STATUS AND THE POTENTIAL OF STINGLESS BEES *(APIDAE: MELIPONINAE)* FOR FOREST CONSERVATION AND INCOME GENERATION: A CASE STUDY OF KAKAMEGA FOREST

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A thesis submitted in partial fulfilment for the Degree of Master of Science in Zoology in the Jomo Kenyatta University of Agriculture and Technology

2008

DECLARATION

This thesis is my original work and has not been presented for degree in any other University.

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This work is dedicated to my late brother, Jack. Thank you for all the support you gave me. You laid a good foundation that has seen me through to this level of education.

ACKNOWLEDGEMENTS

I highly appreciate and acknowledge the financial support for this study from the International Fund for agricultural Development (IFAD), Rome, Italy and UNDP-GEF through the commercial insect project, (*icipe*). It is my pleasure to thank Prof. Christian Borgemeister the Director General, (*icipe*), Prof. Raina, the Project Leader, Commercial Insects for all the research facilities and provisions that I enjoyed during the course of this study and the Director of Training and Capacity Development for awarding me the Dissertation Research Internship Program (DRIP) fellowship. I would like to thank the department of Zoology (JKUAT) for the assistance provided throughout my study period.

My sincere appreciation to Prof. Raina, the Pogramme Leader Commercial Insect project for introducing me into the fascinating world of stingless bees and providing me with the necessary support throughout my study. His constant moral support when the going got tough and keen supervision through out the study gave me a lot of inspiration.

Many thanks to Prof Linus Gitonga, my university supervisor, for his great input, critical comments and his many valuable suggestions that have shaped this research work. He tirelessly and in the shortest time possible reviewed, corrected and brought in critical scissions into the drafts of this thesis. I am also indebted Dr. Kutima for the guidance, encouragement and correction that she provided throughout the study period.

I am highly indebted to Dr. Muli and Dr. Biesmeijer, they were always at hand and ready to help with useful suggestions and constant encouragement.

With pleasure, I wish to express my sincere gratitude to my family members for their love, support and encouragement throughout the study. My sincere appreciation to all colleagues at Commercial Insect Program, for their support. Many thanks to my farmers around Kakamega forest, for their hospitality and support in the field; they always made me feel that I was doing something worthwhile. To all my friends and lecturers, I am deeply indebted to you.

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LIST OF ACRONYMS AND ABBREVIATIONS

ATCC	American Type Culture Collection
NMK	National Museums of Kenya
ICIPE	International Centre of Insect Physiology and Ecology
HMF	Hydroxymethylfurfural ,

ABSTRACT

Stingless beekeeping (meliponiculture) is a unique eco-friendly agro-practice with the potential for environmental amelioration, employment and income generation. Meliponiculture would be ideal for generation of supplementary income to resource-poor farmers around forests, in addition to conservation of stingless bees. Assessment of the knowledge of stingless bees by community around Kakamega forest indicated that most people know stingless bees but they do not undertake any active conservation measures. Results obtained from this study indicated that two genera of stingless bees, *Meliponula* and *Hypotrigona* occur in Kakamega forest. The community around Kakamega forest identifies stingless bees by their morphological features, nesting architecture and taste, smell and colour of their honey. Trials on queen rearing process of *H. gribodoi* indicated that this species rears queens in two ways: (i) using a special queen cell which is bigger than other brood cells (ii) through emergency queen rearing whereby they join two brood cells, destroying one developing larvae. This queen rearing can be used in colony multiplication in stingless bees rearing. Honey from five species (*Hypotrigona gribodoi, Meliponula bocandei, M. ferruginea (black), M. ferruginea (white,), Plebeina spp*) varied in composition. The quality variables analyzed varied as follows: moisture, Hydroxymethylfurfural (HMF), diastase activity, proline, free acidity and pH. Moisture content was higher than that of *Apis mellifera*. Studies on antimicrobial activity of the stingless bees against 5 strains of bacteria; *Pseudomonas aeruginosa*, *Salmonella typhi, Escherichia coli, Staphylococcus aureus* and *Bacillus subtilis*, indicated susceptibility of the bacteria to the honey.

Escherichia coli and *P. auregnosa* were the most susceptible bacteria. Moreover, honey from *P. hildebrandti* and *M. ferruginea* (b) showed the highest antibacterial effect. This study forms a foundation of involving farmers around Kakamega forest in stingless bees keeping as incentive for forest conservation and income generation.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 General Introduction

Wild and managed honeybees seem to be declining rapidly around the world (Biesmeijer *et al.*, 2006). This is causing concern about the future of pollination services for crops and wild plants. Traditionally, agriculture has relied on managed honeybees, *Apis mellifera* (Linnaeus) for pollination of insect-dependent crops. However, recent problems of disease (e.g. varroa, small hive beetle) and defensiveness (e.g. African honeybees in the Americas) honeybee keeping have come under pressure. In addition, it has become clear that other bees do pollinate many crops more efficiently. (Eardley, 2004). Threats to apiculture, have led to the understanding that diversification of the commercially available pollinators is needed to ensure food production in future. Many stingless bees species could be used as surrogates for honey bees (Eardley, 2004).

Stingless bees (Meliponinae) are social bees in the family Apidae. They live in permanent colonies that often contain many thousands of bees (Michener, 2000). There are probably 400-500 species of stingless bees (Apidae, Meliponini) in the world, all of which are highly eusocial. They differ from *A. mellifera* at the subfamily level and are known as Meliponinae (Camargo and Pedro, 1992), which was recently renamed as Meliponini (Michener, 2000).

In Africa, 6 genera of stingless bees comprising of 19 species are known to occur. They are all social. In five of the genera (*Dactylurina*, *Meliponula*, *Plebeina*, *Hypotrigona* and *Liotrigona)*, workers collect pollen and nectar from flowers, and in one genus (*Cleptotrigona* they rob pollen and nectar from the nests of other stingless bees (Eardley, 2004). In Kenya the following species of stingless bees have been found; *Meliponula bocandei* (Spinola), *M. lendliana* (Friese), *Plebeina hildebrandti* (Friesa), *Hypotrigona araujo* (Michener), *Meliponula (Meliplebeina) becarii* (Friese), *M. erythra* (Schletterer), *Hypotrigona gribodoi* (Magretti), *Dactylurina schmidti* Stadelmann, *M. ferruginea* (Lepeletier) in Kakamega Forest, Mwingi District and Arabuko Sokoke (Eardley, 2004; Macharia, 2007; Gikungu, pers. com.).

Stingless bees colonies contain several hundreds to tens of thousands of worker bees, a single egg-laying queen and sometimes a couple of males (Michener, 2000). They also vary greatly in size and appearance: some are slender, some are broad; some are shiny and hairy; their colours are various, and some are metallic (Crane, 1990). The sting is atrophied, the tip of the sting and the venom apparatus having 'dwindled away'. Their defense against man includes biting, irritating by crawling into eyes, ears and by ejecting a caustic fluid (Schwarz, 1948). They nest in cavities, which may be underground or in trees or other enclosed spaces such as termites' nests (Crane, 1990). Stingless bees are relatively old lineage and the species vary widely in their physiology, social organization and ecology. This makes them excellent subjects for comparative studies. They also have a long history in human culture both as providers of honey and wax (or cerumen) and as pollinators of crops (Biesmeijer *et al.*, 2006)

Potential ecological factors that affect population of stingless bees include availability of and competition for food, availability of nest site and predation (Slaa, 2003). Kakamega forest reserve, which is a tropical forest, has the above factors, which has enabled it to support about six species of stingless bees (Macharia, 2007). Like any other rainforests, Kakamega Forest reserve is among the most endangered ecosystems, yet it offers very important direct and indirect values to man. It is now the only surviving rainforest in Kenya (Kokwaro, 1988) The forest is an island of relatively 'natural' habitat in a sea of human-dominated landscape. The forest has been a resource for local people for generations, as a source of fuel wood, building poles and household items (like vines-ropes) and food (honey, bush meat). Many of these traditional uses are outlawed, but they continue, at least partly because local people have no easy alternatives, and also they do not understand how their actions and choices can influence the future of their community and families (Gikungu, 2006). Kakamega forest, as well as other indigenous forests, provides several services and benefits: catchments protection, wildlife conservation, pole and fuel wood conservation and provisions of medicinal plants and animals (Emmerton, 1991). The need to ensure all these benefits on a continued basis has resulted in a zoning management scheme that attempts to reconcile the often-conflicting management goals of conservation and production. At the turn of the 20th century, there were 240,000 hectares of rain forest in Kenya. Now there are only 23,000 ha left due to severe deforestation and fragmentation (Kokwaro, 1988). It is believed that the average annual rainfall in this region may be decreasing due to the deforestation (Kokwaro, 1988). Zimmerman (1972) recorded over 3,500 mm rainfall during 1963, indicating substantial fluctuations. This may be contributed by encroachment of *shambas* (small

farms) and tea plantations as well as legal and illegal government sanctioned harvesting by the timber industry (Mitchel, 2004). Due to the two rainy seasons, two growing seasons occur each year, which accelerates the rate at which nutrients are taken from the soil. This continual depletion of nutrients makes it more difficult for forested land to reestablish when farms are abandoned. For the local families, the forest is extremely important for several reasons. Fuel wood is the dominant energy source for the people around the forest, grazing land and lumbering. These activities endanger stingless bees that nest in tree cavities and collect some of their resources from these trees.

Stingless bees keeping, known as Meliponiculture, is growing fast in some countries like Mexico and Australia, with the later having an estimated 250 stingless beekeepers with more than 400 colonies. (Heard and Dollin, 2000). Some of the reasons for this growth include the realization that stingless bees can be propagated and kept in hives, the rising need of conserving and protecting native bee species from threats such as environmental degradation and competition from honey bees (Heard and Dollin, 2000). In Africa, meliponiculture is uncommon, and harvesting of meliponine honey is mostly destructive. Replacing destructive harvesting with meliponiculture would provide honey for food and medicine and enhance pollination of both commercial crops and indigenous plants (Eardley, 2004). To develop meliponiculture in Kenya, among other things it would require understanding the awareness of the community on importance and conservation of stingless bees.

Natural requeening after queen loss, as it usually occurs in honeybees, has been regarded as highly improbable in stingless bees (Faustino, 2002). This has made it impossible for artificial queen rearing which is important in colony multiplication. Studies were carried out on *H. gribodoi* on emergency queen rearing with the aim of developing a sustainable colony multiplication in stingless bees.

Although several *Apis* species and stingless bees produce honey, widely relished as human food, the official definition of 'honey' is restricted to the honey of *A. mellifera* by the Codex Alimentarius Commission (Patricia *et al.*, 2004). Official methods for honey standards have been developed for *A. mellifera* and these are periodically reviewed by the Codex Alimentarius and the International Honey Commission (IHC) (Bogdanov, 1997). There are no standards for stingless bees honey in worldwide, therefore, this study will broaden the data bank regarding honey produced by Meliponines, for the International Honey Commission, featuring its standardization. Besides promoting knowledge regarding honey from stingless bees, this work is a contribution for the data bank to adjust the quality standards of honey from stingless bees and especially in Africa.

Honey from stingless bees is considered in folk medicine to be more powerful than honeybee honey for use in the treatment of common diseases (Vit, 2001; Garedew *et al.*, 2004). Beekeepers and honey enthusiasts alike have long reported the medicinal qualities of honey (Crane, 1990; Molan, 1997). Much "folklore" has been attached to the putative medicinal effects of honey (Ransome, 1986). Although honey has been used as a medicine since ancient times, its effectiveness as a potential against bacterial

infections has been revealed only a century ago (Molan, 2001). Most of these studies have been conducted using honey produced by *A. mellifera*. However, little has been done to investigate antibacterial effects of stingless bee honey (Vit *et al.*, 1994(a); de Brujin and Sommeijer, 1995; Sommeijer *et al,* 1995).

This study investigated the antibacterial effects of honey from stingless bees namely *M. bocandei*, *M. lendliana*, *M. ferruginea*, *H. gribodoi*, *P. hildebrandti* and *A. mellifera* from Kakamega forest.

1.2 Literature Review

1.2.1 Classification

Together with honeybees (*Apini*), bumble (*Bombini*) and orchid bees (*Euglossini*), stingless bees (*Meliponini*) form a distinct clade (subfamily *Apinae*) within the *Apidae* family (Roig- Alsina and Michener, 1993). *Meliponines* are highly eusocial bees that live in perennial colonies of a few hundred to tens of thousands of bees, normally descendants of a single queen (Michener, 1974; Sakagami, 1982; Willie, 1983)

Classification of stingless bees is complex. Camago *et al.*, (1992) and Crane, (1992), reported that there are 500 species, while Sommeijer *et al.* (1994) reported about 400 species of stingless bees. World-wide, stingless bees (Sub family *Meliponinae*) can be divided into two tribes: *Melipona* and the *Trigonini* with a large number of genera and sub-genera (Sommeijer *et al.*, 1995). The most recent of stingless bees, the Meliponini comprises 23 genera and 18 subgenera, which consist of 374 recognized species (Michener, 2000). A new classification including the stingless bees in the subfamily Apinae, tribe Apini and sub tribe Meliponini (Silveira *et al.*, 2002) continues the taxonomic debate. Stingless bee taxonomy in Africa was reviewed by Eardley (2004).

