

O'Neill, S. L., Giordano, R., Colbert, A. M. E., Karr, T. L., and Robertson, H. M. 1992. 16S rRNA phylogenetic analysis of the bacterial endosymbionts associated with cytoplasmic incompatibility in insects. *Proc. Natl. Acad. Sci. USA*. **89**: 2699-2702

Packer, L., GiIbbs, J., Sheffield, C. and Hanner, R. 2009. DNA barcoding and the mediocrity of morphology. *Molecular Ecology Resources.* **9** (1): 42-50.

Palma, G., Quezada-Euán, J. J. G., Reyes-Oregel, V., Meléndez, V. and Moo-Valle, H. 2008b. Production of greenhouse tomatoes (*Lycopersicon esculentum*) using *Nannotrigona perilampoides*, *Bombus impatiens* and mechanical vibration (Hym.:Apoidea). *Journal of Applied Entomology*. **132**: 79-85.

Pedro, S. R. M. 2014. The Stingless Bee Fauna in Brazil (Hymenoptera: Apidae). *Sociobiology*. **61** (4): 348-354.

Plath, O. E. 1925. The role of bumblebees in the pollination of certain cultivated plants. *Am. Nat.* **59**: 441-451.

Prakash, K. B. 2002. Pollination potentiality of Indian honey bee *viz., Apis cerana* on the production of cucumber (*Cucumis sativus* (Linn.) S. W.: Cucurbitaceae). M. Sc. (Agri.) Thesis, University of Agricultural Sciences, Bangalore.

Rahman, A., Das, P. K., Rajkumari, P., Saikia, J.and Sharmah, D. 2015. Stingless Bees (Hymenoptera: Apidae: Meliponini): Diversity and Distribution in India. www.ijsr.net. Accessed on 27th November, 2018.

Rajasri, M., Kanakadurga, K., Durga Rani, V. and Anurudha, C. 2012. Honey bees-Potential Pollinators in Hybrid Seed Production of sunflower. *International Journal of Applied Biology and Pharmaceutical Technology*. **3** (3): 216-221.

Ramalho, M., Giannini, T. C., Malagodi-Braga, K. S. and Imperatriz-Fonseca, V. L. 1994. Pollen harvest by stingless bee foragers (Hymenoptera, Apidae, Meliponinae). *Grana.* **33**: 239-244.

Rao, M., G. and Suryanarayana, M. C., 1988. Studies on pollination of watermelon (*Citrulus lanatus* (Thumb.) Mansf.). *Indian Bee Journal*. **50**: 5-8.

Rasmussen, C. 2004. A stingless bee nesting with a paper wasp (Hymenoptera: Apidae, Vespidae). *Journal of the Kansas Entomological Society*. **77**: 593-601.

Rasmussen, C. 2008. Catalog of the Indo-Malayan/Australasian stingless bees (Hymenoptera: Apidae: Meliponini). *Zootaxa.* **1935**: 1-80.

Rasmussen, C. 2013. Stingless bee (Hymenoptera:Apidae:Meliponini) of the Indian subcontinent: Diversity,taxonomy and current status of knowledge. http/www.mapress.co/zootax.3647.3.1. Accessed on 20th June, 2018.

Rasmussen, C. and Camargo, J. M. F. 2008. A molecular phylogeny and the evolution of nest architecture and behavior in *Trigona* sp. (Hymenoptera: Apidae: Meliponini). *Apidologie*. **39**: 102-118.

Rokozeno. 2015. Influence of pollinators attractants on quantity and quality of cucumber (*Cucumis sativus* L.). M. Sc. Thesis, Nagaland University, School of Agricultural sciences and Rural Development, Medziphema Campus, India.

Roubik, D. W. 1989. Ecology and natural history of tropical bees, Cambridge Univ. Press, Cambridge, UK. *Journal of Tropical Ecology.* **9**: 248-248.

Roubik, D. W. 1995. Pollination of Cultivated Plants in the Tropics: Food and Agriculture Organization, Rome, Italy. *Agric. Serv. Bull*. **118**.

Roubik, D. W., Moreno, J. E., Vergara, C. and Wittmann, D. 1986. Sporadic food competition with the African honey bee: projected impact on neotropical social bees. *J. Trop. Ecol.* **2**: 97-111.

Roubik, D.W. 1983. Nest and colony characteristics of stingless bees from Panama. *J. Kansas Entomol. Society*. **56:** 327-355.

Roubik, D.W. 2006. Stingless bee nesting biology. *Apidologie.* **37**: 124-143.

