EVALUATION OF APICURE, A PLANT-BASED EXTRACT FOR THE MANAGEMENT OF THE SMALL HIVE BEETLE, *AETHINA TUMIDA*

EDNAH JEBET KOMEN

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Evaluation of Apicure, a plant-based extract for the management of the small hive beetle, *Aethina tumida*

Ednah Jebet Komen

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DECLARATION

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

Signature …… Date: …….

Ednah Jebet Komen

This thesis has been submitted for examination with our approval as the University Supervisors.

Signature …… Date: …….

Dr. Lucy K. Murungi, PhD

JKUAT, Kenya.

Signature …… Date: …….

Dr. Janet Irungu, PhD

***Icipe*, Kenya.**

DEDICATION

To my daughters, Madonna and Amanda for their motivation and love and my family for their love and relentless support

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
BC& ID	Capacity Building and Institutional Development
DRIP	Dissertation Research and Internship Programme
EAG	Electroantennogram
FID	Flame Ionization Detection
GC-EAD	Gas Chromatography-Electroantennographic Detection
GC-MS	Gas Chromatography-Mass Spectrometry
GDP	Gross Domestic Product
GOK	Government of Kenya
ICIPE	International Centre of Insect Physiology and Ecology
IUCN	Internationational Union of Conservation of Nature
JKUAT	Jomo Kenyatta University of Agriculture and Technology
O.I.E	Intergovernmental Organization
SDG	Sustainable Development Goals
SHB	Small hive beetle
UK	United Kingdom
UN	United Nations
U.S.A	United States of America

χ^2	Chi-Square

ABSTRACT

The honey bee, *Apis mellifera* Linnaeus (Hymenoptera: Apidae) is a major pollinator of agricultural crops. However, this role has been threatened by the small hive beetle (SHB) *Aethina tumida* Murray (Coleoptera: Nitidulidae), an invasive pest of honey bees due to their capacity to significantly affect the health of honey bees. This study sought to evaluate the potential of Apicure, a plant -based extract developed at the International Centre for Insect Physiology and Ecology *(icipe)* for the management of honey bee pests. In addition, an in-depth understanding of the volatile organic compounds of Apicure and the mode of action of this novel product against the small hive beetle, *A. tumida* were provided. To achieve the objectives, firstly, headspace volatiles of Apicure were collected using super Q adsorbent traps. Secondly, the volatiles were analyzed using Gas Chromatography–Mass Spectrophotometry (GC-MS) to ascertain the constituent compounds of Apicure. Thirdly, the selectivity and sensitivity of antennal receptors of *A. tumida* adults to the volatile compounds of Apicure was established with the coupled Gas Chromatography-Electroantennographic Detection (GC-EAD). Finally, dual-choice bioassays were carried out in a Y-tube olfactometer to determine the behavioral activity of this product and its electrophysiologically active constituents. GC-MS analyses identified 40 compounds in Apicure. Out of these, 11 compounds that elicited antennal responses to SHB antennae in electroantennography studies. These included camphor, limonene, camphene, cymene, cymenene, *α*-terpineol, geraniol, α-Farnesene caryophyllene oxide, linalool and terpinen-4-ol. Y-tube olfactometer bioassays established that Apicure is a repellant to SHB. Behavioral assays with single synthetic standards showed that linalool, camphor, geraniol, and *α*-terpineol are repellants while limonene is an attractant to SHB. These results signify the potential role of the Apicure and its components as repellants and attractants in SHB management. This product can also be used to mask the in-hive small hive beetle attractants hence reducing the colony invasion by beetles as they use these chemical cues to locate their host.

CHAPTER ONE

INTRODUCTION

1.1 Background of study

Agricultural production and its diversity rely greatly on biotic pollination, predominantly offered by the honey bee, *Apis mellifera* Linnaeus (Hymenoptera: Apidae) (Aizen *et al*., 2009) among other pollinators such as bumble bees and stingless bees. It is approximated that 90% of all insect pollination services are offered by honey bees (Gallai *et al.* , 2009). The overall economic value of global pollination is reported to be €153 billion, which accounts for 9.5% of the world agronomic production used for food (Gallai *et al*., 2009; Lautenbach *et al*., 2012). Honey bee pollination services in the United States alone is estimated at $14.6 billion (Klein *et al.*, 2007). Food crops exhibit improved fruit and seed quality and quantity with animal pollination (Kasina *et al.*, 2009; Ollerton *et al*., 2011).

Biological invasions pose a global threat to both food security and nature (Netwig, 2007; Cook *et al.*, 2011). One of such invasive organisms is the small hive beetle (SHB), a pest of honey bees with the capacity to significantly affect the health of both managed and feral eusocial bee (Page *et al.*, 2016). Originally of sub-Saharan origin, this Nitidulid (sap beetle) has now become established in honey bee colonies in North and Central America (USA, Canada, Mexico, Cuba, and Nicaragua), North Africa (Egypt), Europe (Italy), Australia and South East Asia (The Philippines) (Neumann *et al*., 2016). As its spread is likely to have been facilitated by increased global connectivity through trade, much of Europe and the rest of the world are at risk as its spread continues without appropriate intervention (Paini *et al.*, 2016; Ouessou Idrissou *et al*., 2019).

This pest infests bee colonies as either individuals or swarms with both adult and larval stages known to cause damage as they feed on pollen, honey, brood and young worker bees (Pirk *et al*., 2016). The reported estimated losses attributed to the small hive beetles in the USA in 1998 alone were US$3 million. These losses were in the form of colony destruction and damage to stored honey supers in honey houses.

Aside from honey bees, this beetle has also been observed in stingless bee colonies (Greco *et al*., 2010; Halcroft., 2011) as well as bumblebees (Neumann & Elzen, 2004; Spiewok & Neumann, 2006). In addition to eusocial bees, the beetle has been shown to successfully develop under laboratory conditions on various fruits such as Kei apple *Dovyalis caffra*, cantaloupe *Cucumis melo* , pineapple *Ananas comosus*, mango *Mangifera indica* , banana *Musa spp*, grapes *Vitis vinifera*, oranges *Citrus* × *sinensis* and decaying meat (Ellis *et al*., 2002; Buchholz *et al.*, 2008; Arbogast., 2009). In Africa, SHB has received less attention and has always been considered as a minor pest therefore, minimal progress has been made to evaluate its effect on honey bees. This scenario gave the pest enough time to widely invade new regions in the continent causing undetermined losses (Gela Bayeta *et al*., 2018).

Many management methods aimed at beetle interception both inside and outside the hive environment have been developed with limited success. Most of the tools developed are laborious to deploy and monitor. For instance, the bait like pollen is not cost-effective to small scale beekeepers in the tropics (Fombong, 2012) while chemicals such as the organophosphate, coumaphos pose health risks to both beekeepers and consumers due to residues on products (Tingle *et al.*, 2003). These drawbacks have necessitated the development of affordable and more effective management tools (Fombong, 2012). To date, studies on small hive beetle have focused on exploitation of attractant compounds such as pollen dough inoculated with yeast (Arbogast., 2007), beehive produced volatiles (Suazo., 2003), honey bee hive products acted on by yeast, *Kodamea ohmeri* (Saccharomycetales: Saccharomycetaceae) and small hive beetle larvae (Hayes, 2015), apple cider and yeast-based attractants (Nolan & Hood, 2010). However, none of these studies on SHB explored potential non-host plant-based repellant volatiles that can be used in the management of SHB (Komen *et al*., 2019).

This study reports the chemical composition of Apicure, a plant -based bio pesticide recently developed at *the International Center of Insect Physiology and Ecology (icipe)*. The selectivity and sensitivity of antennal receptors of SHB adults to volatile compounds of this essential oil were determined using the electroantennographic (EAG) technique. This was established to understand the bioactive components of

Apicure and their potential to be used to manage the invasive small hive beetle. This is a step forward to offering an alternative to the synthetic insecticides used to manage the small hive beetle.

1.2 Statement of the problem

The capacity of agriculture to sustain the rapidly growing global population has created anxiety over generations and has persistently become a priority in global policies (Rosegrant & Cline, 2003; Tilman *et al.,* 2011). These policies have led to the development of global, regional and national blueprints that are steered towards achieving zero hunger and malnutrition. The sustainable development goals (SDG), set by the United Nations (UN) assembly in 2015 aims to eradicate hunger and malnutrition by increasing food and nutrition security by 2030 (United Nations, 2019).