There are over 20 stingless bee species in Africa, but much work remains to be done on their biology and behaviour.

1.2.2 Distribution

Stingless bees are considered to have their center of origin in Africa and have dispersed to other tropical and subtropical parts of the world, based on paleontological and biogeographic data (Velthuis, 1997). This hypothesis is also supported by the fact that their primitive species with a well-developed sting system live extensively in Africa (Willie, 1983). Another theory suggests the center of diversity of stingless bees to be South America where meliponiculture is practiced extensively (Eardley, 2004). Stingless bees occur in tropical and subtropical regions of the world. Most stingless bees are in tropical South and Central America- 260 species in Brazil and 20 to 40 each in the tropics of Africa, Asia and Australia (Crane, 1990). *Melipona* species are found only in tropical America (Roubik, 1990). They have the largest body size, 15mm, which is as big as *A. mellifera. Trigona* is an extensive genus of long-winged bees in tropical parts of all continents. (Michener, 2000).

1.2.3 Colony cycle and swarming

New colonies are founded by swarming. In contrast to honey bees, where swarming involves more or less instantaneous departure of the old mother queen and large mass of workers (Michener, 1974), colony establishing is a gradual process in *Meliponines*. After scout bees have located a new nest site, workers from the old colony first translocate nest material and food. Later a virgin queen arrives with actual swarm, makes her nuptial flight, and brood cell construction and oviposition are initiated in the

new nest (Sakagami, 1982). Several weeks or even months can pass until complete independence of the daughter colony to their mother colony (Willie, 1983). Once safely established, individual Meliponini colonies are known to survive for 10 to 26 years (Willie, 1983; Roubik, 1989).

1.2.4 Nesting

Nests of most stingless bees are mainly constructed with cerumen a mixture of wax with resin and gums (propolis) collected from plants and brought to the nest (Sakagami, 1982; Willie, 1983). Different species have varying nest sites, ranging from exposed nests constructed in vegetation, over nests built in underground cavities, to nests in pre-existing cavities in trees (Sakagami *et al.*, 1983). Within this envelope, brood cells are constructed in clusters or combs. Both pollen and nectar (floral and extra-floral) are collected by foraging workers and deposited in special storage pots within the nest. Food storage allows colonies to survive for months without incoming food (Roubik, 1989).

1.2.5 Initial triggers of caste development

In contrast to honeybees, brood rearing in stingless bees is always done through mass provisioning strategy (Sakagami, 1982), in caring for their brood, that is, brood cells built out of wax and resin mixture are filled with well defined amount of larval food regurgitated from the crops of nurse-stage workers (Hartfelder *et al.*, 1987). The system consists of condensed sequence of cell construction, cell provisioning, egg laying and cell enclosure. They also show highly developed social interactions among colonies. They rear brood in mass provisioning whereby

within the cell, the eggs develop into larvae which pupates after consuming the food. In stingless bees, there is no contact between the adult population and the developing larvae (Velthius and Sommeijer 1991). Production of new individuals begins with cell construction. New brood cells are added at the margin of the comb, being built by a number of workers, working independently. A completed cell protrudes above the comb and may remain for several hours until the queen appears in the comb. Upon finding the empty cells, she starts the complex "Provisioning and Oviposition Procession" (POP) (Velthius, 1997). She produces a pheromone by which she attracts workers. This forms a court around the queen and the cell. The queen stimulates the inserting worker to regurgitate food from her crop into the cell. Once a first worker does so, a number of other workers will do the same. Workers that provisioned the cell, quickly move to the other comb where they beg food from other workers. Once enough food is accumulated in the cell, a worker may lay an egg in the cell. The queen invariably eats these worker eggs. The queen also eats some food from the cell provisioning before she herself lays an egg on top of the food. Then she leaves the area and a worker takes position onto the cell to fold the collar inward by rotation, until the collar becomes lid of the cell, thus sealing the cell (Velthius, 1997).

1.2.6 Regulation of colony foraging

Social bee colonies are subject to a continuously changing state in external and internal environment to which they need to respond adequately to survive (Biesmeijer *et al.*, 1999). Roubik (1982) found that when food reserves were low in colonies of *Melipona* at the end of the wet season in tropical Guyana, brood cannibalism occurred and brood

production ceased. In addition foragers did not increase their foraging effort in response to low food reserves. At the same time, lifespan of individual workers doubled. Pollen foraging mainly depended on positive cues related to pollen availability in the habitat, for instance. the number of successful pollen foragers returning to the nest (Biesmeijer *et al,* 1999a). The response therefore, of stingless bees to internal or (perceived) external resource dearth seems more conservative than that of honeybees. This strategy is probably facilitated by the long individual life-spans and long larval development times of Meliponines, as well as by the fact that large amounts of pollen are stored within the developing brood cells (Biesmeijer *et al.*, 1999).

1.3 Statement of the problem.

With the growing pressure on the environment and associated loss of honeybees, the need exists for additional pollinator species to be used in agriculture to maintain resilience in food production and improve yield. Stingless bee keeping is an activity that can be integrated into forestry to diversify income from non-timber products and horticulture for pollination services. Pollination contributes to increase in agricultural production and regeneration of natural vegetation. Beekeeping development programmes in Kenya have largely ignored utilization of stingless bees, mainly due to lack of awareness on their potential for honey production, pollination services and forest conservation. There is also very little knowledge on stingless bees taxonomy, honey and colony multiplication. Despite the richness in biodiversity of Kakamega

forest, there has been a lot of anthropogenic destruction due to uncontrolled harvest of forest resources (Onyango *et al.*, 2004). To mitigate on this, the keeping and utilization of stingless bees as an income generating option can raise the economy of communities around the forest and help conserve the forest.

1.3.1 Justification of the Study

The fundamental goal of biodiversity conservation is to support sustainable development by protecting and using biological resources in ways that do not diminish the world's variety of genes and species or destroy habitats or ecosystems. With the increasing concern for biodiversity and the mounting evidence of irreversible environmental damage, there is need to involve the local communities in the utilization and conservation of their indigenous biodiversity. A solution to this depletion of the biodiversity lies in introducing economic incentives that integrate conservation with economic development of the rural people (Munthali and Mughogho, 1992, Raina, 2000, Raina *et al.*, 2000, Rodgers, 2005, Salehe, 2005). Stingless bees can be utilized for economic incentive for their conservation because they yield highly medicinal honey and provide pollination services both to cultivated crop and wild plants. Very little research has been done on the stingless bees in Kenya. Virtually, nothing has been reported on their conservation, queen rearing process, physio-chemical composition and antibacterial activity of their honey. Hence the need to collect some of this information which will provide basis for meliponiculture development.

1.4 Hypotheses

1. There are no measures to conserve stingless bee species in Kakamega forest.
2. Honey produced by different species of stingless bees found in Kakamega forest differs in physicochemical composition.
3. Honey produced by different species of stingless bees found in Kakamega forest differs in their antibacterial activity.
4. Queen rearing differs in the species of stingless bees found in Kakamega forest.

1.4 Objectives

1.5.1 General objective

To study the conservation status of stingless bees in Kakamega forest and set up queen rearing methods, set preliminary standards and antibacterial effect of their honey.

1.5.2 Specific Objectives

1. To evaluate the conservation status of stingless bees in Kakamega forest.
2. To determine the chemical composition of honey of various stingless bees species
3. To determine the antibacterial activity of stingless bees honey
4. To establish the queen rearing process and colony multiplication in stingless bees species in Kakamega forest.

CHAPTER 2

GENERAL MATERIALS AND METHODS

2.1 Study Area

Kakamega forest reserve is the easternmost remnant of the rainforests of Zaire and West Africa. It is situated in western Kenya (Fig 2.1), between Latitudes 00° 08' and 0° 21' North and 34° 58' Longitude East. The altitude ranges from between 1520 – 1680 meters above sea level (Kokwaro, 1988). Temperature ranges between 10.6 ° C and 27.7 ° C. The entire forest covers an area of approximately 265 km^2 (Muriuki and Tsingalia, 1990). The forest was first gazzeted as a trust land in 1993, although two small Nature reserves, Yala and Isecheno forest totaling about 700 ha had been established within the forest Reserve in 1967 under the management of Kenya Forest Department (KIFCON, 1994). Mutangah *et al.* (1992) recorded the Buyangu block as having the highest tree density of all the Kakamega forest plots. The Kakamega Forest complex lies mostly to the west of Kakakmega town and about 40 km North of L. Victoria (Fig. 2.2). It comprises several separate blocks of forest: Kakamega (13, 878.2ha), Bunyala (825.6 ha), Malava (722.8 ha), Kabiri (3,691.3 ha), Lirhanda hill (52.7 ha), Kisere (471.4 ha), Yala river (2895 ha), Isecheno (415 ha), Ikuywa (380 ha) and Buyangu (3, 997.5 ha). The entire Kakamega Forest area has relatively flat to gently undulating topography, except for a few steep hills like the Buyangu to the north and the Lihanda to the south. To the east, it is bordered by the Nandi Escarpment, which rises up to 2,200 meters above sea level.

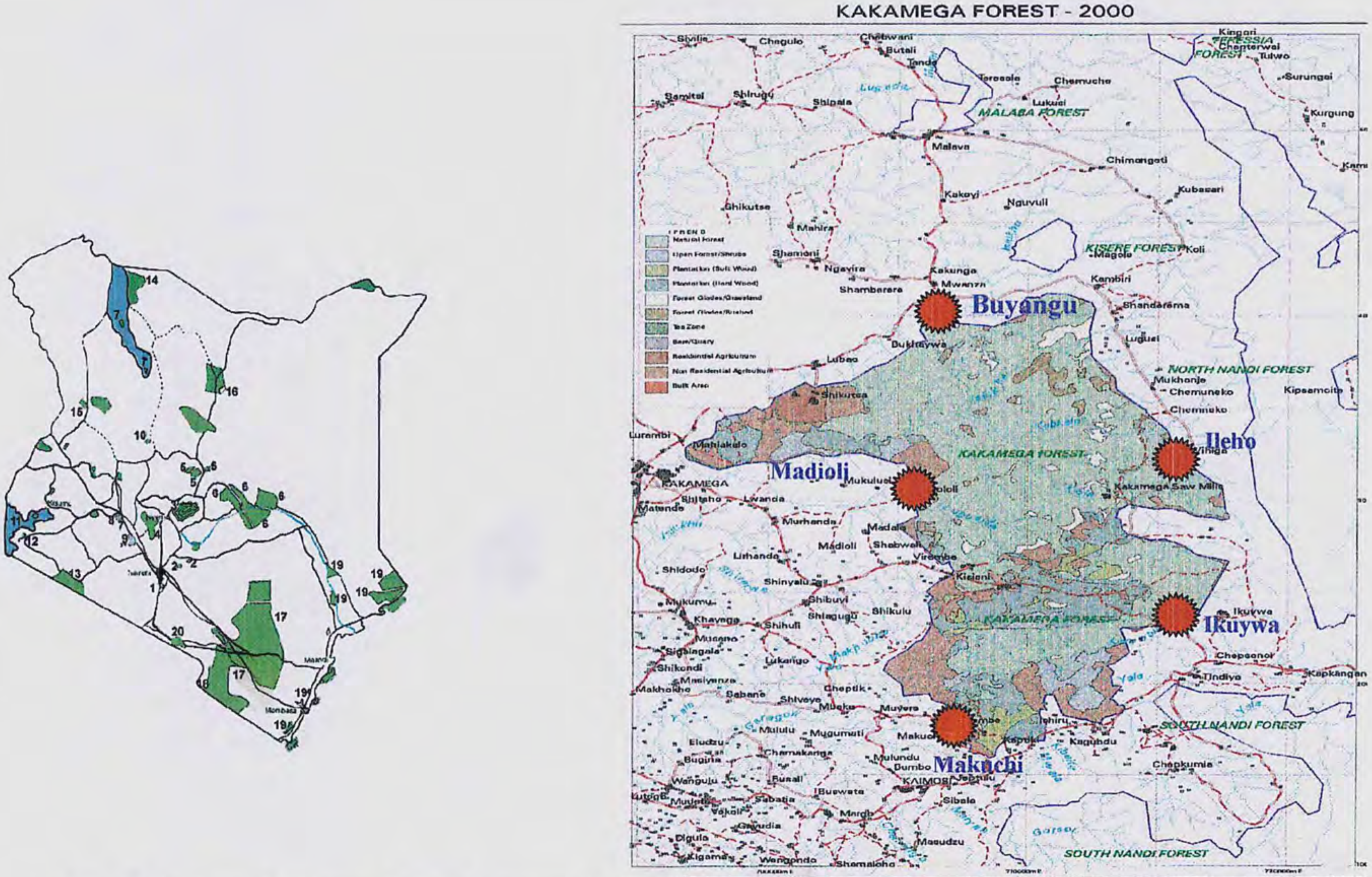

Fig. 2.1. Location of Kakamega Forest in Kenya and the map of the forest

The main vegetation in the Kakamega forest is rainforest vegetation type. There also are other habitats including indigenous forests, colonising forests, disturbed forests and areas of planted forests, glades and riverine forests. Kakamega Forest is classified as a tropical rainforest situated in a fairly wet area of Kenya with an average annual rainfall of 2,500 mm (Emmerton, 1991). The majority of families living around the forest and making use of its resources are of the Luhya tribe. Within the Luhya tribe, the Tiriki and Isukha clans are most abundant in the Kakamega region. Most families live on self-sustaining *shambas*. The primary crops grown by the families in this region are maize, beans, cassava, bananas, and *sukuma wiki* (Kales).