Roubik, D.W., Moreno, J. E. 1990. Social bees and palm trees: what do pollen diets tell us? In *Social Insects and the Environment.* **In** : Proc. 11th Int. Congr. IUSSI, Bangalore India, ed. GK Veeresh, B Mallik, CA Viraktamath, 427-428.

Sakagami, S. F. 1978. "Stingless bees (excl. Tetragonula) from the continental Asia and Sri Lanka (Hymenoptera:Apidae)". J. *Faculty of Science*, *Zoology*. **21**: 165-247.

Sakagami, S. F., Inoue, T. and Salmah, S. 1990. Stingless bees of central Sumatra. **In**: Sakagami, S. F., Ohgushi, R. and Roubik, D. W. (Eds.). Natural History of social wasps and bees in equatorial Sumatra, Hokkaido University Press, Sapporo. pp 13-125.

Sakagami, S. F., Inoue, T., Yamane, S. and Salmah, S., 1983, Nest architecture and colony composition of the Sumatran stingless bee *Trigona* (*Tetragona*) *laeviceps. Kontyu*. **51** (1): 100-111.

Santos, S. A. B dos. 2004b. Pollination of cucumber (*Cucumis sativus*) by stingless bees (Hymenoptera, Meliponini). **In**: *Proc. 8th IBRA Int. Conf. Trop. Bees and VI* Encontrosobre Abelhas. pp 689.

Santos, S. A. B dos., Bego, L. R. and Roselino, A. C. 2004a. Pollination in tomatoes*, Lycopersicon esculentum*, by *Melipona quadrifasciata* and *Apis mellifera* (Hymenoptera, Apinae). **In**: Proc. 8th IBRA Int. Conf. Trop. Bees and VI Encontrosobre Abelhas. pp 688.

Santos, S. A. Bispo dos, Roselino, A. C., Hrncir, M. and Bego, L. R. 2009. Pollination of tomatoe by the stingless bee *Melipona quadrifasciata* and the honey bee *Apis mellifera* (Hymenoptera, Apidae). www.funpecrp.com.br. Accessed on 20th November, 2017.

Sarto, M. C. L del., Peruquetti, R. C. and Campos, L. A. O. 2005. Evaluation of the Neotropical stingless bee *Melipona quadrifasciata* (Hymenoptera: Apidae) as pollinator of greenhouse tomatoes, *J. Econ. Entomol.* **98**: 260-266.

Schwarz, H. F. 1939. The Indo-Malayan species of *Trigona*. *Bulletin of the American Museum of Natural History*. **76**: 83-141.

Schwarz, H. F. 1948. Stingless bees (Meliponidae) of the western hemisphere. Lestrimellita and the following subgenera of Trigona: Trigona, Paratrigona, Schwarziana, Parapartamona, Cephalotrigona, Oxitrigona, Scaura and Mourella. *Bull. Am. Mus. Nat. Hist*. **90**: 1-546.

Seeley, T. D. 1985. Honeybee Ecology, Princeton University Press, Princeton, New Jersey.

Sheffield, C. S., Hebert, P. D. N., Kevan, P.G. and Packer, L. 2009. DNA barcoding a regional bee (Hymenoptera: Apoidea) fauna and its potential for ecological studies. *Molecular Ecology Resources.* **9**: 196-207.

Silveira, F. A, Melo, G. A. R. and Almeida, E. A. B. 2002. *Abelhas Brasileiras*: Sistemática e Identificação. Fernando A. Silveira. pp 253 .

Singh, A. K. 2016. Traditional meliponiculture by Naga Tribe in India. *Indian Journal of Traditional Knowledge.* **15** (4): 693-699.

Singh, H. K. and Chauhan, A. 2018. Pollination potential of stingless bee,*Tetragonula iridipennise* Smith on horticultural crops under protected conditions in Nagaland. In: Souvenir, Nagaland Honey Bee Day 5th December 2018, Nagaland Beekeeping and Honey Mission, Government of Nagaland. 19-20.

Singh, R. and Asrey, R. 2005. Performance of tomato and sweet pepper under greenhouse. *Haryana Journal of Horticulture sciences.* **34** (1/2): 174-175.

Slaa, E. J. 2006. Population dynamics of a stingless bee community in the seasonal dry lowlands of Costa Rica*, Insects Society.* **53**: 70-79.