Among the ways to achieve this is to ensure sustainable food production systems and implement climate smart agricultural practices that increase productivity and production, that help maintain ecosystems, that strengthen capacity for adaptation to climate change, extreme weather, drought, flooding and other disasters that progressively improve land and soil quality (Tomlinson, 2013; Fang & Cao, 2019).

The Kenyan government through the vision 2030 agenda, projects an additional Kshs. 80-90 billion to the Gross Domestic Product (GDP) as a result of increased yields in crops, which is attainable through sustainable and modern agricultural interventions (GOK, 2007). Addressing the above development goals has been challenging (El Bilali *et al*., 2020).

The world economic value of the honey bee colony decline, together with lower crop yields and increased production costs, has been estimated to as high as $5.7 billion per year (Sass, 2011). The honey bee, a major pollinator, has been red listed by the international union of conservation of nature (IUCN) as a threatened organism (IUCN, 2009). The alarming collapse in bee colonies and other pollinators has stressed the need to address this issue. Several factors have been attributed to the decline specifically the honey bee pests like varroa mite and the small hive beetle;

the honey bee pathogens, that are majorly vectored by the pests; pesticides such as neonicotinoids; habitat loss among others (Netwig, 2007; Hein, 2009; Francis *et al.*, 2013). In addition to the agricultural pesticides use, other medical pest control pesticides have been shown to negatively affect the honey bees (Tingle *et al.*, 2003; Munyuli, 2011).

The small hive beetle is an invasive pest that recently has expanded its host range moving from Africa to the USA, Australia, Portugal and Italy in the past 20 years (Mutinelli, 2014). The small hive beetle not only infest the genus *Apis* but also threaten the bumble bees (Hoffmann *et al.*, 2008) as well as stingless bees (Greco *et al.*, 2010). Beekeepers have reported huge colony losses of up to US$3 million attributed to the SHB following its identification in the United States in 1998 (Hood, 2004).

The report of colony losses as a result of Varroa mites and diseases infestation in Madagascar (Rasolofoarivao *et al*., 2013) points out to the possible existence of isolated and undocumented cases of colony collapse disorder in Africa. This pest is not only a threat to the pollinators but also feeds on fruits making it challenging to eliminate from an area posing a threat to fruit production (Buchholz *et al*., 2008b).

Several management strategies to control this pest have been developed. However, most of them are laborious to deploy and monitor, baits developed like pollen is expensive to small scale beekeepers in the tropics while the synthetic chemical pesticides are not sustainable (Karazafiris *et al.*, 2008; Kanga & Somorin, 2012a; Al-Waili *et al*., 2012). Also, none of these tactics has resulted in the total management of the beetle hence, the necessity for the development of sustainable and more effective management tools (Fombong, 2012; Kajobe *et al.*, 2016). The purpose of this study is sought to evaluate the bioactive chemical components of apicure, a novel plant-based extract, that showed efficacy in preliminary field trials against the small hive beetle.

1.3 Justification

Inevitably, the application of acaricides to manage honey bee pests has led to residues being regularly found in hive products (Martel & Zeggane, 2002; Abd El-Wahab *et al*., 2021). Most of the synthetic chemicals are lipophilic, therefore, their residues are identified in the beeswax, whereas residues in honey are comparatively low (Karazafiris *et al*., 2008b; Valdovinos-Flores *et al*., 2017). The global concern for pesticide residues in hive products has led to the implementation of the maximum residue limits (MRLs) in several countries to minimize the health risk it poses to the consumers. Coumaphos is a major acaricide used to control the small hive beetle and other pests and reports of its residues have been documented in honey (Martel *et al.*, 2007). Moreover chronic exposure to coumaphos can lead to reduced foraging ability of honey bees hence threatening their ability to pollinate (Tihelka, 2018). Boric acid also commonly used to control pests in hives has been reported to be highly toxic to bees posing direct threat to honey bee population and other non-target species (Stuhl, 2020). With the setbacks that come with the use of synthetic chemicals, this calls for development of other strategies that are environmentally safe and conserve the pollinators.

In Africa, there's limited information on managing this pest to the beekeepers limiting the strategies they deploy to traditional methods such as applying ashes around apiaries, hanging the hives, applying used engine oil to hive stands and smoking the hives when infestation is high (Kajobe *et al*., 2016). The enormous losses caused by SHB in the Western hemisphere and its occasionally severe damage to African apiaries have demanded the development of tools and strategies to monitor and manage its damage. Trapping has shown to be a promising sustainable method to control small hive beetle (Hood & Miller, 2003; Nolan Iv & Hood, 2010; Torto *et al*., 2010a). Optimization of traps has been observed when attractants were incorporated showing the importance of semiochemicals (Arbogast *et al*., 2009; Stuhl, 2020). Therefore, exploiting non-host volatiles from plants that are highly foraged by bees will enable discovery of useful semiochemicals that can be used to disrupt the host finding since honey bee odors are known to attract the small hive beetle and other honey bee pests (Bobadoye *et al.*, 2018). It is essential to carry out

more research on the SHB ecology and control to develop integrated parasite management tools to curb the alarming honey bee population decline (Di Prisco *et al.*, 2013). Apicure is a novel plant-based extract developed by Lwande *et al.*, (2016) for the management of honey bee pests and diseases. Preliminary field trials by these authors against the SHB showed that the product might be repelling these pests. However, the bioactive components of this product against the small hive beetles and other hive pests is unknown. This study, therefore, sought to provide an in-depth understanding of these components and to evaluate the potential of Apicure in pest management.

1.4 Objectives

1.4.1 General objective

To evaluate the bioactive components in Apicure, a plant-based extract, and their potential in management of the small hive beetle, *Aethina tumida*, a major pest of honey bee colonies

1.4.2 Specific objectives

i. To identify the bioactive volatile chemical components of Apicure against the small hive beetle.
ii. To establish the behavioral activity of small hive beetle to the electrophysiologically active components of Apicure

1.5 Hypotheses

The null hypotheses are:

i. There are no bioactive volatile chemical components in Apicure against the small hive beetle.
ii. The small hive beetle does not exhibit any behavioral activity to the electrophysiologically active components of Apicure

CHAPTER TWO

LITERATURE REVIEW

2.1 Origin and distribution of honey bees

The honey bee, *A. mellifera,* is considered to originate from tropical Africa and spread throughout the continent to Northern Europe to India and China (Ellis & Ellis, 2016) (Fig 2.1). In Africa, honey bees are primarily wild population while the European honey bees are majorly managed with extinct wild populations.

2.2 Economic importance of honey bees

Loss of the honey bees does not only affect the beekeeping industry, but also the agricultural sector because most cross-pollinated crops are dependent on honey bees for pollination. The overall economic value of global pollination is reported to be €153 billion, which accounts for 9.5% of the world agronomic production used for food (Gallai *et al*., 2009; Lautenbach *et al.*, 2012). The direct benefits of honey bees include the value of honey bees pollination services and its products that include honey, beeswax, pollen, royal jelly, bee venom, and propolis in cosmetics and medicines (Gebrekristos, 2015). The key insect group in managed animal pollination services is the bees, particularly the honey bee. Honey bee pollination in the United States alone is estimated at $14.6 billion (Klein *et al*., 2007). It is estimated that 75% of the global food crops exhibit improved fruit and seed quality and quantity with animal pollination (Ollerton *et al.*, 2011).

In Kakamega, Kenya honey bee pollination was shown to improve yields in crops such as beans, cowpeas, green grams, Bambara nuts, tomatoes, capsicum, passion fruit, sunflower and squash. The range of increase in yields was from 25% in tomatoes to more than 99% in squash (Kasina *et al.*, 2009). In the same study, significant improvement in seed quality of sunflower oil by 21%; and fruit sizes of capsicum increased by 29%, this correlates to higher market prices. Despite this huge contribution of the honey bees to agriculture, biodiversity and the economy, the honey bees especially the wild bees are under threat to extinction (Nieto *et al*., 2014).

This decline in population and extinction by some species has been attributed by a myriad of factors.

2.3 Factors that contribute to the global bee colony losses

Various factors have been anticipated to explain the reported honey bee population decline. These factors include habitat fragmentation and its loss, intensified agriculture, overuse and poor handling of pesticides such as neonicotinoids, pathogen spillover from managed pollinator species, invasive species, global climate change and genetic factors that can lead to species extinction (Steffan-Dewenter *et al*., 2005; Le Conte & Navajas, 2008; Zayed, 2009).