2.2 Status of stingless bees

To document the knowledge that communities have on the ecology of stingless bees, informal interviews using a questionnaire were administered to 76 people. The members were selected from community-based organizations around Kakamega by random selection during the group meetings. The data collected included: local names used in identification of different stingless bees species, medicinal uses of honey, conservation measures on stingless bees and importance of stingless bees. Colonies of stingless bees were easily found through conversation with local farmers, honey hunters, woodcutters or beekeepers and honey hunters. Most of the colonies were identified and described with honey gatherers in Kakamega forest during the course of the study. They were then identified using the key developed by Eardley, (2004) and in the National Museums of Kenya.

2.3 Queen rearing

2.3.1 Emergency queen rearing/replacement

Three colonies of *H. gribodoi* queens were used. They were transferred from a natural colony (tree cavity) to a rational hive. The queens were removed from the colonies observations made on construction of new brood cells daily for three months. Search of any new queen cells was made to confirm that there were no new developing queens or queen cells (which are bigger compared to worker cells) in all three colonies. The time taken for the construction of the queen cells, the number of royal cells and time taken for the queen to hatch were recorded.

2.4 Physiochemical analysis of honey

A total of 120 samples of honey samples from the five different species of stingless bees were collected between August and December 2006 around the Kakamega forest. Target sampling was used in nest location for honey collection. The honey was collected directly from the pots in the nests using sterile syringes then transferred to 4ml glass vials fitted with rubber septa and stored away from the sunlight and analyzed within two weeks.

The methods used for analysis were based on those of the Association of the Official Analytical Chemists (AOAC, 1990) and International Honey Commission (Bogdanov *et al.*, 1997; Bogdanov, 1999). The percentage of moisture in honey was determined using a hand-held honey refractometer (HHR – ATAGO, Model REF 106c). Hydroxymethylfurfural (HMF) content was determined using the spectrophotometric

method (White, 1979) on UV absorbance at 284nm (CECIL CE 3041 3000 Series, CECIL Instruments, Cambridge, England). Diastase activity of each honey sample was quantified by spectrophotometric method (Schade *et al.*, 1958) at UV absorbance 660nm (CECIL CE 3041 3000 Series, CECIL Instruments, Cambridge, England). Free acidity was quantified by volumetric - titration of a honey sample titrated with 0.1M NaOH until a pH of 8.3 was attained and the result expressed in milliequivalents of acid per 1000g of honey. Proline was quantified using spectrophotometric method and absorbance determined at 510nm (CECIL CE 3041 3000 Series, CECIL Instruments, Cambridge, England).

2.5 Antibacterial activity of stingless bees honey

2.5.1 Bacterial Cultures

Five bacterial strains were used in the study; *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhi* (ATCC 2202), *Escherichia coli* (STD 25922), *Staphylococcus aureus* (ATCC 20591) and *Bacillus subtilis* (ATCC 6633) were obtained from Incline International, Nairobi, Kenya. The bacteria were used for the five species of stingless bee honeys.

2.5.2 Antibacterial activity tests

Agar disc diffusion method (Allen, 1991) was employed to test the antimicrobial activity of the stingless bee honey. The inoculum was prepared with fresh cultures of bacterial strains, cultured on nutrient agar. A loopful of the bacterial culture was inoculated into a nutrient broth medium and incubated for 24 hours at 37^0C. The size was adjusted to 0.5 McFarland standard turbidity, approximately 10^8 colony-forming

units (CFU/ml). The cell suspensions (100µl of target strain) were introduced into the nutrient agar plates and spread thinly on the plates using a glass spreader. The discs were then placed on inoculated agar plates. The plates were incubated at 37^0C for 24 hours under aerobic conditions. The diameter of the inhibition zones around the discs was measured (in millimeters) after 24 hours. Tests were performed in duplicate.

2.6 Data analysis

2.6.1 Physio-chemical composition of honey

Moisture, Acidity, Hydroxymethylfurfural (HMF), Diastase and Proline content in the honey samples collected from different species and locations were compared using two-way analysis of variance (ANOVA). The Tukey-Kramer multiple comparison test was used to separate significant means at 5% level of significance.

2.6.2 Antibacterial activities

Analysis of Variance (ANOVA) was employed to compare antibacterial effect of the different honeys. Significant means were separated using the Tukey-Kramer multiple comparison test was used to separate significance means at 5% level of significance.

CHAPTER 3

STINGLESS BEE AND FOREST CONSERVATION IN KAKAMEGA FOREST

3.1 Introduction

Kenya's forests contain a large number of endemic and globally threatened species. Many rural communities are dependent on forest resources for water, energy, poles, medicinal plants and a variety of other products, to augment their incomes. This dependence is particularly strong where communities adjacent to forests are poor (since forest resources are treated as free goods), and it often leads to forest degradation because resource extraction rates are unsustainable (Raina *et al.*, 2006). With the increasing concern on the loss of biodiversity, and the mounting evidence of irreversible environmental damage, there is growing need for serious steps towards conservation. Heightened environmental awareness, population reduction and provision of alternative livelihoods for the communities adjacent to forests may reduce pressure on forest resources from the same communities and provide an opportunity for conservation. There is need to involve the local population in utilization and sustainable conservation of their indigenous biodiversity especially the bees that can also generate income.

Few studies have been done in Africa on the folk knowledge of insects, yet this taxonomic knowledge can be extremely a useful component of ecological records or resource use data. In Brazil, Posey (1983), made detailed records of Kayapo Indian taxonomy of stingless bees showing the use of folk taxonomy in the naming

of stingless bees. In Uganda, Baranyunga (1996) demonstrated the accuracy of Batwa (Abayanda) pygmies systematics (or folk taxonomic) knowledge. Local peoples' concepts and beliefs have been used by western scientists as epic (internal or indigenous) guides for their research designs (Posey, 1983). Eder *et al.* (1987) argues that data collection utilizes indigenous categories for biological inventories, while ecological concepts (often couched in myth and natural symbols) establish the basis for interdisplinary dialogue and research. They take the view that indigenous knowledge of biological communities and ecological relationships can be studied and compared. In this case, the knowledge of the communities around Kakamega forest was key to investigation on the ecology and traditional uses of honey from stingless bees. This information was used for laying a foundation for meliponiculture and to consolidate conservation efforts in Kakamega forest. It is possible to develop stingless beekeeping by combining the folk knowledge and cultural practices intimately bound with stingless bees and their products. It is against this background that a study was carried out on folk knowledge on stingless bees in Kakamega forest.

3.2 Materials and Methods

3.2.1 Community awareness and folk knowledge

To document ecological knowledge of the communities on stingless bees, informal interviews using a questionnaire (Appendix 1) was distributed in five villages around the Kakamega forest (Fig 2.1 and Plate 3.1). The questionnaire focused on:

community awareness/opinion on stingless bees, importance and identification. The knowledge was classified in the following levels:

- ***a*** – just knows stingless bees
- ***b*** – knows stingless bees by local names but can not differentiate the species and knows the honey uses
- ***c*** – knows stingless bees by local names; knows honey uses; can differentiate the species
- ***d*** - knows stingless bees by local names; can differentiate the species; knows honey uses; has tried to domestic the stingless bees.

From the five villages, Ileho, Madioli, Makuchi, Ikuywa and Buyangu (Fig 2.1), 74 people aged 15-80 years were randomly chosen from each village for the survey.

Five honey hunters each from Ileho and Makuchi village, which harvested honey in the forest and sold it to the community members, were used in collecting folk knowledge of stingless bees. Search in the forest was done for the nest entrance in tree trunks, between butterness or ground. Inspection of trunks was essential so as not to miss out on nests.

3.3 Results

3.3.1 Community awareness

Of the 74 people interviewed 24% were in category ***b*** (knows stingless bees by local names and can not differentiate them but there has been no effort to conserve them), 34% just knows stingless bees and a total of 82% have not tried to conserve

stingless bees but they know the stingless bees (Fig 3.1). The honey hunters harvest the honey from feral colonies and sell it to other community members (Plate 3.15 – 3.16). The medicinal uses attributed to stingless bees honey are presented in Table 3.1 This made the honey to be sold at prices t three times more than that of *A. mellifera.*

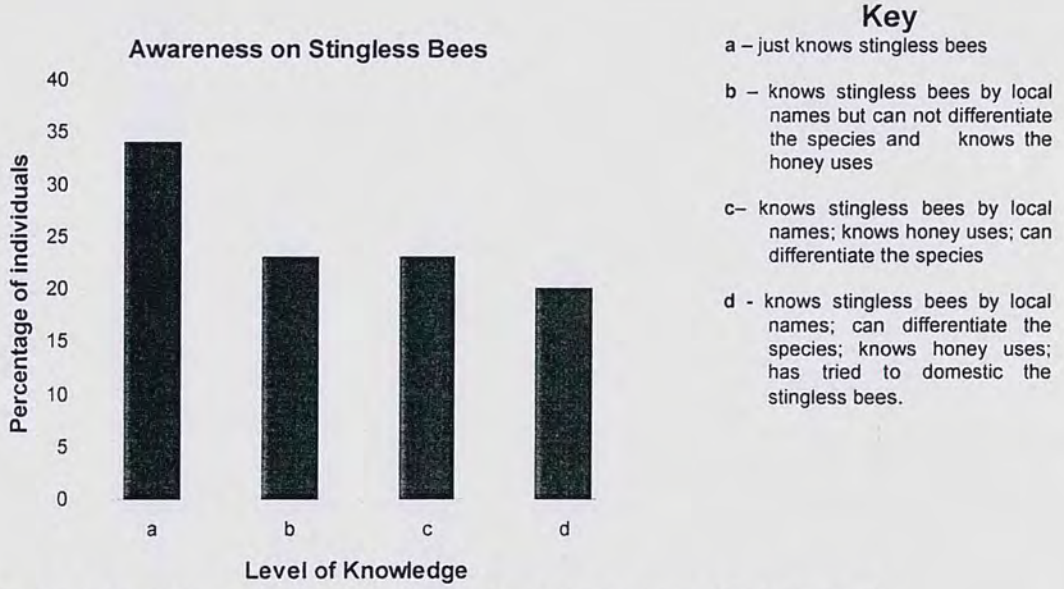

Fig 3.1. A graph showing community awareness on stingless bees of five villages of Kakamega forest

3.2 Folk taxonomy

Nest entrances were at the bases of trees, between butterness or many meters above the ground. The major Luhya dialects round Kakamega forest, Isukha and Tiriki, identified the different stingless bees species into five main species, which they have given local names such as 'Ikore', 'Iwele', 'Inanasa', 'Viuyiya' and 'Vusitsi' (Plates 3.1-3.17) These communities use different characteristics in identifying stingless bees. These characteristics include: body size, colour shape of the nest entrance, taste and quantity of honey produced by different species (Fig 3.1 and

Table 3.1). Most of the bee and nest characteristics were described with the assistance of the honey hunters in Kakamega forest during the course of the study (Table 3.2).

Plate 3.1.'Vuiyiya' (*Plebeina hildebrandti)* bee

Plate 3.2. 'Vuiyiya' (*Plebeina hildebrandti)* entrance on the ground.

Plate 3.3. 'Vuyiya' (*Plebeina. hildebrandti*) nest

Plate 3.4. *Meliponula. lendliana* colony

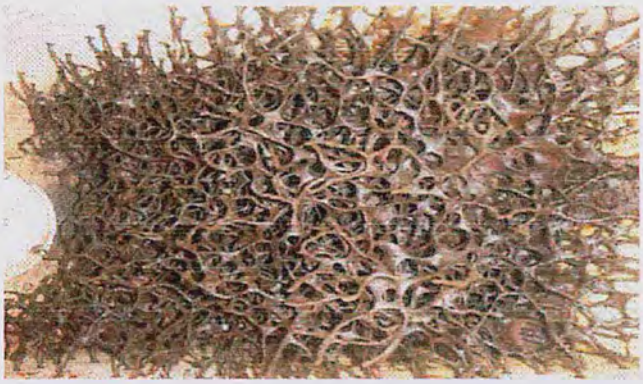

Plate 3.5 *M.lendliana* nest at Ikuya village

Plate 3.6. The wide opening of *Melinonula. lendliana* entrance

Plate 3.7. 'Ikore'(*M. Bocandei*) bee

Plate 3.8. 'Ikore'(*Meliponula Bocandei*) nest

Plate 3.9. 'Ikore'(*Meliponula. Bocandei*) entrance

Plate 3.10. ***Meliponula. ferruginea*** **nest in a cavity**

Plate 3.11. ***Meliponula. ferruginea*** **entrance**

Plate 3.12. ***Hypotrigona. gribodoi*** **nest in an old shoe.**

Plate 3.13. ***Hypotrigona. griboidoi*** **double entrance**

Plate 3.14. Destructive honey harvesting from tree nests.