Solimon, M. K., Hatem, M. M., Abd elfatah, H. B., Maysa, S. E. and Mahmoud, M. 2013. Foraging Activity of four bee species on sesame flower two successive seasons in Ismailia Governorate, *Egypt.Pestic. Phytomed (Belgrade*). **28** (1): 39-45

Sommeijir, M. J . 1999. Beekeeping with stingless bees: a new type of hive. *Bee World.* **80** (2): 70-79.

Sreejith, K. and Sebastian, C. D. 2015. Molecular phylogeny and genetic analysis of green leafhopper- *Nephotettix virescens* (Distant) using mitochondrial COI gene. *Indian J. Sci. Technol.* **8** (1): 61-64.

Staden, R., Beal, K. F. and Bonfield, J. K. 2000. The Staden package. *Methods in Molecular Biology.* **132**: 115-130.

Starr, C. K. and Sakagami, S. F. 1987. An extraordinary concentration of stingless be colonies in the Philippines, with notes on nest structure (Hymenoptera: Apidae:*Trigona* spp.), *Insect Sociaux*. **34** (26): 9-107.

Steffan-Dewenter, I., Potts, S. G. and Packer, L. 2005. Pollinator diversity and crop pollination services are at risk, *Trends Ecol. Evol*. **20**: 651-652.

Stuchi, A. L. P. B. , Arnaut de Toledo, V., Lopes, D. A. , Cantagalli, L. B. and Ruvolo-Takasusuki, M. C. C. 2012. Molecular marker to identify two stingless bee species: *Tetragonisca angustula* and *Tetragonisca fiebrigi* (Hymenoptera, Meliponinae). *Sociobiology*. **59** (1).

Suriawanto, N., Atmowidi, T. and Kahono, S. 2017. Nesting sites characteristics of stingless bees (Hymenoptera: Apidae) in Central Sulawesi, Indonesia. *Journal of Insect Biodiversity.* **5:** 1-9.

Syafrizal, T. D. and Yusuf, R.. 2014. Biodiversity and habitat of trigona at secondary Tropical Rain Forest of Lempake Education Forest, Samarinda, Kalimantan Timur. *Jurnal Teknologi Pertanian.* **9** (1): 34-38.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. and Higgins, D. G. 1997. The CLUSTAL_X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research.* **25**: 4876-4882.

Vijayakumar, K. 2014. Nest and colony characters of *Trigona (Lepidotrigona) ventralis* var. *arcifera* Cockerell from North East India. *Asian Journal of Conservation Biology*. **3** (1): 90-93.

Vijayakumar, K., Muthuraman, M. and Jayaraj, R. 2013. Propagating *Trigona iridipennis* Colonies (Apidae: Meliponini) By Eduction *Method Scholars Academic Journal of Biosciences* (SAJB) *Sch. Acad. J. Biosci*. **1** (1): 1-3.

Villanueva-G.R., Roubik, D. W. and Colli-Ucán. 2005. Extinction of *Melipona beecheii* and traditional beekeeping in the Yucatán peninsula. *Bee World.* **86**: 35-41.

Visscher, P. K. and Seeley, T. D. 1982. Foraging strategy of honeybee colonies in a temperate deciduous forest. *Ecology.* **63**: 1790-1801.

Wargovich, M. J. 2000. Anticancer properties of fruits and vegetables. *Hort Science.* **35**: 573-575.

Watanabe, M. 1994. Pollination worries rise as honeybees decline. *Scienc*e. **265**: 1170.

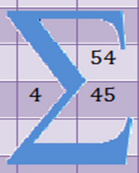
Wille, A. 1979. Phylogeny and relationships among the genera and subgenera of the stingless bees (Meliponinae) of the world. *Rev. Biol. Trop*. **27** (2): 241-277.

Wille, A., Orozco, E. and Raabe, C. 1983. Polinizaci´on del chayote *Sechium edule* (Jacq.) Swartz en Costa Rica. *Rev. Biol. Trop.* **31 (**5): 145.

Winston, M. L. and Michener, C. D. 1977. Dual Origin of highly social behavior among bees. Proc. *Nat. Acad. Sci. Wash*. **74**: 1135-1137.

Yankit., Rana, K., Sharma, H. K., Thakur, M. and Thakur, R. K. 2018. Effect of bumble bee pollination on quality and yield of tomato (*Solanum lycopersicum* Mill.) grown under protected conditions. *International Journal of Current Microbiology and Applied Sciences.* **7** (01).