A honey bee hive has maintained conditions of optimal temperature, humidity, and carbon dioxide level, availability of the honey bees (host), proteinaceous (pollen), carbohydrate (honey), and wax which are a suitable habitat for various parasites and pathogens (Pirk *et al*., 2016). Among the parasitic mites attacking honey bees are the tracheal mites, tropilaelap mites and the *Varroa* mite. The honey bee is also attacked by pests such as the small hive beetle, ants, greater and lesser wax moth and other lepidopterans (Spivak, 2010). Small hive beetle is a scavenger and facultative predator in honey bee colonies, it is an invasive pest of honey bees in the United States and Australia, where it feeds on pollen, honey, and bee brood (Arbogast *et al*., 2012).

Recent colony losses like in North America have raised concerns of synergistic effects of the above factors as a cause for honey bee damage or colony losses (Higes *et al*., 2016). The eminent example of a potential interaction of several factors is the Colony Collapse Disorder (CCD) where up to 50% of colony winter losses have been recorded in the United States (Oldroyd, 2007). There is the lack of enough information to estimate the colony levels losses in Africa. A study in South Africa reported 29.6% (2009-2010) and 46.2% colony losses (2010-2011), within a sampled population of beekeepers, these levels are higher than acceptable levels in Europe (Pirk *et al*., 2014).

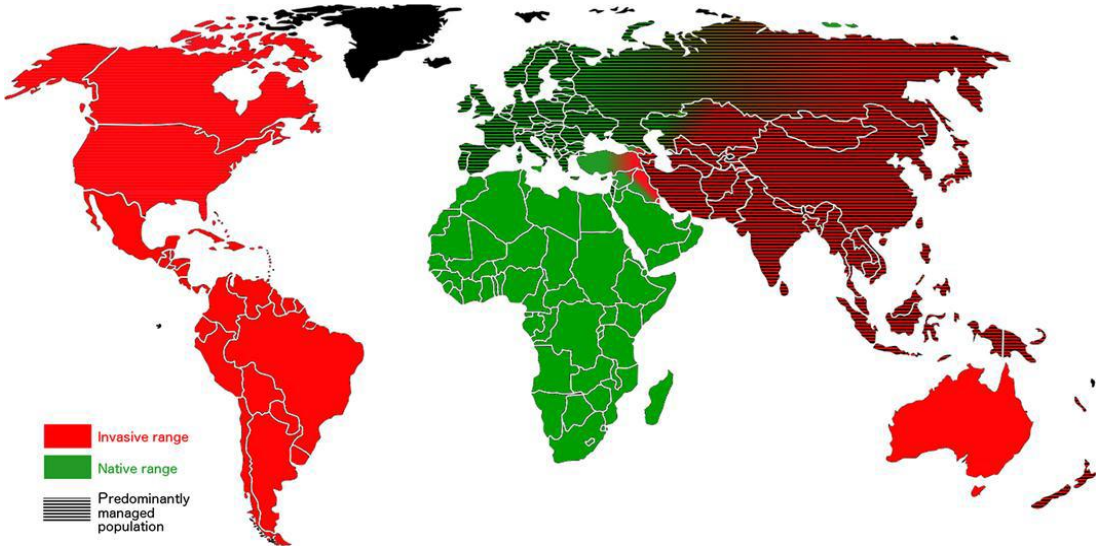

Figure 2.1: Worldwide distribution of *Apis mellifera*.

The native distribution (green), anthropogenic-assisted range expansion (red), relatively small bee-keeping (managed) population or equality between wild and managed (plain) and population predominantly consisting of managed colonies (stripes). Source: (Pirk *et al.*, 2017).

2.4 The small hive beetle *Aethina tumida* Murray

2.4.1 Taxonomy, description and distribution

The small hive beetle *A. tumida*, first described in 1867, is a coleopteran of the family, Nitidulidae. It has approximately 2,800 described species in 172 genera globally (Habeck, 2002). Nitidulidae differs from other beetles because of their transverse procoxal cavities, grooved metacoxae, dilated tarsal segments, small fourth tarsi and three-segmented antennal club (Habeck, 2002). They can feed on fresh, rotten and dried fruits, plant juices and crops but occasionally on flowers as well (Fadamiro *et al*., 1998; Wolff *et al*., 2001; Hepburn & Delaplane, 2003;). Since 1998, the SHB has raised international concerns because it has become an invasive species in European honey bee populations. Before June 1998, the small hive beetle was well-known to occur only on the African continent but it has spread out of its endemic range (Fig 2.2).

2.4.2 Life cycle of the SHB

The SHB completes its life cycle (egg, larva, pupa and adult) within 4 to 6 weeks (Fig 2.3). There may be as many as six generations in 12 months under moderate climatic conditions (Hood, 2004). The eggs are small, whitish, rod-shaped and about 1.4 mm long and 0.26 mm wide and exhibit notable similarity to that of its host except for their smaller size. They oviposit in clusters of 10-30 in open or capped brood cells or crevices and cracks within the hive, with females known to layover 1000 eggs during their entire life.

The eggs hatch after 2-4 days into young larvae (Elzen *et al*., 2009). The larval stage lasts for 10-14 days after which it goes into a 'wandering' stage where they move out of the hives late evening with its peak activity at 21.00 h in search of a favourable pupation substrate (Elzen *et al.*, 1999).

Figure 2.2: World distribution and introductions of small hive beetle.

Endemic distribution range in sub-Saharan Africa (dark grey areas), well-established invasive populations (medium grey areas), personal observations (dark grey circle) not well established (light grey); new records in endemic range (black) and introductions (white circles) (Source: Neumann *et al*., 2016).

The larva is cigar-shaped, pale yellow with a light brown head and the presence of a pair of distinct dorsal spines on each body segment which is used for distinguishing small hive beetle larvae from larvae of other insects within the hive.

The larvae tunnel 10-20 cm and occasionally up to 30 cm into a favourable substrate usually moist soil and build a smooth-walled, earthen Chamber where pupation and development take place. Early-stage pupae of SHB are pearly white, having distinctive projections on the thorax and abdomen. Later-stage pupae darken as their exoskeleton develops and hardens (Ellis & Ellis, 2016). An adult usually emerges 15-60 days post pupation with most adults emerging after 21 to 28 days (Elzen *et al.*, 1999; Ellis & Ellis, 2016). Newly emerged adults are brown-black in colour on the dorsal side and reddish-brown to black on the ventral side. Adult SHB are known to reproduce on a range of diets which include bee brood, pollen, honey, fresh and rotten fruits, empty bee combs (Ellis *et al.*, 2000; Buchholz *et al.*, 2008; Arbogast *et al*., 2010) thus higher chances of survival as they appear as facultative parasites of honey bees.

2.4.3 Host-parasite interaction

2.4.3.1 Host finding

Small hive beetles are active flyers and can invade colonies by individually or occasional swarms (Elzen *et al*., 1999). It has been noted by that SHB can detect stressed colonies (as a result of diseases or management strategies like splitting) from about 13-16 km distance (Neumann & Elzen, 2004). Stressed colonies detection may be adaptive in Africa and reproduction is favoured in such colonies than in strong ones however the actual mechanism for detection is unclear.

Previous studies elucidate that honey/pollen and adult bees' combination is highly attracts flying beetles, while adult bees alone are less attractive. Hive products and infested combs alone are not attractive (Elzen *et al*., 1999). Honey bee colony with food storage is a favourable place for breeding by the SHB.

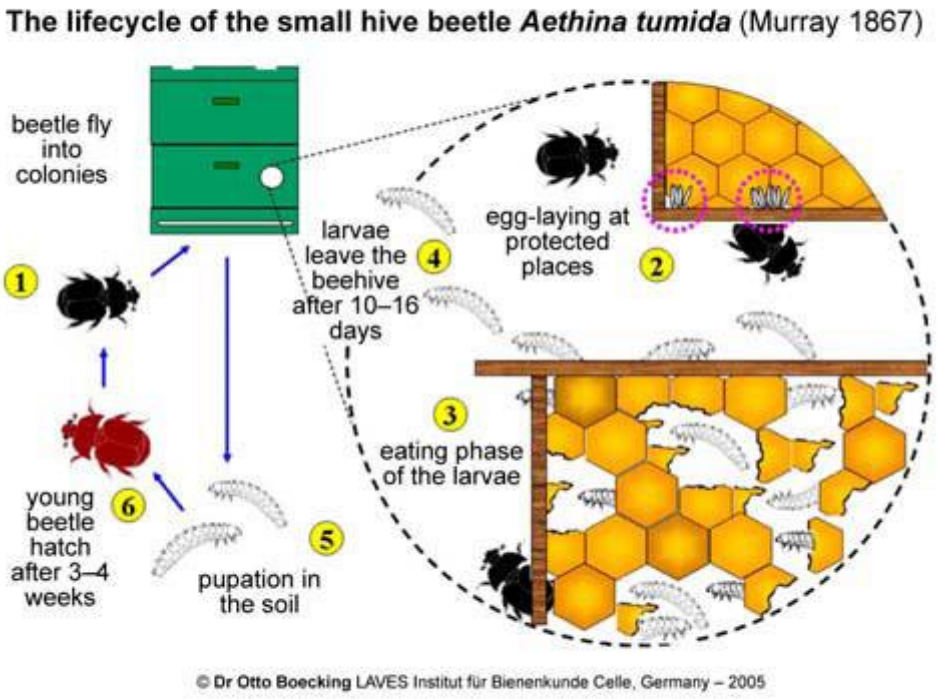

Figure 2. 1:The life cycle of the small hive beetle.