Plate 3.15. Destructive honey harvesting from ground nests.

Plate 3.16. An interviewing session with one of the local farmers in Ikuya village.

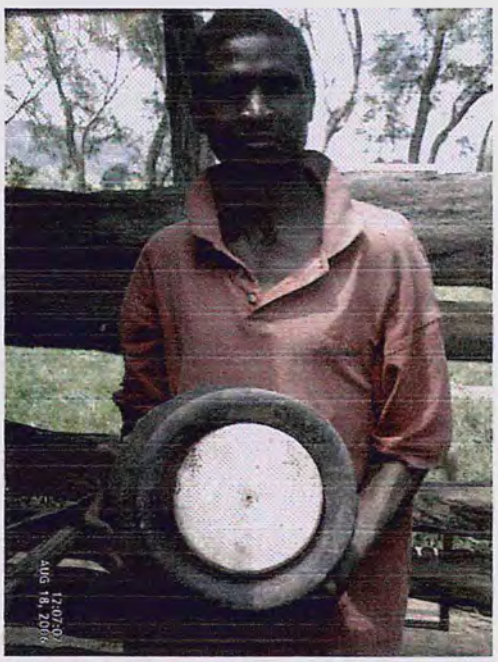

Plate 3.17. A farmer who has taken the intiave to hive stingless bees in a pot in Ileho village.

Table 3.1 Characteristics features (local knowledge) around Kakamega forest used in identifying stingless bees (Apidae: Meliponinae)

Local name	Literal meaning	Scientific name	Characteristics features	Honey characteristics	Medicinal uses of honey
Ikore	'It had been lost'	*M. bocandei*	Big in size (7.0 –9.0mm) compared to others Brown in color Does not have a protruding entrance.	Honey has a characteristic smell	Digestive disorders, respiratory infections, wound healing, post-birth recovery, fatigue, skin problems
Inanasa	Msasa-man	*M. ferruginea*	Reddish brown in color 6.0mm in size The entrance protrudes and opens wide like a bell The honey pots are irregularly arranged	Honey is sweet	Increase libido
Iwele	Brought by God	*M. lendliana*	Black and smaller in size (4.0mm) Entrance is circular but tapers at the lower side. Arranges the pots in a regular pattern. Has smaller honey pots with a regular pattern	The honey has a characteristic smell. Induces diarrhea to some people	Constipation, stomach ache
Vuyiya	Stays with people	*H. gribodoi*	Smallest species of stingless bees Clustered type of brood. 2.0 – 3.0 mm in size	Very sweet honey	Cold treatment
Vusitsi /inyira	Plantain	*P. hildebrandti*	Black with Yellow markings at fore head. Prefers nesting in the base of the plantain Honey used in cultural ceremonies	Honey has a smell similar to honey from M. *bocandei* used to relief chest pain.	Wound healing, curse healing, chest pains

3.4 Discussion

Assessment of the knowledge on stingless bees conservation by the community around Kakamega forest indicated that most people (74%) know about stingless bees but do not domestic them. This is an indication of what has been reported earlier by Cortopassi *et al.*, (2006) that meliponiculture has not been practiced much in Africa. The farmers in their daily lives interact a lot with nature and this could explain their awareness of the existence and the naming of stingless bees, but there were no conservation measures. There was evidence of destructive harvesting of stingless bees (Plates 3.14 and 3.15). Similar observations were made by Marilda, (2006). Even though the locals around Kakamega forest recognized the importance of stingless bees in the production of highly medicinal honey, the majority 74% have not tried to conserve stingless despite the knowledge they have on stingless bees. However, the initiative by some members of the community especially the honey hunters in keeping some stingless bees in pots is encouraging (Plate 3.17). This only calls for demonstration and the training of farmers on stingless beekeeping.

In the Brazilian Amazon, factors determining the Kayapo Indian taxonomic system for stingless bees included: habitat, preferred substrate or niches, defense behaviour, size, forms and colour of adults and smell of bees (Posey and Camargo, 1985). Similarly the same taxonomic approach was used by (Abayanda) pygmy in Uganda (Byarugaba, 1998). In Kakamega forest, the Luhya community use characteristic features of the insects and their behaviour and nesting ecology as the characteristic guiding them to systematic naming. Three community members in

Kakamega were using hollow logs or clay pots as hives, and harvest the honey in a more sustainable way. In Tanzania and Angola, meliponiculture exists and an interest to develop meliponiculture has been identified in Ghana, Kenya, Botswana and South Africa (Armour, 2005).

A wide range of attributes may suggest that the honey from stingless bees enhance several systems to control digestive, respiratory and wound healing. *Plebein hildebrandti* is the most important stingless bee in Kakamega on the basis of reported medicinal properties of this honey. For this reason, honey from stingless bees to fetch up to 3 times the honey from *A. mellifera.* This is an indication of the potential of rearing stingless bees as an income generating activity to the community living around the forest.

Extensive agriculture and population growth are among the main causes of the rapid dwindling of the rich biodiversity of Africa (Raina, 2005) including stingless bees farming could diversify the rural people's economic base, and therefore, encourage them to be allied with the current world efforts in promulgating conservation-based development. It had been reported earlier that, stingless bees occur in Africa and have commercial value but not fully utilized (Eardley, 2004; Gikungu, 2006; Macharia, 2007). Much equally, stingless bees farming has been recognized as an important practice in sustainable income generation and environmental amelioration (Heard, 2000, Cortopassi, 2002, Cortopassi *et al.*, 2006, Macharia *et al.* 2007). The fact that stingless bees occur in Kenya indicates a very good potential for their farming (meliponiculture) in this country.

3.5 Conclusion

This study has highlighted the need of utilizing the knowledge and skills of community especially the honey hunters to establish sustainable use of stingless beekeeping. This knowledge forms foundation for their conservation and sustainable management of stingless bees and the forest at large. Stingless bees keeping in Kenya is still in a stage that demands greater effort of investigation, as well as the increase in the diffusion and socialization of the existing knowledge between the communities and the researchers. It is also necessary to control activities such as cutting down of trees that diminishes resources and nesting sites, so as to take advantage of sustainable use of stingless bees farming

CHAPTER 4

STINGLESS BEE QUEEN REARING

4.1 Introduction.

Stingless bees, like honeybees, have evolved special adaptations that enable a colony to overcome queenlessness. Honey bees rear emergency queens by selecting one of the young larvae to receive extra portions of royal jelly (Crane 1990). The involved cells are subsequently enlarged and transformed into royal cells. This process is feasible because larval feeding in the honeybees is progressive and the brood is kept open during most of the larval feeding period. Stingless bees in contrast, are mass-provisioners, and their cells are sealed right after the queen's oviposition (Faustino *et al.*, 2002).

Caste determination in stingless bees is still not completely understood (Ribeiro, 2004). In *Melipona* there is genetic influence, although trophic aspects are also important (Velthuis and Sommeijer, 1991; Kerr, 1997). Queens and workers are reared in identical cells. *Trigona,* (African stingless bees) on the other hand present three possibilities for queen production. The caste determination is, in principle, trophic. However, more complexity is involved in the process of the production of new queens, depending on the genus (Riberio, 2004). In *Frieseomellita* and *Leurotrigona,* a female is able to perforate the wall of a contiguous cell and eat extra larval food and hence, becomes a queen (Faustino *et al.*, 2002). In all other genera, the workers build special royal cells. These cells are lager than cells from

where the workers (and males) emerge, and therefore, contain more food (Engels and Imperatriz-Fonseca, 1990).

Besides, in several species new queens can emerge from the normal sized cells as well. These queens, ingesting less food as larvae, are of smaller size: they are called "miniature" or "dwarf" queens. This suggest that the amount of food theory is not enough for explaining the transformation of female larva to queen. In this case the quality of food could also have some influence (Ribeiro, 2004). But studies on food quality (Hartfelder, 1987; Hartfelder and Engels, 1989), discarded this possibility, leaving the problem unsolved. These authors found no differences in the amount of protein in the larval food of different cells. According to Ribeiro, (2004), the amount of protein varied in individual cells in *Schwarziana quadripunctata* Lepeletier. Although this finding does not prove that the quality of food is important for caste determination, it reinforces the idea of quality of food and keeps the discussion open.

For these reasons, natural requeening after queen loss, as it usually occurs in honeybees, has been regarded as highly improbable in stingless bees. Observations by Faustino, (2001) of orphan colonies of *Trigona* (*Friesmelitta*) producing gynes motivates this study of queen replacement in species found in Kakamega forest. The aim here was to suggest management systems of propagating the stingless bee colonies through queen rearing process for colony division.

4.2 Materials and Methods

4.2.1 Emergency queen rearing

Four colonies of *H. gribodoi* in hives which had been developed (Macharia *et al.*,2007) (C1, C2, C3, C4) were used. The colonies were collected from Kakamega and transferred to *icipe* bee yard, Nairobi, and left to adapt for 2 months. In the 3rd month, queens from two colonies (C1 and C2) were removed. Observations were made on the changes in the brood construction of queen new queen cells, which is an indication of queen rearing. This was done for 4 months. This being a clustered type of brood it was easier to note any change in the brood. Observations on construction of new cell were made daily in the morning and evening. Photos were taken for any change and records made on any change. Colonies C3 and C4 were the control. This was replicated in two colonies.

4.3 Results

The observations made is summarized in table 4.1. In C1 colony, the bees constructed 3 queen cells 4 days after the removal of the queen (Plate 4.1-4.2) and in the C2 colony 4 queen cells were constructed. In C1 colony the queen cells were on the edges of the brood (Plate 4.2) and in the C2 colony there was a queen cell at the center of the brood (Plate 4.3). The cells were destroyed on the 3rd and 4th day. In C1 colony, two brood cells were noted being joined together to form one brood cell that was larger than the other worker brood cells after 15 days. They were then destroyed after three days. After 6 days they started constructing another queen cell, which on the 8th day was also destroyed.

In the C1 colony normal brood cell with larvae was extended and joined with another worker brood cell (Plate 4.4). On the 48 days a queen was observed after the removal of the queens. The queen later disappeared from the nest. It was assumed that the queen disappeared after the mating flight since she was a virgin queen. In the C2 colony joined queen cells were noted after 20 days. After 21 days a virgin queen was noted (Plate 4.5). There was no change in queen rearing of C3 and C4 colonies (control) where queens were not removed.

Day	Colonies	
	C1	**C2**
4	Queen cells constructed at the edge of the brood.	
5		Queen cells constructed at the center of the brood
7	One queen cell destroyed.	
8	The second queen cell destroyed.	Queen cell destroyed.
15	Initial stage of joining two brood cell noted and destroyed after 3 days	
20		Initial stage of joining two brood cell noted.
24	A new pair of brood cells joined together to form one queen cell.	
41		A virgin queen noted
48	A virgin queen noted which disappeared.	

Table.4.1. Changes in the brood of the two colonies of *H. gribodoi* during queen rearing process.

During the period of queenlessness, oviposition was carried out by the workers. The duration of joining the two brood cells could not be exactly be determined but it was less than 3 days. The expansion of the royal larval cell was a gradual process. Of the three mode of queen rearing in stingless bees, *H. gribodoi* proved to rear queen in two ways through normal construction of a queen cell (Plate 4.2 – 4.4) and joining of two brood cells (Plate 4.5.) during the emergency queen rearing.