Σ	23	33	14	56	76
	3	8	80	5	
	2	9	0	45	
		0			
	5	32		54	
	2	2	4	45	
	24				
	3	54			45



APPENDICES

ANOVA Ia: Two factor with replication for establishment and growth of colony (2016-17)

SUMMARY	Ho	H1	Total
Mo			
Count	3	3	6
Sum	703	1098	1801
Average	234.3333333	366	300.1667
Variance	6864.333333	1	7946.967
M1			
Count	3	3	6
Sum	210	577	787
Average	70	192.3333	131.1667
Variance	1900	14636.33	11104.17
M2			
Count	3	3	6
Sum	975	480	1455
Average	325	160	242.5
Variance	1225	19600	16497.5
Total			
Count	9	9	
Sum	1888	2155	
Average	209.7777778	239.4444	
Variance	15028.69444	17764.53	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	88563.11111	2	44281.56	6.007446	0.015567	3.885294
Columns	3960.5	1	3960.5	0.5373	0.477629	4.747225
Interaction	85329.33333	2	42664.67	5.788092	0.017388	3.885294
Within	88453.33333	12	7371.111			
Total	266306.2778	17				

ANOVA Ib: Two factor with replication for size of honey and pollen chamber (2016-17)

SUMMARY	Ho	H1	Total
Mo			
Count	3	3	6
Sum	335	621	956
Average	111.6667	207	159.3333
Variance	1758.333	63	3455.067
M1			
Count	3	3	6
Sum	210	347.5	557.5
Average	70	115.8333333	92.91667
Variance	100	5689.583333	2946.042
M2			
Count	3	3	6
Sum	895	685	1580
Average	298.3333	228.3333333	263.3333
Variance	37008.33	92808.33333	53396.67
Total			
Count	9	9	
Sum	1440	1653.5	
Average	160	183.7222222	
Variance	20806.25	27318.06944	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	88538.03	2	44269.01	1.932757	0.187225	3.885294
Columns	2532.347	1	2532.347	0.110561	0.745243	4.747225
Interaction	21601.36	2	10800.68	0.471551	0.635122	3.885294
Within	274855.2	12	22904.6			
Total	387526.9	17				

ANOVA Ic: Two factor with replication for honey production (2016-17)

SUMMARY	Ho	H1	Total
Mo			
Count	3	3	6
Sum	351.1	1520	1871.1
Average	117.0333333	506.6667	311.85
Variance	40705.12333	933.3333	62199.62
M1			
Count	3	3	6
Sum	1.2	1	2.2
Average	0.4	0.333333	0.366667
Variance	0.07	0.023333	0.038667
M2			
Count	3	3	6
Sum	551.4	650.3	1201.7
Average	183.8	216.7667	200.2833
Variance	100576.83	140768.3	96864.11
Total			
Count	9	9	
Sum	903.7	2171.3	
Average	100.4111111	241.2556	
Variance	41782.59111	83832.78	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	298871.3233	2	149435.7	3.168429	0.078548	3.885294
Columns	89267.20889	1	89267.21	1.8927	0.194033	4.747225
Interaction	140084.2011	2	70042.1	1.485077	0.265296	3.885294
Within	565967.4467	12	47163.95			
Total	1094190.18	17				

ANOVA Id: Two factor with replication for durability and portable of hives (2016-17)

SUMMARY	Ho	H1	Total
Mo			
Count	3	3	6
Sum	23	36.3	59.3
Average	7.666666667	12.1	9.883333
Variance	14.33333333	0.01	11.63367
M1			
Count	3	3	6
Sum	15.5	17.1	32.6
Average	5.166666667	5.7	5.433333
Variance	1.083333333	0.07	0.546667
M2			
Count	3	3	6
Sum	30.1	27	57.1
Average	10.03333333	9	9.516667
Variance	12.20333333	7	8.001667
Total			
Count	9	9	
Sum	68.6	80.4	
Average	7.622222222	8.933333	
Variance	11.34694444	9.4525	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	73.22111111	2	36.61056	6.330355	0.013276	3.885294
Columns	7.735555556	1	7.735556	1.33756	0.26997	4.747225
Interaction	23.77444444	2	11.88722	2.055427	0.170756	3.885294
Within	69.4	12	5.783333			
Total	174.1311111	17				

ANOVA IIa: Two factor with replication for establishment and growth of colony (2017-18)