(Source: Blueebees.com)

2.4.3.2 Host invasion

Adult beetles are attracted to the hive through volatiles (Suazo *et al*., 2003) .The guard bees prudently inspect incoming individuals (Carreck, 2016). Adult beetles can invade strong honey bee colonies as well as weak ones with equal effect (Lundie, 1940). Nonetheless, reduced entrances minimize the number of intruding beetles (Ellis *et al.*, 2002), signifying that this can be used to minimize SHB invasion though this has been reported to affect the thermoregulation of the hive. Beekeeping management practices like regular inspections seem to enhance intrusion of the beetle into host colonies.

2.4.3.3 Host strategies

Honey bee species defend themselves through active aggression to both the adults and larvae (Neumann *et al*., 2001). The bees try to bite or sting the adults but usually with only little success (Neumann & Elzen, 2004). In the few cases, when the adult beetles are decapitated or extremities are removed, they are thrown out of the hive (Neumann *et al*., 2016a). Observations that small hive beetles can live for long periods even in strong colonies with relative effect also suggest that aggression is not very effective in killing the beetles. This may be partially due to their hard exoskeleton but also due to the defense tactics of the adult beetles.

However, aggression is not very effective in killing the beetles, it may contribute to resistance. African honey bees show more investigative contact and aggression behaviour to the adults than European ones. As a defense against the SHB, honeybees construct cells of propolis (plant resins) into which they drive the beetles and imprison them (Ellis *et al*., 2002) . Removal of eggs is another tactic deployed by honey bees whereby the SHB eggs are eaten by the worker bees (Neumann & Hartel, 2004), both protected underneath cell capping (Ellis *et al*., 2003) or in cracks and exposed ones. Moreover, the bees remove the SHB larvae out of the hive (Neumann & Hartel, 2004; Spiewok & Neumann, 2006), this is mostly effective in strong colonies. Absconding occurs in the case of high infestation of SHB, both African and European honey bee colonies abscond (Hood, 2000).

2.4.3.4 Parasite defense tactics

The SHB is known to deploy a variety of tactics for its defence. First, is the defense posture during the attack, the adults can make a turtle-like defense posture (Neumann *et al*., 2001). The beetle stays motionless and keeps its head underneath the pronotum with the legs and antennae pressed tightly to the body. Secondly, SHB ordinarily moves very fast out of the range of bees (Neumann & Elzen, 2004). In addition, the beetles can purposely drop from the honeycombs to escape pursuit by the honey bees (Hood, 2015). The SHB also hide in the nest cavities with the adults hiding in little cracks below the bottom board of commercial hives or cells. While hiding in cells, small hive beetles usually stay motionless at the bottom. During field colony

inspection, they are observed moving around the hiding places this is the case in the observation hives.

2.5 Economic impact of SHB

The SHB is a major threat to destabilized or stressed colonies of honey bees, especially in its native range. Following its introduction, the SHB has become a major pest for commercial beekeepers in the southeastern United States due to their ability to infest even strong colonies of European honey bees (Hood, 2004). In the USA, this invasive pest feeds on bee brood, pollen, honey and bee combs causing severe damage estimated at US$3 million (Hood, 2000). Similar but negligible damage patterns have been observed within its African host and several studies have accredited these observed difference in infestation levels between African and European honey bee colonies to the greater hygienic behavior of the African honey bees as opposed to the European honey bees (Neumann & Hartel, 2004).

Recently, the realistic effects of the small hive beetle on honeybee colonies and bee products was established in Ethiopia that ranged from bee population, honey yield, brood area ,this calls for more studies that estimate the losses by this pest in Africa (Gela Bayeta *et al.*, 2018).

In their introduced range, SHB promotes a decrease of the brood area is highly infested colonies. This is possible in the following two ways. First, the adult and larval beetles may consume bee brood, hence reducing the brood area; even though each of the populations may be high before a quantifiable reduction in brood area is realized. Female beetles bite openings in the wax capping of brood cells (or along the cell wall) after which they oviposit on bee pre-pupa/pupa in the cell. This can be detected by the honey bees and they remove brood oviposited on in this way (Ellis *et al*., 2003a; Ellis *et al.*, 2004). SHB oviposition on brood stimulates the removal of infected brood hence reducing the brood area. Intra-colonial destruction has been accredited to the feeding behaviours of adult and larval beetles. As the SHB feeds, it defecates on the honey which has been postulated to promote its fermentation (Elzen *et al*., 1999; Hood, 2000). Additionally, bee brood and pollen reserves are consumed, consequently weakening or destroying a colony. In the case of high

infestation of beetles may prompt honey bee colonies to abscond, though the population of beetles per frame of bees has to be high to cause absconding (Ellis *et al*., 2003a). Beetle populations may decrease flight activity hence reducing colony production. This is possibly related to absconding by honey bees. The loss in flight activity may be that bees guarding beetle confinement sites are foraging age bees (Ellis *et al*., 2003a), with growing intra-colonial populations of beetles diverting bees from foraging to guarding.

During migration from colony to colony, beetles possibly may transmit bee pathogens mechanically as the pathogen may stick to beetle bodies hence the possibility of horizontal pathogen transmission to other colonies or apiaries. The SHB might be a possible vector for viruses such as deformed wing virus and sac brood virus (Eyer *et al.*, 2009) increasing the risk of colony collapse and lesser productivity in infected colonies. The SHB also consume and reproduce on numerous varieties of fruit, such as bananas, mango, grapes, and strawberries (Buchholz *et al*., 2008a) as well as avocado, cantaloupe, pineapple, honeydew, and star fruit (Ellis & Hepburn, 2006) hence the potential threat to the fruit industry.

2.6 Management strategies for SHB

Management strategies are still being explored, with minimal progress made since the initial *A. tumida* discovery, especially in the chemical controls (Ellis & Ellis, 2016). Additionally, there is not a well-established economic threshold for *A. tumida*, making treatment decisions difficult.

2.6.1 Cultural control

There are various cultural methods that beekeepers may use to manage the small hive beetle. Minimizing colony stress conditions and conserving strong productive colonies are highly recommended, more so in regions where beetles is a challenge. Any practice that aids in maintaining well-populated honey bee colonies that decrease the comb-to-bee ratio and eliminates beetles from the brood area is recommended (Roth *et al.*, 2022).This also includes good management practices that reduce the possible occurrence of brood diseases, mite infestation, wax moth

survival, queens failure, and excessive swarming, over supering and colony starvation. Sugar patties increase beetle incidence, therefore, more caution should be taken during feeding colonies with sugar water or corn syrup inside hives (Westervelt *et al.,* 2001).

Push-in screened queen introduction cages are not recommended in heavily beetle-infested areas as the SHB adults and larvae go into the cage and are secured. Sanitation should be maintained around apiaries to inhibit SHB damage to stored comb (Hood& Taber, 2000) Beekeepers should remove wax capping and other wax materials and equipment containing bee pollen. Pollen baited traps should not be left on colonies over lengthy periods because the unprotected pollen will nourish the beetles for regeneration. Maintenance of relative humidity of 50% or less in honey houses will promote beetle egg desiccation (Somerville, 2002). Moreover, Selection of apiary locations with drier soil conditions (open, sunny) is recommended for small hive beetle control.

2.6.3 Chemical control

Chemical control includes in-hive use of coumaphos and fluvalinate (Mostafa & Williams, 2002b) and soil treatments using permethrin (Hood, 2000) The SHB adults are susceptible to fenitrothion, chlorpyrifos and methomyl (Kanga & Somorin, 2012b). Fenitrothion is most toxic to SHB larvae. Most of them like permethrin are lethal to non- target species and more so the honey bees and can lead to building up resistance by the beetles.