Plate 4.1. ***Hypotrigona gribodoi*** **colony (C1)**

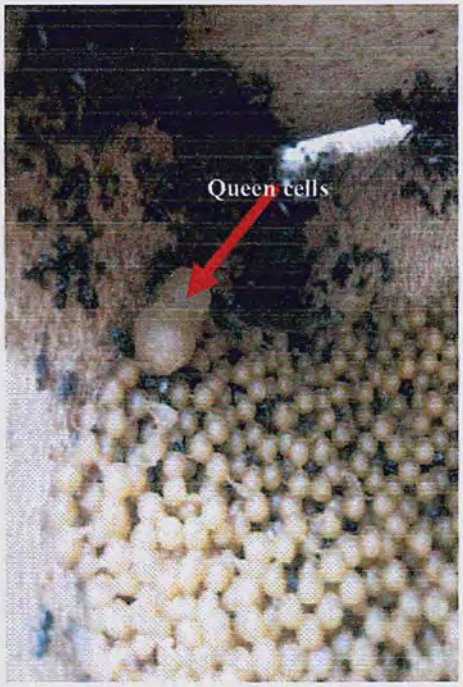

Plate 4.2. ***Hypotrigona gribodoi*** **queen cells at the edge of brood.**

Plate 4.3. ***Hypotrigona gribodoi*** **queen cell at the center of the brood.**

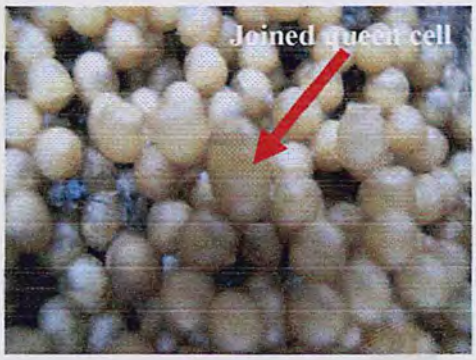

Plate 4.4. ***Hypotrigona gribodoi*** **joined queen cell.**

Plate 4.5. Virgin queen of *Hypotrigona gribodoi*

Plate 4.6. Mated queen of *Hypotrigona gribodoi*

4.4 Discussion

The mode of selecting a worker larva to become a queen, as realized in honey bees, would be unlikely to evolve in stingless bees due to three kinds of bionomic constraints: Firstly, mass provisioning of brood cells requires that all the nutrients necessary to support immature's ontogogeny are deposited at once, before the queen's oviposition. Secondly, brood cells are sealed right after the queen oviposits and thirdly, the strong socio-behavioral disorganization that usually occurs when colony is deprived of the queen's influence (Lercerda and Zuchi, 1999). This is the reason why the queen cells were destroyed in our study since they were sealed before food and eggs were deposited. Emergency queen rearing has been described in *Trigona (Fieseomelitta) varia* Lepeletier (Faustino, 2002) whereby the larva constructed a feeding connection. Faustino (2002) states that occurrence of equivalent patterns under contrasting colonial conditions suggest that mediators of such complex events are similar either under queen hood or queenless. From this study, it showed that *H. gribodoi* under emergency condition rear queen by joining two brood cells to have one lager queen cell rather than a feeding connection.

Caste differentiation in social insects is often influenced by the amount of food the developing larva obtains. This has been described for termites, ants, wasps and honey bees (Wilson, 1971). Like in other Trigonini, *H. gribodoi* exhibited the quantity of food as the determinant of caste differentiation due to the bigger sized queen cell compared to brood cells of workers. Earlier reports by Darchen, (1971) indicate that *H. gribodoi* rears queen by constructing a special queen cell which

was also found in this study by the initial queen cells constructed. For a successful meliponiculture in Kenya the developed method of emergency queen rearing can be applied for colony multiplication.

4.5 Conclusion

From this study it can be concluded that *H. gribodoi* rears queen in two different ways as compared to earlier studies by Darchen (1974); (i) using a special queen cell which is bigger than other brood cells (ii) through emergency queen rearing whereby they join two brood cells, destroying one developing larvae. This queen rearing can be used in colony multiplication in stingless bees rearing. The queenless daughter colony is able to raise a new queen and get established as fully-fledged new colony.

CHAPTER 5

PHYSIO-CHEMICAL COMPOSITION OF HONEY FROM STINGLESS BEE

5.1 Introduction

The keeping of bees by different communities for purposes of harvesting honey and wax has a long-standing tradition and history. Most research reported on honey has been from honey produced by *Apis* spp. This honey is undoubtedly the most widely and massively collected and used by people for different purposes. But regionally, especially in the tropics, there are other honeys made by different species of bees which are sometimes collected in substantial quantities (Krell, 1996). One group of those bees are the stingless bees that produce a considerable amount of honey and hence can be used in beekeeping. When compared to honey which is produced by *A. mellifera*, honey from stingless bees is little known by the general public. Also, analysts and supervision agencies are faced with difficulties due to the lack of standards for the honey which is produced in "pots" instead of "honeycomb" (Vit, 2006). Honey from stingless bees is a valuable product with a long tradition of consumption, to which several medicinal uses are attributed. Due to the scant knowledge about the product, Meliponini honey is not included in the international Codex Alimentarius Commission standards (Codex, 2001) and the food control authorities do not regulate it. Thus there is no assurance for consumers on is quality.

Honey standards have been established only for honey from *A. mellifera* from many parts of the world, following the guidelines of international standards of the *Codex Alimentarius* Commission (Codex, 1969, 1987, 2001). There are no standards for *Meliponinae* honey production and different quality criteria based on physicochemical parameters have been used to test stingless bee honey (Bruno, 2006). Countries like Mexico, Guatemala and Venezuela already posses proposals for the quality control of the honey of its stingless bees (Vit *et al.*, 1994a, 2004; Bogdanov *et al.*, 1996). There are no similar proposals for honey from the African stingless bees.

The composition of honey depends not only on the floral sources from which it is derived but also on other factors like the species of the bee, the physiological state of the colony, the state of maturation of the honey, and the season of harvesting among others (Marcos *et al.*, 2005). The large number of stingless bees species producing honey and the species of bee flora that need pollen identification to assign a botanical origin, increase the complexity of an attempt to set quality standards and bioactive properties of the stingless bee honey. For this purpose, besides a review of the already published data, a new honey database also needs to be created with agreeing methods to confirm the composition of honey which is produced by different species of stingless bees.

5.2 Materials and methods

5.2.1 Honey collection

Five grams of honey sample from each of the species were collected between August and December 2006 in the Kakamega forest. Colonies of stingless bees were located with the help of local farmers, hunters and woodcutters. Populous nests were located by the dense forager traffic near their entrances. Trees were felled and nests opened with machete, hatchet or small cross cut saw. Cuts were made above and below the nest entrance, and this section of tree was removed after carefully cutting through the tree (wood surrounding the nest). The honey was collected directly from the pots within the nest using sterile syringes (plate 4.0), then transferred to 4ml glass vials fitted with rubber septa and stored away from the sunlight (plate 4.1). Analysis on the different parameters was done within two weeks. Some honey was also collected from 3 farmers who had domesticated the stingless bees.

Composition data from 120 stingless bee (*Meliponini*) honey samples were compiled from 5 different species from Kakamega forest, which included: *H. gribodoi, M bocandei, M. ferruginea (black), M. ferruginea (white,)* and *P. hildebrandti.* They were evaluated with a view to propose a quality standard for this product. Since honey from stingless bee has a different composition from that of *A. mellifera,* some physiochemical parameters are presented according to stingless bee species. All the experiments were repeated at least three times (triplicates)

5.2.2 Physiochemical analysis

The parameters analyzed were moisture, hydromethylfurfural (HMF), diastase, proline content and free acidity. Moisture was determined using a honey refractometer, HMF and Diastase content was determined through spectrophotometry, and proline was determined through spectrophotometry and free acidity quantified by volumetry – titration technique.

Plate 5.1. Harvesting honey from stingless bees honey pots.

Plate 5.2 Honey samples ready for analysis.

5.2.2.1 Moisture

Five grams of honey sample from each of the species were weighed and placed in a flask in water bath at 50°C until all the sugar crystals had dissolved. The solution was cooled to room temperature and stirred to homogenize. The sample was evenly spread on a clean and dry prism of hand held refractometer avoiding any bubbles and covered tightly. Readings were done and recorded after 2 minutes against light. This was repeated three times to each honey sample.

5.2.2.2 pH and Free acidity

Ten grams of honey samples from each of the species were dissolved in 75 ml of distilled water in 250 ml beaker. The solution was stirred with a magnetic stirrer, the pH electrode emerged in the solution and the pH read and recorded. After reading the pH free acidity was determined by titration with 0.1M sodium hydroxide solution to pH 8.30 (when solution was completely neutralized).

5.2.2.3 Hydroxymethylfurfural (HMF)

Five grams of honey samples from each species were measured and dissolved in 25ml of water, and then quantitively transferred into a 50ml volumetric flask. 0.5ml Carrez sol (I) was added and mixed thoroughly, then 0.5ml of Carrez sol (II) was also added and mixed thoroughly. The solution was made to the mark with water (50ml flask). It was then filtered, rejecting the first 10ml (which is mostly water) of the filtrate. In two test tubes (A and B) 5ml of water were added to test tube A and 5ml of Sodium bisulphate solution was added to test tube B and in both A and B, 5ml of the sample filtrate solution was added. The solution in tube B was

used as the reference solution. Then absorbance was determined at 284 and 336 nm. The expression of HMF in mg/kg

$$HMF = (A_{284} - A_{336}) \times 149.7 \times 5 \times D/W$$

Where;

A_{284} is the absorbence at 284nm;

A_{336} is the absorbence at 336 nm

D is dilution factor

$$D = \frac{\text{final volume sample}}{10}$$

W weight of the sample

5 is a theoretical nominal sample weight

5.2.2.4 Diastase

Ten grams of each honey sample from different species were weighed and dissolved in 15 ml of water and then 5ml of acetate buffer was added. The solution was transferred to 50 ml volumetric flask, which had 3ml of Sodium Chloride solution. The volume was then adjusted to the mark with water. Starch solution was calibrated by measuring 20, 21, 22, 23, 24, and 25mls of water and 5ml dilute Iodine solution. Starting with the first measurement of water 0.5ml of a mixture of water and 5ml of starch solution was added and mixed thoroughly. Absorbance

was read at 660nm and water was used as the reference (blank). This was done until absorbance obtained was in the range 0.770 – 0.745. The amount of water which gave this range was taken as the standard for every determination carried out using starch solution. Ten milliliters of the honey and 10ml of starch were measured separately and placed in water bath at 40°C. After 15 minutes, 5ml of starch solution was put in the honey solution and mixed. The timer was immediately started and at intervals of 5 minutes, aliquots of 0.5 ml were removed and mixed with 5ml diluted Iodine solution then added to 23mls of water (which was determined showing the calibration of starch solution). The absorbance of each separate solution at 660nm was read and recorded.

5.2.2.5 Proline

Five grams of honey from each species was weighed and dissolved in water to make 100ml solution and put in three test tube labeled (i), (ii) and (iii) and 0.5ml of the sample solution was placed each of these test tube (i) of 0.5ml water in another test tube (ii) and 0.5ml of proline in another test tube (iii). One milliliter of formic acid and one ml of ninhydrin solution were added to each tube. The tubes were corked and shaken vigorously for 15 minutes. They were then placed in boiling water bath for 15 minutes. They were transferred to water baths at 70°C for 10 minutes. Five milliliters of 2-Propanol were added to each tube and corked and placed under room temperature and absorbance was determined after 45 minutes at 510 nm.

5.3 Data analysis

All the determinations were repeated at least three times (triplicates), and the means and standard errors determined. The effect of species and location on Moisture, pH, Acidity, Hydroxymethylfurfural (HMF), Diastase and Proline was analyzed using two-way analysis of Variance (ANOVA). The Tukey-Kramer multiple comparison test was used to detect significant differences between means in the event of a significant F value. All analysis were carried out at 5% level of significance.

5.4 Results

The means for the quality variables analyzed (moisture, HMF, diastase, proline, free acidity) are summarised in table 4.1. Significant differences were found between the species in water content ($F_{3,29}$=5.59, P=0.0037) and diastase activity ($F_{3,29}$ =3.95, P=0.0178) and no difference in the other parameters Moisture varied from 21.1-27.1 g.100g^{-1}. *Plebeina schimdnti* had the highest moisture content of 27.1±0.41 while *H. gribodoi* had the lowest moisture content of 21.1±1.70. HMF ranged from 2.1-8.14 mg/kg^{-1}. *Plebeina schimdnti* had the lowest (2.1±3.14 mg/kg^{-1}) while *M. bocandei* had the highest (8.14±4.29) mg/kg^{-1}. Diastase activity levels varied from 8.88-35.7 DN whereby, *M. ferruginea* had the highest (35.7±7.22) Schade units and *H. gribodoi* had the lowest (8.88±1.06). Other parameters varied as follows; proline (70.6 – 412 mg/kg^{-1}); free acidity (30.2-46.0) meq/kg^{-1} and pH (3.24-4.09).

Table 5.1: Means (±SE) of physiochemical composition of honey samples obtained from stingless bees from Kakamega forest.