SUMMARY	Ho	H1	Total
Mo			
Count	3	3	6
Sum	644	1098	1742
Average	214.6666667	366	290.3333
Variance	17190.33333	1	13747.07
M1			
Count	3	3	6
Sum	118	122	240
Average	39.33333333	40.66667	40
Variance	26.33333333	104.3333	52.8
M2			
Count	3	3	6
Sum	1099	1098	2197
Average	366.3333333	366	366.1667
Variance	2.333333333	1	1.366667
Total			
Count	9	9	
Sum	1861	2318	
Average	206.7777778	257.5556	
Variance	24388.94444	26487.03	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	349604.3333	2	174802.2	60.53638	5.38E-07	3.885294
Columns	11602.72222	1	11602.72	4.018181	0.06811	4.747225
Interaction	22752.77778	2	11376.39	3.939799	0.048377	3.885294
Within	34650.66667	12	2887.556			
Total	418610.5	17				

ANOVA IIb: Two factor with replication for size of honey and pollen chamber (2017-18)

SUMMARY	Ho	H1	Total
Mo			
Count	3	3	6
Sum	675	645	1320
Average	225	215	220
Variance	2275	25	950
M1			
Count	3	3	6
Sum	180	193	373
Average	60	64.33333333	62.16667
Variance	100	16.33333333	52.16667
M2			
Count	3	3	6
Sum	1325	1420	2745
Average	441.6667	473.3333333	457.5
Variance	19308.33	10408.33333	12187.5
Total			
Count	9	9	
Sum	2180	2258	
Average	242.2222	250.8888889	
Variance	32900.69	34702.11111	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	475212.1	2	237606.1	44.36674	2.86E-06	3.885294
Columns	338	1	338	0.063113	0.805893	4.747225
Interaction	1344.333	2	672.1667	0.12551	0.88319	3.885294
Within	64266	12	5355.5			
Total	541160.4	17				

ANOVA IIc: Two factor with replication for honey production (2017-18)

SUMMARY	Ho	H1	Total
Mo			
Count	3	3	6
Sum	351	1660	2011
Average	117	553.3333333	335.1667
Variance	40716.76	2533.333333	74416.07
M1			
Count	3	3	6
Sum	0.6	0.7	1.3
Average	0.2	0.233333333	0.216667
Variance	0.01	0.023333333	0.013667
M2			
Count	3	3	6
Sum	1710	1980	3690
Average	570	660	615
Variance	7900	400	5750
Total			
Count	9	9	
Sum	2061.6	3640.7	
Average	229.0667	404.5222222	
Variance	80094.6	94807.01944	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	1136913	2	568456.7	66.16357	3.3E-07	3.885294
Columns	138530.9	1	138530.9	16.12383	0.001713	4.747225
Interaction	159199.2	2	79599.62	9.264724	0.003688	3.885294
Within	103100.3	12	8591.688			
Total	1537744	17				

ANOVA IId: Two factor with replication for durability and portable of hives (2017-18)

SUMMARY	Ho	H1	Total
Mo			
Count	3	3	6
Sum	28	36.3	64.3
Average	9.333333	12.1	10.71667
Variance	9.333333	0.01	6.033667
M1			
Count	3	3	6
Sum	16.5	17.1	33.6
Average	5.5	5.7	5.6
Variance	0.25	0.07	0.14
M2			
Count	3	3	6
Sum	36.4	27	63.4
Average	12.13333	9	10.56667
Variance	0.023333	7	5.754667
Total			
Count	9	9	
Sum	80.9	80.4	
Average	8.988889	8.933333333	
Variance	10.71861	9.4525	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	101.7411	2	50.87056	18.29145	0.000227	3.885294
Columns	0.013889	1	0.013889	0.004994	0.944826	4.747225
Interaction	26.25444	2	13.12722	4.720136	0.03074	3.885294
Within	33.37333	12	2.781111			
Total	161.3828	17				

ANOVA IIIa: Single Factor ANOVA on mean tomato yield per plant/season (2016-17)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Controlled green house	7	9400	1342.857143	55323.80952
green house with stingless bee	7	9670	1381.428571	19380.95238
Open condition	7	7410	1058.571429	29680.95238

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	435266.67	2	217633.3333	6.2546873	0.008661	3.554557
Within Groups	626314.29	18	34795.2381			
Total	1061581	20				

CV	14.793169
SEM	70.503534
CD 5%	243.78431

ANOVA IIIb: Single Factor ANOVA on mean tomato yield per plant/season (2017-18)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Controlled green house	7	9230	1318.571429	52647.619
green house with stingless bee	7	9190	1312.857143	23657.143
Open condition	7	7300	1042.857143	26723.81

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	347552.38	2	173776.1905	5.0600388	0.018043	3.554557
Within Groups	618171.43	18	34342.85714			
Total	965723.81	20				

CV	15.130962
SEM	70.043718
CD 5%	242.19437