A reduction of larval infestation with formic acid treatment has been demonstrated whereas acetic acid recorded high mortality of adult beetles (Schäfer *et al*., 2009; Buchholz *et al*., 2011). In-hive treatment with CheckMite+ Strips^{TM} containing coumaphos by attaching the traps made of corrugated cardboard and CheckMite+ strips (10% w/w coumaphos) to the hive bottom boards has reported mortality of upto 90 % (Neumann & Hoffmann, 2008).

Development and commercialization of the harbourage has been done with the trade name, Apithor™ (Levot, 2012) and is used widely all over Australia and other regions for SHB management. More lately, Paradichlorobenzene has been put forward also as a fumigant for beetle control in the stored comb (Mostafa & Williams, 2002). Soil treatment by numerous materials has been investigated during pupation in the soil. Such treatments include the use of HCH (benzene hexachloride), carbaryl, chlordasol and salt solutions in South Africa where Chlordasol was reported to be the most effective in this trial. GardStar® (40% permethrin), registered for over a decade in several beetles infested states in the USA, is a soil drench that is used to kill beetle larvae and pupae (Delaplane, 1998). Slaked lime and diatomaceous earth are potential control materials for SHB (Buchholz *et al*., 2009). Slaked lime prevented wandering larvae from pupating and the diatomaceous earth was toxic to both adults and larvae. Application of slaked lime leads to absorption of water from the soil and thus disturbing SHB larvae pupation. Despite the efficacy of the chemicals, there's the concern of its residues in the hive products that is a global health concern to humans.

2.6.4 Biological control

Biological control has been investigated for the suppression of the SHB (Rong *et al*., 2004). Use of microbial pathogens precisely entomopathogenic fungi is a prospective substitute to chemical insecticides (Lacey *et al.*, 2001). Fungal species have been identified in a complex isolated from the pathogen-killed SHB pupae: two of these were *Aspergillus niger* van Tieghem and *Aspergillus flavus* (Richards *et al.*, 2005). Both species are cosmopolitan soil fungi that appear to infect the SHB pupal stage when post-feeding larvae exit the host honey bee colony and burrow into the surrounding soil for pupation. Various isolates of both *Metarhizium* and *Beauveria* are efficient against larvae and adult SHB in laboratory assays. Generally, the *Metarhizium* isolates performed best against larvae killing more than 70% of larvae by day 7 while the *Beauveria* isolates produced 99 and 100% mortality of adult beetles respectively 14 days after treatment (Leemon & McMahon, 2009).

Recent studies have demonstrated that the generalist entomopathogenic nematodes, *Steinernema riobrave, Steinernema carpocapsae, Steinernema kraussei* and *Heterorhabditis indica* have the potential to control larval stages of the SHB after a single soil application (Ellis *et al.*, 2010; Cuthbertson *et al.*, 2012). The nematodes *S.carpocapsae* and *S. kraussei* each provided total mortality of pupating larvae in sand pots and that nematodes readily emerged from dissected larvae (Cuthbertson *et al*., 2013). The fire ant, *Solenopsis invicta*, infests much of the current beetle-infested range in south eastern USA. Here, the ant has been observed feeding on mature SHB larvae as they enter the soil to pupate (Hood, 2000). Fire ants may reduce beetle activity in some areas but little is known about this predator- prey relationship (Torto *et al*., 2010).

2.6.5 Physical/Mechanical/trapping

Numerous methods are available for the control of the SHB, these methods are very time consuming due to regular visits to the same colonies, especially when the infestation is high. Frequent changing of apiary sites has been reported to decrease beetle problems (Hood and Taber, 2000) but this is time-consuming and labour intensive. Selection of sites that are exposed to full sunlight has been recommended for beetle control, this is because of its relation to low soil moisture content hindering pupation.

Various traps have been developed to manage the SHB numbers without disturbing the honey bees or contaminating the honey. Several attempts have been put forward to exclude beetles from entering or exiting a beehive. Modification of the entrance into the colony has been studied to minimize the invasion by beetles (Ellis *et al.*, 2002). The reduction of the entrance size resulted in reduced brood area, impaired in-hive thermoregulation and poor water drainage, which adversely affected the honey bee colony(Hood & Miller, 2005). A SHB trap with a plastic bucket was developed. Holes were made on the bucket big enough for the SHB but too small to permit entrance by bees (Elzen *et al.*, 1999). The buckets were placed randomly throughout the apiaries with known infestations of SHB and inspected at 24 and 48-hour intervals. The traps were baited with various hive product combinations, and the

most attractive combination being the honey, pollen, and live honey bee mixture. Competing odors from surrounding colonies were put forward as a limitation of this trap to manage SHB.

Two types of in-hive traps was evaluated by Torto *et al*., (2007). One trap was a modified Langstroth bottom with a rectangular opening in its center. The opening was covered with four-mesh aluminium screening to block the bees from entering the trap. The modified bottom board was then attached to a three-sided frame, with the missing side positioned toward the rear or side of the hive, with runners to let the trap slide into position. A lid of an egg container was placed below the hole and held the bait while the two openings in the middle were fixed with polymerase chain reaction plates, and the tray was painted black since beetles prefer the dark. Traps baited with inoculated pollen dough trapped significantly more beetles than the unbaited traps, suggesting the significance of attractants in controlling SHB. A refuge trap that uses a corrugated cardboard insert treated with fipronil and encased in plastic resulted to 60% SHB mortality within 6 weeks of treatment application was detected (Levot, 2008).

Development of other traps with different levels of effectiveness is documented. One of these traps is the Hood beetle trap. The trap is a three-chambered plastic box that can be fixed firmly to the bottom bar of a frame and placed in the bee colony in place of a normal frame in either a honey super or brood chamber with relatively little difference in the number of SHB trapped that is filled with attractants usually cider vinegar (Nolan & Hood, 2008) However, this trap does not eliminate the SHB rather reduces its infestation (Nolan & Hood, 2008).

The Cutts Trap is another trap which is a disposable plastic trap that has square openings in the top to let beetles enter the trap preventing bee entry. The trap is thin and placed in between frame top bars, with the top resting on the top of two adjacent frames. The trap is half-filled with vegetable oil, which has a similar effect to the mineral oil in the Hood trap.

The Freeman trap comprises a specially designed screened bottom that allows a plastic tray to slide into it and under the colony (Roth *et al*., 2022). The screen

attempts to prevent bees from being trapped while allowing beetles to enter. The tray is partially filled with vegetable oil to drown beetles that run to the bottom of the hive to escape attack by honey bees. However, this trap allows the beekeeper to monitor the SHB in a colony with a limited disturbance on the bees since the trap can be inspected without opening or excessively disturbing the colony. The trapping methods above are mainly optimized using potential attractants but no possible repellant has been reported. The potential of deploying a push-pull strategy into the small hive beetle management is worthwhile to evaluate.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

Laboratory studies were carried out at the International Centre of Insect Physiology and Ecology (ICIPE) Duduville campus, Nairobi Kenya (1º 16’ 60’’ S; 36º 49’ 0’’ E) while the field collection of SHB was conducted in experimental apiaries at Karura forest located at Latitude: 1° 14' 15.00" S, Longitude: 36 ° 49' 14.99" E.

3.2 Insects

Using a modified standard methodology, a colony of the small hive beetle, *A. tumida*, was reared from beetles collected in Karura forest in August 2017(Neumann *et al*., 2013). Female beetles were placed in a plastic container (11 cm long, 11 cm wide, 11 cm high) with a 1 mm mesh insert in the center of the lid to promote aeration. The containers were half- filled with sliced ripe bananas as a feeding substrate, as well as moistened cotton wool to maintain humidity and supply water for the beetles. The same substrate (bananas) was used to feed the emerging larvae, which was supplied as needed.

One week old larvae that had entered the wandering stage (i.e. crawled away from the food source in search of pupation substrate) were removed from the container and transferred to a new plastic container measuring (18.5 cm 14 cm 9.5 cm) filled to a depth of 6 cm with autoclaved and moistened sandy loam soil collected from the Bee health farm at ICIPE.

To pupate, the larvae burrowed into the soil. Adult beetles emerged from the soil after three weeks and were put to plastic containers with the banana diet. When the beetles were fully developed, they were used in bioassays a week later (when they turned black). Rearing at all stages was done at the laboratory at ambient conditions.

3.3 Headspace volatile collection

The Environmental Health Department at the International Center for Insect Physiology and Ecology provided one sachet of Apicure, which included a sponge with 3.5 g of essential oil *(icipe).* The contents of the sachet were poured into a 500-ml cylindrical glass flask (Sigma Scientific, Gainesville, FL, USA) using a scalpel.