Species *Standard*	Moisture *[≤21%]	HMF [≤40 mg/kg^{-1}]	Diastase [≥8Schade units]	Proline [≥180 mg/Kg^{-1}]	Free Acidity *[≤50meq/kg^{-1}]	pH
H. gribodoi (n=21)	21.10±1.70^{a}	6.98±2.56^{a}	8.88±1.06^{a}	70.60±48.10^{b}	46.00±1.41^{a}	4.09±0.11^{a}
M. bocandei (n=30)	26.40±0.42^{a}	8.14±4.29^{a}	19.80±3.89^{ab}	412±132^{a}	34.60±5.01^{a}	3.41±0.11^{a}
M. ferruginea (b) (n=28)	24.20±0.77^{a}	5.18±1.33^{a}	13.10±2.07^{a}	206±36.10^{b}	38.70±3.58^{a}	3.51±0.26^{a}
M. ferruginea (w) (n=35)	25.60±0.41^{a}	6.08±2.33^{a}	35.70±7.22^{ab}	234±65.90^{b}	31.00±3.69^{a}	3.40±0.11^{a}
P. schimndti (n=6)	27.10±0.41^{a}	2.10±3.14^{a}	30.70±4.12^{a}	186.12±0.60^{b}	30.20±2.41^{a}	3.24±0.12^{a}

Different letters within columns **(a-b)** indicate significant difference

*Honey quality standards of the Codex Alimentarius for floral honeys. HMF = hydroxymethylfurfural

5.5 Discussion

Moisture content is the criterion that determines the capability of honey to remain stable and resist spoilage by yeast fermentation. Moisture content of the samples of stingless bee honey from Kakamega was higher than the standard for *A. mellifera* honey (max 20% of moisture content). These observations agree with other findings reported by Vit *et al.* (1994), Vit *et al.* (2004), Torres *et al.* (2004), Bruno *et al,* (2006) and Lubertus *et al.* (2006). The combination of Hydroxymethylfurfural (HMF), diastase and invertase enzyme levels indicate the extent of heat and storage damage of honey and can be used as markers of honey freshness and adulteration (White, 1994). HMF is produced in honey to some degree all the time, and is a breakdown product arising from the action of normal honey acidity on sugars (glucose and fructose) at ambient temperatures. A maximum content of $\leq$ 40mg/Kg is allowed in *A. mellifera* honey in the international market. Amounts in excess of this minimum are considered a main indicator of honey deterioration (White, 1979; White and Siciliano 1980; Bogdanov and Martin 2002) either through heating or long periods of storage (White *et al.*, 1962). All the samples in this study had varying HMF within the acceptable limits, agreeing with other results of Vit *et al.*,(2004) an indication that the honey had neither been stored for long periods nor adultered.

The measurement of proline is used as an indication of honey ripeness – its quantity being an indication of adulteration when it falls below a certain limit of 180 mg/Kg^{-1} . With the exception of *H. gribodoi,* honey exceeded the minimum limit of 180mg/kg^{-1}, an indication that all the honey was ripe. Acidity varied

according to the samples; all of the samples fulfilled the maximum of 50 meq.kg^{-1}. All the honey samples had the recommended free acidity level allowed by the Codex and Council of the European Union (EU) for the *A. mellifera.* The predominant acid in honey is glucconic acid, a derivative from dextrose (Stinson *et al.*, 1960). The gluconic acid present in all honeys originates largely from the activity of glucose, added by the bee during the ripening (Ruiz-Argueso and Rodriquez- Navarra 1973).

5.6 Conclusion

Comparing the data of stingless bee honey analyzed with the international standards for *A. mellifera* honey (Codex, 2001), the honey from different stingless bees varied in their composition. It is hoped that the results of this study would constitute a starting point for creating a solid database of stingless bee honey, including all parameters useful for honey quality control. A honey quality control campaign directed to stingless beekeepers is needed, for harmonization of analytical methods. This will allow the control of this valuable product, leading to the setting of quality standards. It can also be concluded that the five species of stingless bees found in Kakamega varies in their physiochemical composition.

CHAPTER 6

ANTIBACTERIAL ACTIVITIES OF STINGLESS BEE HONEY

6.1 Introduction

Beekeepers and honey enthusiasts alike have long reported the medicinal qualities of honey (Crane 1990; Molan, 1997). Honey from stingless bees is considered in folk medicine to be more powerful than that of honeybee for use in the treatment of common diseases (Vit, 2001; Garedew *et al.*, 2004). For community around Kakamega forest, honey from stingless bees is highly valued as a panacea for many ailments and is believed to have more value compared to other honey from *A. mellifera* (Macharia *et al.*, 2007). Although honey has been used as medicine since ancient times, its effectiveness against bacterial infections was revealed only a century ago (Molan, 2001). Many authors (Molan, 1988; Bogdanov 1997; Taormina *et al.*, 2001; Selcuk and Nevin 2002) have confirmed the antimicrobial activity of honey. Most of these studies have been conducted using honey produced by *A. mellifera*. However, little has been done to investigate antibacterial effects of stingless bee honey (Vit *et al.*, 1994; De Brujin and Sommeijer, 1995; Sommeijer *et al.*, 1995).

The aim of this study was to investigate the antibacterial effects of honey from; *M. bocandei*, *M. lendliana*, *M. (Trigona) ferruginea*, *H. gribodoi*, *P. hildebrandti* and *A. mellifera* collected from Kakamega forest, Kenya.

6.2 Materials and Methods

6.2.1 Honey samples

Ten honey samples from each species of stingless bees were collected from closed honey pots in the nests of different species viz *M. ferruginea (b), M. ferruginea (w), H. gribodoi P. hildebrandti* and *M. bocandei* in each of the five regions in Kakamega forest. This honey from stingless bees was collected using sterile syringes and stored in sterile dark glass bottles and the honey samples were kept at temperatures of -7°C in the refrigerator.

6.2.2 Bacterial Cultures

Five bacterial strains were used in the study viz; *Pseudomonas aeruginosa* (ATCC 27853); *Salmonella typhi* (ATCC 2202); *Escherichia coli* (STD 25922); *Staphylococcus aureus* (ATCC 20591) and *Bacillus subtilis* (ATCC 6633), obtained from Inoclaine International, Nairobi, Kenya.

6.2.3 Antibacterial activity tests

The agar disc diffusion method was employed (Allen *et al.*, 1991) to evaluate the antibacterial activity of honeys from stingless bees and the honeybee against the test bacteria, which included both the Gram-positive and Gram-negative bacteria. The bacteria inoculum was prepared from fresh overnight cultures of bacterial strains. A loopful streak of the bacterial culture was inoculated into a nutrient broth medium and incubated for 24 hours at 37°C. The size was adjusted to 0.5 McFarland standard turbidity, approximately 10^8 colony forming units (CFU/ml). The cell suspensions (100µl of target strain) were introduced into the nutrient agar

plates using the spread plate technique. Sterile filter paper discs of 6mm diameter were impregnated with 25µl of each honey sample. The discs were placed aseptically on the agar plates inoculated with the test bacteria and then incubated at 37°C for 24 hours under aerobic conditions. The diameter of the inhibition zones around the discs was measured after 24 hours. Tests were performed in duplicate.

6.2.4 Data analysis

Analysis of Variance (ANOVA) was employed to compare antibacterial effect of the different honeys. The Tukey-Kramer multiple comparison test was used to separate significant means at 5% level of significance.

6.2.5 Results

A summary on the antibacterial activity (expressed as inhibition zones) of stingless bees honey are presented in table 6.1 and figure 6.1. Generally, larger zones of inhibition were exhibited on Gram-negative bacteria *P. aeruginosa* and *E. coli* as compared to the Gram-positive bacteria, *B. subtilis* and *S. aureus*. Among the Gram-positive bacteria, *S. aureus* had the largest inhibition zones compared to the inhibition zones recorded for *B. subtilis*. These differences were not significant (p=0.05). In the Gram negative bacteria, the highest antibacterial activity was recorded in *E. coli* and *P. aeruginosa* compared to inhibition zones recorded for *S. typhi*. However, the differences in the sizes of the zones of inhibition were not statistically significant (p = 0.05). Table 6.1

There were variations in antibacterial activity of honeys obtained from the different bee species, with honey from *P. hildebrandti* and *M. ferruginea* exhibiting highest level of inhibition in all the bacteria. Honey from *H. gribodoi* had no inhibitory effect on *B. subtilis* and *S. typhi*, but had the highest inhibitory effect on *E. coli*. Honey from *M. bocandei* had no inhibitory effect on *B. subtilis*. Streptomycin had inhibitory effects on all the five bacteria strains.

Significant differences were obtained on the effects of each honey on the different bacteria strain; *M. ferruginea* (black) ($F_{4, 43}$=4.48, P=0.0041), *M. ferruginea* (white) ($F_{2, 3}$=69.5 P=0.0031), *H. gribodoi* ($F_{2, 17}$=6.13, P=0.0099) and *A. mellifera* (F2, 27=5.78 P=0.0082). There was no significant difference on the effect of bacteria in *P. hildebrandti*, *M. bocandei* and Streptomycin (P=0.05). Honey from *M. ferruginea* (w), *H. gribodoi*, *M. bocandei* and *A.m. scutellata* did not cause inhibition on bacteria *B. subtilis* isolate. The same honeys, with an exception of *M. bocandei*, did not cause inhibition on *S. typhi* ATCC 2202.

Table 6.1. A summary on the antibacterial activity (expressed as inhibition zones) of stingless bees honey from from Kakamega forest against 5 bacteria strains (*B. subtilis, S. aureus, P. aeruginosa, E. coli,* and *S. typhi)*

	Inhibition Zones (in mm) ±SE				
Species	***B. subtilis*** **Local isolate**	***E. coli*** **STD 25922**	***S. aureus*** **ATCC 20591**	***S. typhi*** **ATCC 2202**	***P. aeruginosa*** **ATCC 27853**
M. ferruginea (b)	8.25 ± 0.16^{Bb}	12.20 ± 1.44^{Ab}	9.70 ± 0.15^{Bb}	9.25 ± 0.37^{Bb}	12.00 ± 0.77^{Aba}
M. ferruginea (w)	–	15.51 ± 0.50^{Aba}	9.00 ± 0.00^{Bb}	–	10.50 ± 0.50^{Bba}
H. gribodoi	–	17.31 ± 1.93^{Aba}	9.50 ± 0.50^{Bb}	–	13.62 ± 1.53^{BAba}
P. hildebrandti	9.33 ± 0.67^{Ab}	10.00 ± 0.41^{Ab}	10.31 ± 0.25^{Ab}	8.75 ± 0.25^{Ab}	9.00 ± 0.41^{Ab}
M. bocandei	–	10.00 ± 0.91^{A}	9.75 ± 0.25^{Ab}	10.00 ± 0.41^{Ab}	12.83 ± 1.11^{Aba}
Streptomycin	21.00 ± 3.00^{Aa}	22.51 ± 0.50^{Aa}	20.32 ± 0.67^{Ab}	16.30 ± 0.33^{Aa}	17.24 ± 1.69^{Aa}

Different letters within rows (**A-B**) and within columns (**a-b**) indicate significant difference ($p<0.05$). A dash (–) indicates zero inhibition.

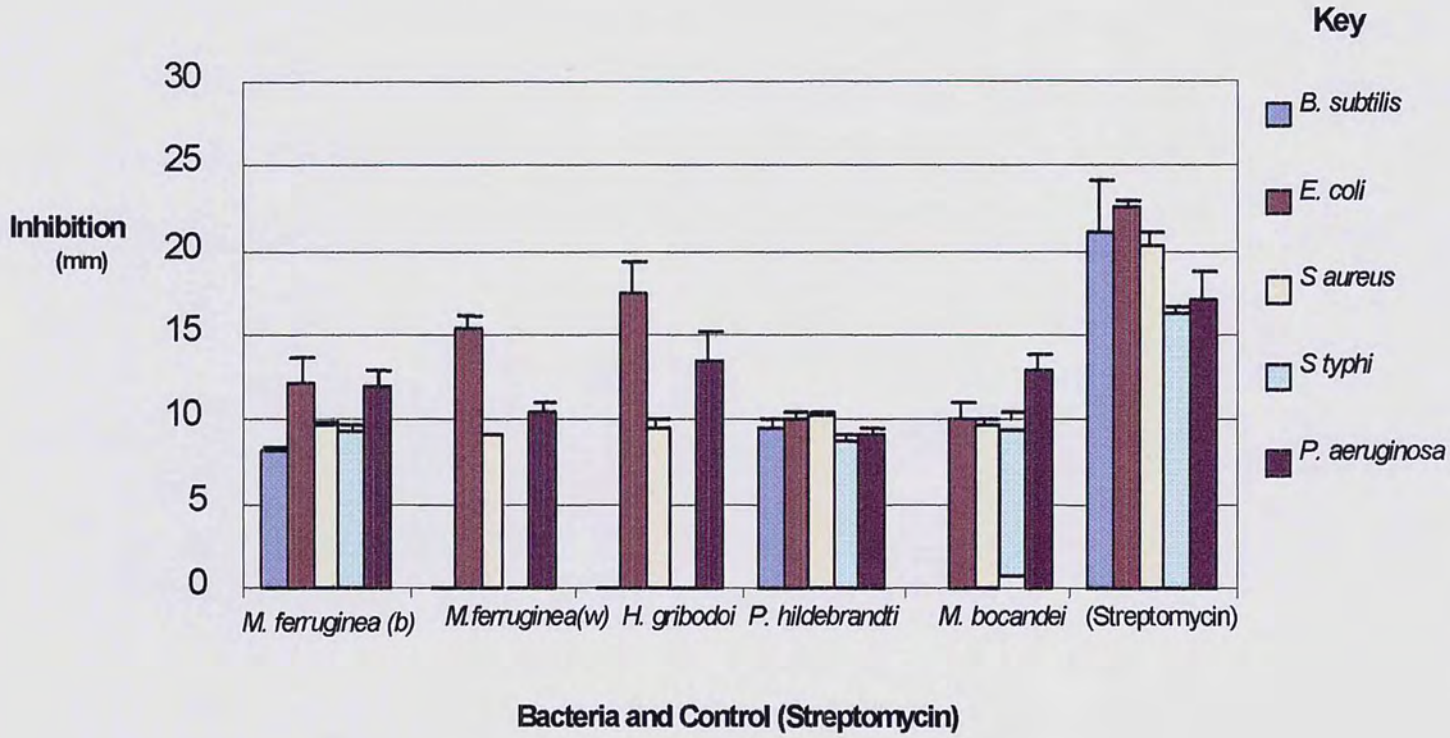

Fig.6.1. Graph of antibacterial activity of stingless bees honey.

6.3 Discussion

All honeys exhibited antibacterial properties against the test bacteria strains of *E. coli, S. aureus* and *P. aeruginosa*; but the inhibition effects were varying. Honeys from *M. ferruginea (b)* and *P. hildebrandti* tested positive to bacteria strain *B. subtilis, E. coli, S. aureus, S. typhi* and *P. aeruginosa.* This finding agrees with earlier studies done by Sommeijer *et al.*, (1995) and Vit *et al.*, (1994).

Of all the bacteria tested, Gram-negative bacteria (*E. coli* and *P. aeruginosa*) exhibited higher sensitivity to different honeys compared to the Gram-positive bacteria (*S. aureas* and *B. subtilis*). These results are consistent with those of Vorlora *et al.*, (2005). The result indicated that pure honey has some antibacterial activities against many pathogenic organisms, as previously described (Jeddar, 1985). The response of the different bacterial strains to the treatment with certain honey sample may not be due to the composition of the cell wall, which determines the Gram reaction of the bacteria since both the Gram-positive and Gram-negative bacteria were susceptible to all the treatments. The mode of action of honey from *M. ferruginea* and *P. hildebrandti* against *P. aeruginosa* and *S. aureus* which are the majority bacteria causing wound and burn infections is of interest to clinicians. These results were similar to those reported by Jeddar *et al.*, (1985) and Agbaje *et al.*, (2006).

The results suggest that various kinds of honey significantly differ in their antimicrobial strength, which is in agreement with the earlier studies (Vorlova *et al.*, 2005). The reasons for their antibacterial effects are controversial (Jeffrey *et*

al., 1996). Some authors suggest that it may be due to the physical and chemical factors that can be related to their different botanical origins and bees from different origin (Weston, 2000; Demera and Angert, 2004; Vorlova *et al.*, 2005; Kajobe, 2006). This is supported by Cortopassi and Gelli, (1991) who observed that strongest antimicrobial characteristics in pollen from the Mimosa (*Melipona subnitida* Ducke) and eucalyptus (*Eucalyptus globules* Labill). Honey viscosity also provides a barrier against the infections, and on the other hand its osmolarity (Yatsunami and Hartfoot, 1984) causes the exit of liquids creating a septic humid atmosphere (Molan, 1992) that inhibits pathogenic microorganisms. It can also be attributed to the activity of several aromatic acids (Molan, 1992b), to the acidity and volatile compounds (Weston, 2000). Hydrogen peroxide, which is more in stingless bee honey, also plays a significant role in bactericidal action. (Torres *et al.*, 2004)

6.4 Conclusion.

Based on the results of this study it can be concluded that Gram-negative bacteria (*E. coli* and *P. aeruginosa*) and Gram positive bacteria (*S. aureus*) are susceptible to stingless bee honey, but in varying degrees. *Bacillus subtilis* is only susceptible to honey from *M. ferruginea* (b) and *P. hildebrandti*. It can also be concluded that different honey from stingless bees have different strengths in antibacterial effect.

The traditional use of stingless bees honey in Kakamega as a panacea against different illnesses is rational if the infection to be treated is caused by bacteria. Stingless bees honey offers many possibilities as a broad spectrum-healing agent against both gram positive and gram-negative bacteria. The current prevalence of

antibiotic-resistant microbial species has led to a re-evaluation of the therapeutic use of ancient remedies, including honey. However, this study indicated that honey may not offer a total solution to the current challenges facing bacterial chemotherapy. Stingless bees honey being a natural product, may be used as a multifaceted drug, as claimed by the local people.

CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

The conservation status of stingless bees is very low or never thought of in Kakamega forest. This threatens the nesting sites of stingless bees. There is great potential in stingless bees farming due to the highly valued medicinal honey and the indirect benefit of pollination. For successful conservation of stingless bees the local community knowledge should be included in any sustainable farming of stingless bees. The honey produced by stingless bees around Kakamega forest varies in its composition. Traditional use of stingless bee honey in the community around Kakamega forest as a panacea against different illness is rational if the infection is caused by the tested bacteria. A colony multiplication method through emergency queen rearing is possible and important for meliponiculture. Providing education for the people resulted in supportive stewardship action from them in return; what has emerged in this study is a science, public education, and stewardship cycle that continues to aid conservation, and has been invaluable to science and management decisions in the area

7.2 Recommendations

Despite the potential of stingless bee keeping in Kakamega forest, conservation and utilization for income generation has been very low or never thought of in this area. Therefore, to further the potential and conservation of stingless bees for income generation the following are recommended:

- Meliponiculture should be encouraged in the region, through training the community on how to rear the stingless bees in a rational hive.
- There is need to improve the methods of harvesting the stingless bee honey without destroying the colony.
- There is need to carry out a general resource inventory of stingless bees in Kakamega forest.
- Conservation education should be strengthened. In particular scientific concepts like pollination not familiar to farmers should be introduced.
- Further studies should be carried out to document folk forest ecology of stingless bees known to the elderly people around the forest.

REFERENCES

Agbaje, E. O., Ogunsanya, T., Aiwerioba, O. I. (2006). Conventional use of honey as Antibacterial agent. *Annals of African Medicine.* **5**.(2); 78-81.

Allen, K., Molan, P.C., Reid, M., (1991). A survey of the antibacterial activity of some New Zealand honeys. *Parm. Pharmacol* **43:** 817-822.

AOAC 1990: Association of the Official Analytical Chemists, Official Methods of Analysis No 980. Edition 15 Washington, DC, USA

Armor, M. (2005) Stingless Bees in Angola, [online] http://www.beesfordevelopment.org/info/stingless/stingless-bees-in-angola.shml (accessed on 17 February 2006).

Biesmeijer, J. C., Richter, J.A.P., Smeets, M.A.J.P. & Sommeijer, M.J. (1999) Niche differentiation in nectar-collecting stingless bees:the influence of morphology, floral choice and interference competition. Ecol. Entomol. 24, 1–9.

Biesmejer, J. C., Hartfelde R L., Imperatriz-Fonseca, V. L. (2006). Stingless bees: biology and management. *Apidologie* **37**: 121-123.

Bogdanov S., Vit P., Kilchenmann V. (1996) Sugar profiles and conductivity of stingless bee honeys from Venezuela. *Apidologie 27*: 445-450

Bogdanov, S. (1997). Nature and origin of antibacterial substances in honey *Lebensm. Wiss Technol.* **30**:748-753.

Bogdanov, S. (1999): Harmonised methods of the International Honey Commission. Swiss Bee Research Center, FAM, Liebefels, Bern, Switzerland

Bogdanov, S., Martin, P., Lulmann, C. (1997). Harmonized methods of the European Honey Commission. *Apidologie* (extra issue) 1-59.

Bogdanov, S., Vit P., Kilchenmann, V. (1996). To suck profiles and conductivity of stingless bee honey from Venezuela. *Apidologie* **27**, 445-450

Byarugaba, D. (1998). Stingless bees (Hymenoptera, *Apidae, Mliponinae*) and Abayanda honey-hunters: A study linking bee Ecology and Indigenous knowledge. MSc Thesis, Makerere University, Faculty of Science.

Camago J. M. F. & Menezes Pedro S. R. (1992). Systematics, phylogeny and biogeography of the Meliponinae (Hymenoptera, Apidae): a mini-review. Apidologie **23**: 509– 522

CODEX (1987). *Revised Codex Standard for Honey*. CODEX STAN 12-1981. Codex Alimentarius Commission. FAO/OMS. Rome, Italy. 17 pp

CODEX (2001). *Revised Codex Standard for Honey*. CODEX STAN 12-1981. Codex Alimentarius Commission. FAO/OMS. Rome, Italy. 7 pp.

CODEX, (1969). *Norma Regional Europea para Miel*. CAC/RS 12-1969. Codex Alimentarius Commission. FAO/OMS. Rome, Italy. 29 pp.

CODEX, (2001). *Revised Codex Standard for Honey*. CODEX STAN 12-1981. Codex Alimentarius Commission. FAO/OMS. Rome, Italy. 7 pp.

Cooper, R.; Molan P., Harding, K. (1999). Antibacterial activity of honey against strains of Staphylococcus aureus from infected wounds. *J. R. Soc. Med.* **92**:283-285.

Cortopassi -Laurino, M. ; Imperatrizfonseca,V.; Roubik, D.W.; Dollin, A.; Heard, T.; Aguilar, I.; Venturieri, G.C.; Eardley, C.;Nogueira-Neto

(2006). P. Global meliponiculture: Challenges and Opportunities. *Apidologie*, .**37**:.275-292

Cortopassi, L. M. and Gelli, D.S. (1991). Pollen analysis phyisco-chemical properties and antibacterial action of Brazilian honeys from *Apis mellifera*. *Apidologie*, **22**, 61- 73.

Crane, E. (1990). Bees and beekeeping: science, practice and world resources, Heinemann Newnes, Oxford, 614 p.

Crane, E. (1992). The past and the present status of beekeeping with stingless bees. *Bee world* **73** (1): 29 – 42

Darchen, R. (1971). Description d'une abeille sociale de tres petite taille Trigona (Liotrigona) parvula n.sp. Darchen (Apidae, Trigoninae) trouvee dans la savane de Lamto (Cote d'Ivoire). Biologia Gabonica **7**: 403–406.

De Brujin, L. L., Sommeijer, M. J. (1995). The composition and properties of honeys of stingless bees (Melipona), in: Properties for honey production in the Tropics Proc. V Int. Conf. On apiculture in tropical climates IBRA, pp. 149-168.

Demera, J. H., Angert, E. (2004). Comparison of the antibacterial activity of honey produced by *Tetragonisca angustula* (Meliponinae) and *Apis mellifera* from different phytogeographic regions of Costa Rica. *Apidologie* **35** *411-417.*

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

Emerton L. (1991). Licensed utilization of Kakamega Forest. Kenya indigenous Forest Conservation Programmme, Forest Department, Nairobi.

Engels, W., Imperatriz-Fonesca, V. L. (1990). Caste development, reproductive strategies, and control of fertility in honey bees and stingless bees. In: Engels W (Ed) *Social Insects: An evolutionary approach to castes and reproduction.* Springer- Verlag; Heidelberg, Germany; pp 167- 230

Faustino, C. D., Silva-Matos, E. V., Mateus, S., Zucchi, R. (2001). First record of emergency queen rearing in stingless bees (Hymenoptera, Apinae, Meliponini). *Insectes soc.* **49:** 111-113

Garedew, A., Schmoltz, E., Lamprecht, I. (2004). - Microcalorimetric investigation on the antimicrobial activity of honey of the stingless bee *Trigona* spp and composition of some parameters with those obtained with standard methods. *Therochimica acta.***415** (1-2), 99-106.

Gikungu, M. W. (2006). Bee diversity and some Aspects of their Ecological Interactions with Plants in a Successional Tropical Community. PhD Dissertation. Universitat Bonn - Germany.

Hartfelder, K. (1987). Caste differentiation in stingless bees: comparative analysis of larval food from 7 species. In Eder, J. & Rembold, H. (Eds.). *Chemistry and Biology of Social Insects*. Munchen, Verlag J. Peperny, 174.

Hartfelder, K., and Engels, W. (1989). The composition of larval food in stingless bees: evaluating nutritional balance by chemosystematics methods. *Ins. Soc.,* **36**: 1-14.

Heard, T. and Dollin, A. (2000). Stingless Beekeeping in Australia: snap shot of an infant industry. *Bee World*, **81**, 116-124

Jeddar, A., Kharsary A., Ramsaroop U., Bhamjee A., Haffejee I., Moosa R.; Molan, P (1985). The antibacterial action of honey. An invitro study. *S afri Med J;* **67**:257-258.

Jefrey, E. and Echazarreta, M. (1996). Medicinal uses of Honey. Rev Biomed; 7:43-49.

Kajobe, R. (2006). Botanical sources and sugar concentration of the nectar collected by two stingless bee species in a tropical African rain forest. *Apidologie* **38**:1-12.

Kerr, W. E. (1997). Sex determination in honeybees (Apidae and Meliponinae) and its consequences. *Genetics in Tropical and Molecular Biology* **20**(4): ISSN 0100-8455

KIFCON, (1994). Kenya Indigenous Forest Conservation Programme. Phase 1 Report. Karura Forest Station, Center for Biodiversity, Nairobi.

Kokwaro, J. O. (1988). Conservation status of Kakamega forest in Kenya. The Eastern most relic of the equatorial rain forest of Africa. *Monographs of Systematic and Botanical Gardens,* **25**:471-489.