Headspace volatiles were collected for 24 hours by aeration and adsorption on charcoal filter adsorbents (5 mg, Brechbuhler, Schlierensee, Switzerland). Each filter was connected to a mobile battery-operated pump (PAS-500 Personal Air Sampler, Supelco, Bellefonte, PA, USA) by PVC tubing (Masteflex. 06409-15 Tygon mfg by St. Gobain), which supplied a continuous flow of clean air through the sample and also pulled the volatiles to the filter at a flow rate of 348 mlmin-1. All the filters were eluted with 100 ml of GC-grade dichloromethane (Sigma Aldrich, Gillingham, UK) into vials. The eluates were stored in amber screw-capped glass vials at -80°C until they were used.

3.4 Analysis of volatiles

On an Agilent 7890A gas chromatograph with an HP-1 column (30 m x 0.32-mm diameter x 0.25 mm thickness), coupled GC/EAD studies were performed (Agilent, Palo Alto, California, USA). At a flow rate of 1.2 ml min-1, nitrogen was used as the carrier gas.

At 280°C, the injection was splitless, with a 3-minute split valve delay. The oven temperature was set at 35°C for 5 minutes, then increased to 280°C at a rate of 10°C/min for 10 minutes. The column effluent was split 1:1 for flame ionization detection (FID) and EAD detection at the same time. The column effluent was combined with humidified air (200 mlmin-1) before being sprayed on the EAD preparation. (Njihia *et al*., 2017 ; Murungi *et al.,* 2018). The reference and recording electrodes for EAD were silver wires in glass capillary electrodes filled with ringer solution. The entire head of SHB was chopped with a scalpel to prepare the antennae; the reference microelectrode was placed in contact with the basal segment of the head of SHB, and the recording electrode was linked to the distal end of the

antennae. An amplifier (INR-II, Syntech, Hilversum, The Netherlands) detected the antennal signal, which was then acquired and processed by a data Acquisition controller (IDAC-4, Syntech, Hilversum, The Netherlands), and then analyzed with GC/ EAD 2000 software (Syntech). An aliquot (3 µl) of the charcoal filter-adsorbed volatile extract of Apicure was analyzed with either fresh male or female antenna in four replicates.

On an Agilent 7890A gas chromatograph coupled to a 5795C mass spectrometry, equipped with MSD Chemstation E.02.00.493, and Wiley 9th/NIST 2008 MS library, GC/MS studies of the charcoal filter-adsorbed volatile extract of Apicure were performed. In GC-EAD analysis, the same GC/MS column and temperature parameters were utilized as mentioned above. The retention time and mass spectral fragmentation of similar authentic standards in the library were compared to identify substances in Apicure.

Plate 3.1: Gas Chromatography- Electroantennodetection set up. 1 = antennal mount; 2 = Gas Chromatography – Mass Spectrophometry 3=amplifier; 4= Display computer

3.5 Bioassays with Apicure and synthetic components

A Pyrex glass Y-tube olfactometer (internal diameter 10 mm; stem 85 mm; arms 75 mm at a 60° angle to the stem) (Analytical Research System INC, Gainesville FL, USA) was used to test the behavioral response of SHB to Apicure and its components (Njihia *et al*., 2017). The olfactometer's Y-arms were connected to a sealed glass odor source chamber (internal volume 50 ml) supplied with charcoal-filtered and humidified air via PVC tubing (Masteflex. 06409-15 Tygon mfg. by St. Gobain, Paris, France) (90 % RH).

A battery-powered pump (USDA/ARS-CMAVE, Gainesville, FL, USA) kept the airflow through each arm of the Y-tube at 30 ml min-1. To avoid volatiles build-up in the test arena, a PVC tube was connected at the base of the Y-tube to the pump's vacuum source at 60 ml min-1. A positional bias of the Y-tube was performed prior to behavioral tests with Apicure and synthetic standards (blank vs blank). Using a micropipette, approximately 40µl of the extracts were applied onto 2.5cm x 2.5cm filter papers No.1 Whatman Int Ltd. Maidstone, England). Before placing the solvent into the holding chambers of the Y-tube, it was allowed to evaporate for 2 minutes. An adult SHB was individually introduced at the entrance of the main vertical arm of the Y-tube and considered to make a choice after walking beyond the Y-tube intersection in 1 min. A screen mesh barrier at the openings of each arm kept small hive beetles from escaping through the arms of the olfactometer. Each trial (N = 75) used twenty-five adult females and was replicated three times. During the bioassays, each individual was used only once. To avoid positional bias, the positions of the test and control odor sources were reversed after every three tests. Following the experiments, glassware was washed with Teepol® (multipurpose detergent; Teepol® products, Kent, UK), rinsed with acetone, distilled water and then baked at 80 ºC for 2 hours. Apicure and the electrophysiologically active compounds' synthetic standards were tested at three concentrations at 10 ng/µl, 100 ng/µl and 1000 ng/µl.

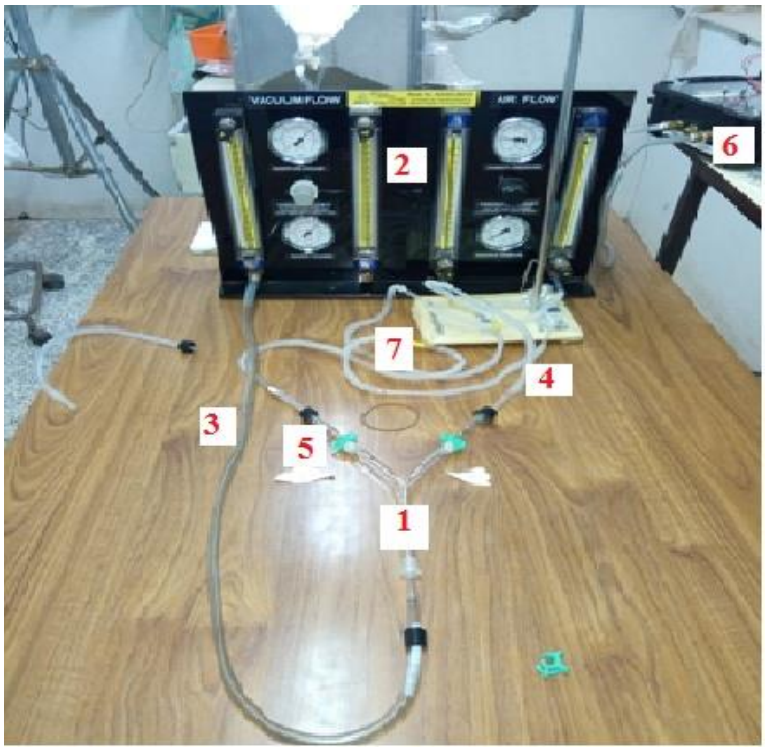

Plate 3.2: Y-tube olfactometer set up in a laboratory bench. 1 = Y-tube olfactometer; 2= flow meter; 3=vacuum; 4= air supply; 5= Odor dispensers (filter papers) placed inside glass chambers; 6= pump; 7= Teflon tubes

3.6 Chemical standards and reagents

Synthetic standards of camphor, α-terpineol, limonene geraniol and linalool used in behavioral bioassays were obtained from Fluka® Analytical. Before analysis, all chemical standards were prepared in dichloromethane (Sigma Aldrich) and stored in amber screw-capped glass vials at -20°C.

3.7 Statistical analysis

The response of the beetle to the treatment i.e., Apicure volatiles and synthetic standards of the electrophysiologically active compounds compared to the control (solvent/blank) was analyzed using Chi-square (χ^2) goodness-of-fit tests, assuming a

distribution ratio of 1:1 to compare responses of the test individual to odor sources and control. Non- responders were not included in the analysis. Data analysis was done using R version 3.4.1 software (R Core Team, 2017).

CHAPTER FOUR

RESULTS

4.1 Identification of Apicure volatiles

GC-MS analyses identified a total of 40 compounds that varied in their relative abundance in Apicure. The most abundant constituents were monoterpenes and their derivatives (34.16%) and sesquiterpenes, (26.83%). Of the monoterpenes, camphor (41.4%) was the most abundant followed by terpinen-4-ol, an isomer of terpineol (7.9%) and terpineol (7.4%) (Table 4.2; Fig 4.1). The most abundant sesquiterpenes in Apicure® was (Z) - β -farnesene (3.2%) followed by α- copaene (2.1%) and trans-calamenene (1.6%). Of the 40 components, nine monoterpenes (camphene, cymene, limonene, cymenene, linalool, camphor, terpin-4-ol, α-terpineol, and geraniol) and two sesquiterpenes (β-farnesene and caryophyllene oxide) stimulated the antennae of the SHB (Fig 4.2). The electrophysiological activity of synthetic standards of limonene, geraniol, linalool, camphor and α-terpineol was confirmed with the positive recording of the SHB antennae (Fig 4.3).