Krell, R. (1996). Value added products from Beekeeping. *FAO Agricul. Serv. Bull.* No. 24.

Lacerda, L. M. and **Zuchi, R. (1999).** Behavioral alterations and related aspects in queenless colonies of *Geotrigona mombuca* (Hymenoptera, Apidae, Melioninae). *Sociobiology* **33** 277-288.

Lubertus, B., Luc, L. M., Martens, E. P., Sommeijer, M. J. (2006). Water content of stingless bee honeys (*Apidae, meliponini*): interspecific variation and comparison with honey of *Apis mellifera Apidoligie* **37** 480-486

Macharia, J. K. (2007). Distribution of stingless bees in selected Kenyan forests: Potential for meliponiculture enterprises, pp. 188–191. In Development of Sericulture and Apiculture Products for the Poor in Fragile Ecosystems Using the Value Chain Approach (Proceedings) (Raina S. K Eds. et al.). *icipe* Science Press, Nairobi. ISBN 92 9064 191 6.

Macharia, J. K., Raina, S. K., Muli, E. M. (2007). - Stingless bees in Kenya. *Bees for Development Journal issue* **83** pp.

Marcos, R. A., Lopes C. C., Almeida, B. S., Sodre, G. S., Marchini, L. C. (2005). Physico-chemical characteristics of honey samples of stingless bee *Melipona mandacaia* (Hymenoptera: Apidae) Cienc. Technol. Aliment. Vol.25

Marilda, C. L., Impertriz, V. L., Roubik, D. W., Dollin, A., Heard, T., Aguilar, I., Venturieri G. C., Eardley C., Neto P. (2006). Global meliponiculture: challenges and opportunities. *Apidologie* **37**: 275 - 292

Michener, C. D. (1974). The Social Behaviour of the Bees; a Comparative Study. Cambridge, Mass, Havard Univ. Press, pp 404.

Michener, C. D. (2000) *Bees of the world*, Johns Hopkins Press, Baltimore.

Mitchel, N. (2004). BIOTA East report No. 1. The exploitation and disturbance history of Kakamega forest, Western Kenya.

Molan, P. C. (2001). Why honey is effective as a medicine, II. The scientific explanation of its effects. Bee world, **82** (1): 22-40.

Molan, P. C (1997). The antibacterial activity of honey. 2. Variation in the potency of antibacterial activity, *Bee World* **73** *59 – 76*

Molan, P. C. (1992a). The antibacterial activity of honey.2 variation in the potency of the antibacterial activity. *Bee world* **73**:59 –76.

Molan, P. C. (1992b). The antibacterial activity of honey. 1. The nature of the antibacterial activity. *Bee world* **73**:5 –28.

Molan, P. C., Russel K. M (1988). Non- peroxide antibacterial activity in some New Zealand Honeys. *J. apic. Res.*, **27** (1): 62-67.

Munthali, S. M. and Mughogho, D. E. C. (1992). Economic incentives for conservation; An experiment on beekeeping & Saturniidae caterpillar utilization by rural people. Biodiversity & Conservation 1: 143-154.

Muriuki, J. H., and **Tsingalia, M. H. (1990).** A new population of De Brazza's monkey in Kenya. Oryx, **24**:157-162.

Mutangah, J. C.; Mwangangi, O. M. and **Mwaura, P. K. (1992).** Kakmega Forest vegetation Survey. Kenya Indigenous Forest Conservation Programme, National Museums of Kenya, Nairobi, Kenya.

Noguera-Neto, (1970). A criac°a¬o de abelhas ind|genas sem ferra¬o Melipo(ninae), 2nd edn. Sao Paulo: Chäcaras e Quintais.

Onyango, J. C., Nyunja, R. A. O., Bussmann, R. W (2004). Conservation of Biodiversity in the East African tropical forest; *Lyona* **7** (2).

Patricia, V., Medina, M., Maria, E. E. (2004). Quality standards for medicinal uses of Meliponinae honey in Guatemala, Mexico and Venezuela. *Bee World* **85**(1): 2-5.

Posey, D. A. and Camargo, J. M. F.. (1985).Additional notes on the classification and knowledge of stingless bees by the Kayap´o Indians of Gorotire, Par´a, Brazil. Ann. Carnegie Mus. 54: 247–274.

Posey, D. A. (1983) Folk apiculture of the KayapoÂ Indians of Brazil. Biotropica, 15, 154 - 158.

Raina, S. K (2004). Commercial insects: A practical Guide for Raising and Utilizing Silk moths and Honey Bees in Africa. IBRA press, U.K, Volume 1. pp 16 –22

Raina, S. K. (2000). The economics of apiculture and sericulture modules for income generation in Africa. IBRA Press, 18 North Road, Cardiff, CF10 3DT. UK.57pp

Raina, S. K.; Kioko, E.; Adolkar, V.; Muiru, H.; Nyagode, B. A.; Kimbu, D.; Lumumba, J; Waruiru, R. and Nguku, E. (2000). ICIPE'S Groundwork in Building African Commercial Insects Farming Industries for the New Millennium, pp. 1-16. In: *Sericulture and Apiculture: Prospects for the New Millennium*. Proceedings of the Second International Workshop on Conservation and Utilization of Commercial Insects, 28th November-1st December, 1999. Riana, S.K; Nyagode, B; Adolkar, V; Kioko, E. and Mwanycky, S.W. (Eds.). ICIPE Science Press, Nairobi, Kenya.

Ransome, H. M. (1986). The sacred bee in ancient times folklore, Burrowbridge Somerset, England 308 p.

Ribeirio, F. (2004). Miniature queens in stingless bees species - A review. Proceedings of the 8th IBRA International Conference on Tropical Bees and VI Encontro sobre Albelhas.

Rodgers, A. W. (2005). Crossborders Conservation in East Africa, pp.3-8. In: *Integrating Sericulture and Apiculture Technologies with Regional Development Operations*. Raina, S.K and Nguku, E.K. (Eds.). Proceedings: Trainers Course and Third International Workshop on Conservation and Utilization of Commercial Insects, 13th November - 8th December, 2000, Nairobi Kenya.

Roig-Alsina and, Michener C. D. (1993). Studies of the phylogeny and classification of long-tongued bees (Hymenoptera: Apoidea). *Univ. Kans. Sci. Bull.* 55:124–62.

Roubik, D. W. (1979). Nest and colony characteristics of stingless bees from French Guiana (Hymenoptera: Apidae). Department of Entomology, University of Kansas. *Journal of the Kansas entomological Society* **52**(3), pp. 443-470

Roubik, D. W. (1989). *Ecology and natural History of Tropical bees*. Cambridge University Press, Cambridge, New York. 514p

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

Sakagami, S. F., (1982). *Stingless Bees*. In: *Social Insects* (H.R Hermann, Ed.) Academic Press, New York, pp. 361-423.

Salehe, J. Y. (2005). Biodiversity Conservation Through Income Generation Initiatives: A case of the East African Crossborders Biodiversity, pp.20-25. In: *Integrating Sericulture and Apiculture Technologies with Regional Development Operations*. Raina, S.K and Nguku, E.K. (Eds.). Proceedings: Trainers Course and Third International Workshop on Conservation and Utilization of Commercial Insects, 13th November - 8th December, 2000, Nairobi Kenya.

Schade J. E., Marsh G. L., Eckert J. E. (1958). Diastase activity and hydroxymethylfurfural in honey and their usefulness in detecting heat adulteration. Food Res 23, 446-463.

Schwarz, H. F.(1948). Stingless bees (Meliponidae) of the Western Hemisphere. Bull.

Selcuk, H.; Nevin, K. (2002). Investigation of antibacterial effect of honey collected from various regions of Turkey. Pakistan *J. Biol. Sci.* **5**:325-328.

Silveira, F. A., Mello, G. A., Almeida, E. (2002) *Abelhas brasileiras. sistemática e identificação*. Fundação Araucária. Belo Horizonte, Brasil. 253 pp.

Slaa, J. (2003). Foraging ecology of stingless bees; from individual behaviour to community ecology. Universiteit Utrechta. *Sociobiology* **33**:277-288.

Sommeijer, M (1999). Beekeeping with stingless bees: a new type of hive. *Bee world* **90** (2) 70 – 76.

Sommeijer, M. J., De Brujin, L. L. (1994). Intranidal feeding, trophallaxis, and sociality in stingless bees. *Nourishment and evolution in insect societies. Westview* **13**: 391-418

Sommeijer, M. J., Beetsma, J., Boot, W. J., Roberts, E. J., De Vries, R. (1995). Perspective for Honey Production in the Tropics, Proc. Symp. Organized by the Netherlands Expertise Centre for Tropical Apiculture Research (NECTAR), Utrecht, Netherlands.

Stinson E. E., Subers M. H., Petty J, White J. W. (1968): The composition of honey. V. Separation and identification of the organic acids. *Arch Biochem.* Biophys **89** (1): 6 – 12.

Torres, A., Garedew A., Schmolz E., Lamprecht I., (2004) Calorimetric investigation of the antimicrobial action and insight into the chemical properties of 'angelita' honey – a product of the stingless bee *Tetragonisca angustula* from Columbia. *Thermochim, Acta* 415, 107-113.

Velthius H. H.W (1997) The Biology of Stingless Bees. Utrech University, Netherlands, pp 3.

Velthius, H H W. and **Sommeijer, M. J. (1991).** Roles of morphogenetic hormones in caste polymorphism in stingless bees. In: Gupta A P (Ed) *Morphogenetic hormones of arthropods*. Rutger University Press, pp: 346-383.

Vit P., Medina M., Enríquez M. E (2004) Quality standards for medicinal uses of Meliponinae honey in Guatemala, Mexico and Venezuela. *Bee World* 85(1): 2–5

Vit, P. (2001). Stingless bees honey and the treatment of cataracts in: Munn P. and Jones R. (Eds) Honey and healing, IBRA Cardiff UK, pp 49.

Vit, P., Bogdanov, S., Kilchenmann V (1994) (a). Composition of Venezuelan honeys from stingless bees (Apidae: Meliponinae) and *Apis mellifera. Apidologie* **25**, 275-288.

Vit, P., De Selgrad, R., Nonovoa, M. L., Reinosa, F., Carmargo J. (1994)(b). Antibacterial activity and mineral content of Venezuelan stingless bee honeys, Proc. V Int. Conf. On apiculture in tropical climates IBRA, pp. 254-258.

Vit, P., Odd L P; Marano M L. (1998). Analysis of Venezuelan stingless bee honeys characterized by multivariate of physicochemical properties. *Apidologie* **29**, 377- 389.

Vit, P., Rodriguez A., Almelida D., Souza B., Marchini C., Diaz F., Trichio K., Heard T. (2006). A scientific event topromote knowledge regarding honey from stingless bees: Physical-Chemical composition. *Magistra, Cruz das Almas- BA, v.18, n. 4, p. 270-276.*

Vorlova, L., Karpiskova, R., Chabiniokova, K., Kalabova K., Brazdova Z. (2005). The antimicrobial activity of honeys produced in the Czech Republic. *Czech J. Anim. Sci.,* **50** (8): 376-384

Waser, N. M (1983) Competition for pollination and floral character differences among sympatric plant species; a review of evidence. In: CE Jones and RJ little [eds.] Handbook of Experimental Pollination Biology, 277-293. Van Nostrand Reinhold Company Inc., New York

Weston, R. (2000). The contribution of catalase and other natural products to the antibacterial activity of honey. A Review: Food Chem **71**:235 –239.

White J. W. (979). Spectrophotometric method for hydroxymethylfurfural in honey. *J. Ass. Off. Anal. Chem* **62**: 509.

White J. W (1994). The role of HMF and diastase assays in honey quality evaluation. *Bee World* **75:** 104 – 117.

White J. W, Tech. Bull. IU.S.Dept. Agric. 1242 p.

White J. W and Siciliano J (1980) Hydroxymethylfurfural and honey adulteration. *J. Ass. Off. Anal. Chem* **63 (1)**: 7–10

Willie, A. (1983). Biology of the stingless bees. *Ann. Rev. Entomol.*, **28**, 41-64.

Wilson E.O. (1971) The insect societies, Harvard University Press, Cambridge, MA, USA

Yatsunami, K., Echigo, T., (1984).Antibacterial activity of royal jelly and honey. *Honey Bee sci* **5**:125-130.

Zimmerman, D. A. (1972). The avifauna of the Kakamega Forest, Wesetern Kenya including a bird population study. *Bull. Amer. Mus. Nat. Hist.* **149**: 255-339.

Appendices

Appendix 1:
Questionnnaire

Area	Date					
Name of correspondence	**Age**	**Knowledge level**				
		a	**b**	**c**	**d**	**Remarks**

Level a: Just Knows stingless bees

Level b:
Knows stingless bees by local names
EITHER,
Can differentiate the species.
OR
Knows honey uses

Level c;
Knows stingless bees by local names
Can differentiate the species.
Knows honey uses

Level d:
Knows stingless bees by local names
Can differentiate the species.
Knows honey uses
Has tried to keep them