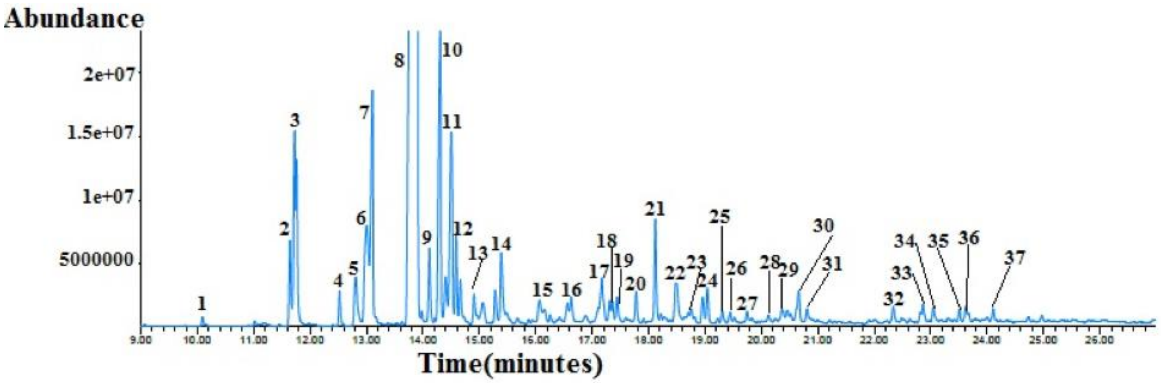

Figure 4.1: A representative chromatogram showing identified Apicure® volatile compounds collected with super Q adsorbent trap

Table 4.1: Identification and quantification of volatile compounds by Gas Chromatography – Mass Spectrophometry analysis in Apicure

Pea	RT	Compound Name	Chemical	Abundan
1	10.	Camphene	Monoterpe	0.2
2	11.	Myrcene	Monoterpe	0.2
3	11.	*O*- Cymene	Monoterpe	1.5
4	11.	Limonene	Monoterpe	6.0
5	11.	Lavender Lactone	Lactone	0.1
6	12.	(Z)-Linalool oxide	Monoterpe	0.7
7	12.	Camphenilone	Monoterpe	0.0
8	12.	*p*- Cymenene	Monoterpe	1.5
9	13.	Linalool	Monoterpe	5.5
10	13.	Bicyclo [2.2.1] heptan-2-ol, 1,3,3-trimethyl-,	Others	0.2
11	13.	Camphor	Monoterpe	41.4
12	14.	Borneol	Monoterpe	1.6
13	14.	Terpinen-4-ol	Monoterpe	7.9
14	14.	α-Terpineol	Monoterpe	7.4
15	14.	Trans- carveol	Monoterpe	0.8
16	15.	Geraniol	Monoterpe	3.1
17	15.	cis-1,4-Dimethyl-2-methylenecyclohexane	Others	0.4
18	16.	1,2-Cyclohexanediol, 1-methyl-4-(1-	Others	1.6
19	17.	α-Copaene	Sesquiterp	2.1
20	17.	Bicyclo [4.3.0] nonane, 7-methylene-2,4,4-	Others	1.0
22	17.	(Z)-Caryophyllene	Sesquiterp	0.8
23	18.	(Z)-beta-Farnesene	Sesquiterp	3.2
24	18.	α-Muurolene	Sesquiterp	1.2
25	19.	trans-Calamenene	Sesquiterp	1.6
26	19.	beta-Calacorene	Sesquiterp	0.7
27	19.	Cyclooctene, 3-(1-methylethenyl)-	Others	0.7
28	19.	Spathulenol	Sesquiterp	0.9
29	20.	(Z)-Cadina-1(6),4-diene	Others	0.8
30	20.	1-Methyl-6-methylenebicyclo [3.2.0]heptane	Others	1.1
31	20.	Caryophyllene oxide	Sesquiterp	1.2
32	21.	Premnaspirodiene	Sesquiterp	0.2
33	22.	1-Methylbicyclo [3.2.1] octane	Others	0.9
34	23.	4,4,8-Trimethyltricyclo [6.3.1.0(1,5)]	Others	0.7
35	24.	3-Octyne, 2,2,7-trimethyl-	Others	0.5
36	25.	Neopentylidenecyclohexane	Others	0.5
37	25.	Farnesol isomer	Sesquiterp	0.6
38	26.	Thunbergol	Diterpene	0.3
39	29.	α-Farnesene	Sesquiterp	0.3
40	29.	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-	Others	0.3
41	29.	Octadecane, 1-iodo-	Others	0.2

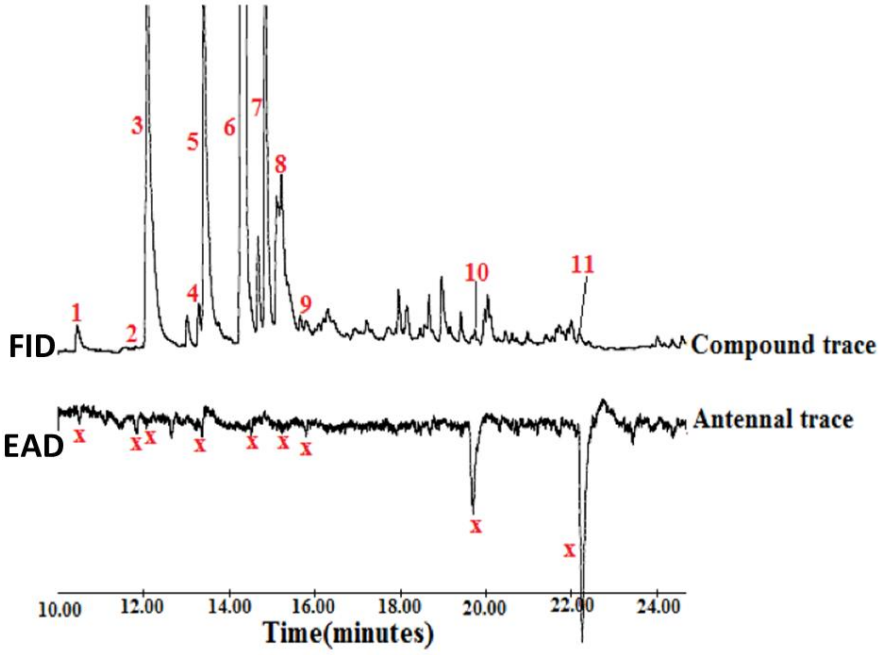

Figure 4.2: Electroantennography detection of Apicure volatiles by Aethina tumida. 1*- camphene; 2*- cymene; 3- limonene; 4*- cymenene; 5- Linalool; 6- camphor; 7- terpin-4-ol; 8- α-terpineol; 9- geraniol; 10*- β farnesene; 11*- caryophyllene oxide

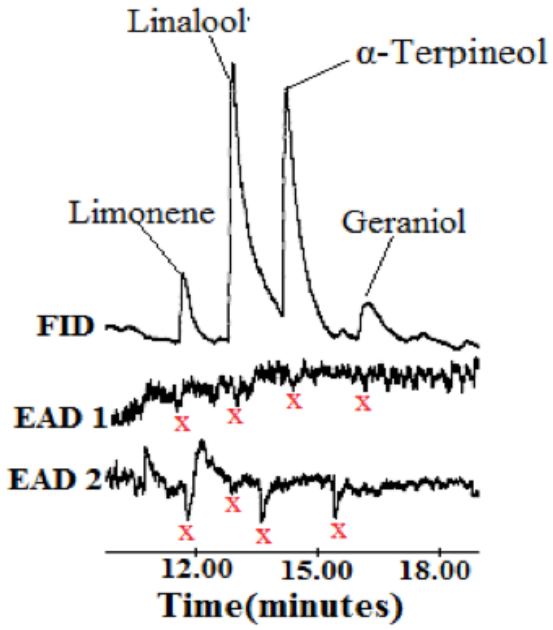

Figure 4.3: The electrophysiological response of *Aethina tumida* antennae to a synthetic blend of limonene, linalool, α-terpineol, and geraniol

4.2 Olfactory response of *A. tumida* to Apicure volatiles

Beetles significantly avoided the Apicure across all the concentrations tested at: 10 ng/µl ($\chi 2$ = 6.45, d.f = 1, P <0.05); 100 ng/µl ($\chi 2$= 17.28, d.f = 1, P<0.001) and 1000 ng/ µl ($\chi 2$ = 38.30, d.f = 1, P <0.001) (Fig. 4.4). About 60% of beetles preferred the control more than Apicure across all the concentrations. Similarly, linalool, geraniol, α-terpineol and camphor showed a significant repellency to SHB compared to the control (Fig. 4.5). Interestingly, limonene attracted 2.7 times more beetles than the control at 100 ng/ µl ($\chi 2$= 61.65, d.f = 1, P<0.001) but an avoidance behavior was observed at 1000 ng/µl ($\chi 2$ = 15.41, d.f = 1, P = 0.1659).

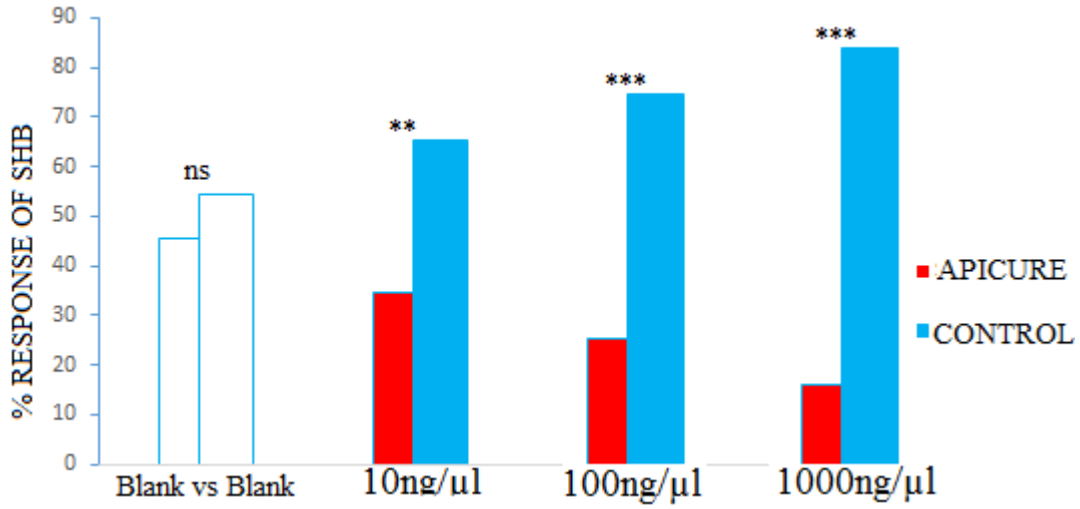

Figure 4.4: Olfactory response of *Aethina tumida* to Apicure volatiles relative to a control (dichloromethane). N = 75; *P<0.05, **P<0.01 and ***P<0.001

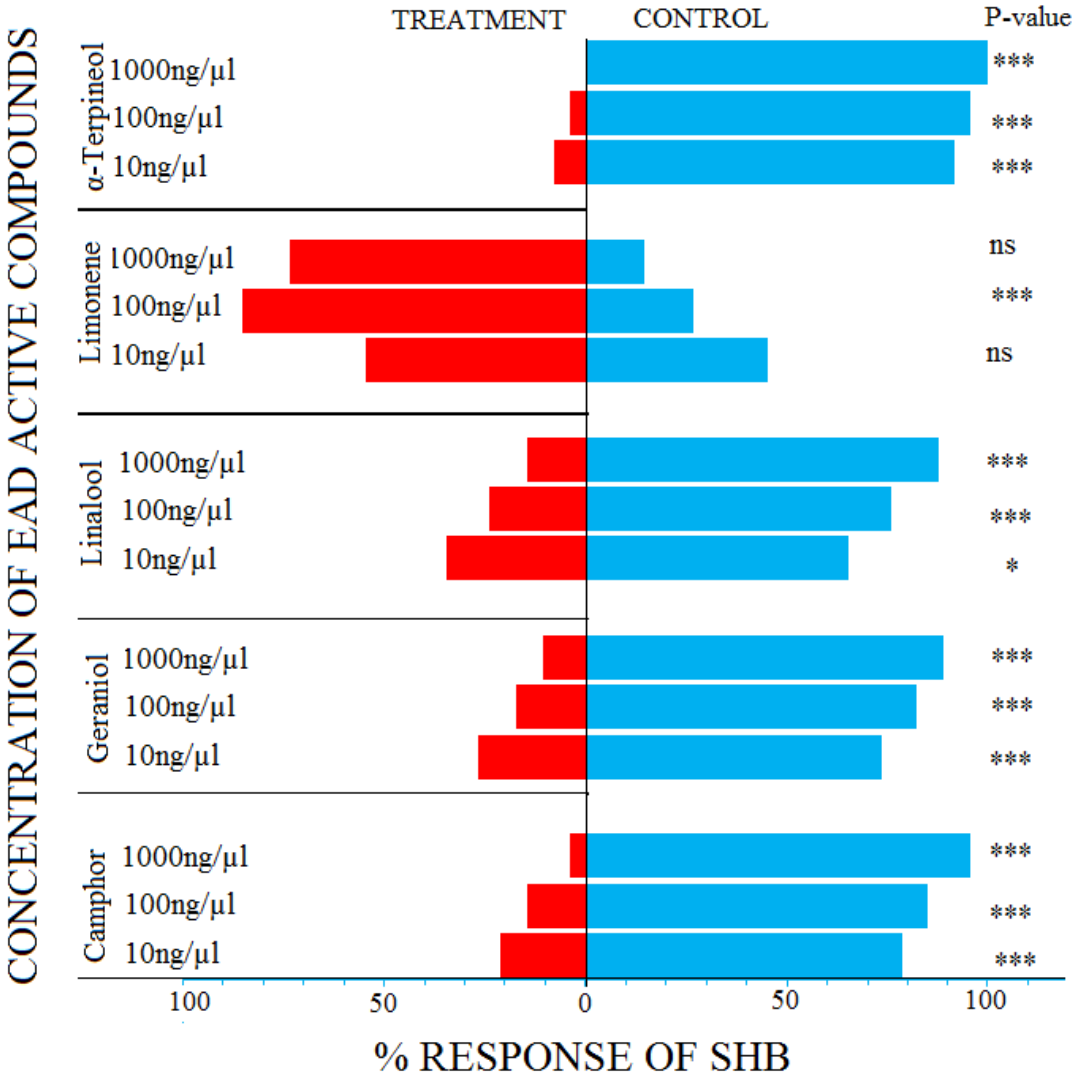

Figure 4.5: Olfactory responses of *Aethina tumida* to electrophysiologically active compounds of Apicure (N = 75). The asterisks designate the significance levels: *P<0.05, **P<0.01; ***P<0.001; ns = not significant

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

The exploitation of natural products that are effective and are environmentally safe is a promising alternative to synthetic chemicals. Among these potential products, essential oils from several species of plants have been extensively researched to ascertain their repellent activities as a prospective natural resource (Maia & Moore, 2011). In this study, we primarily summarize the phytochemical and bioactivity studies of Apicure, a plant-based bio pesticide as a potential non-chemical resource to manage the small hive beetle. While Lwande *et al*., 2016 demonstrated the potential of this product in field experiments to significantly repel beetles, our study is to provide an in-depth knowledge of the chemical composition and its bioactivity against the small hive beetle.

5.1.1 Behavioral response to Apicure

Essential oils have demonstrated a wide range of activity against pests and pathogens ranging from, repellent, antifeedant, oviposition deterrent, insecticidal, growth regulatory and antivector activities (Koul *et al*., 2008). From our olfactometer bioassays, it was ascertained that Apicure is a repellant to the small hive beetle and repellency increased with concentration. This result concur with preliminary field results reported by Lwande *et al*., (2016) that reported significant repellency of beetles from beehives when one sachet of Apicure was applied.

5.1.2 Analysis of volatiles

Our GC-MS analysis of volatile organic compounds of Apicure collected with super Q traps revealed that this product is a complex of 40 compounds present at varying proportions. Essential oils are described by 2 or 3 major compounds (Pandey *et al*., 2014), our results, therefore, suggests that Apicure an essential oil-based product, can be described as a major camphor containing oil as it constituted of 41.4% total ion abundance with no other constituent representing more than 7.9%.

5.1.3 Bioassays with synthetics standards

Bioactivity of essential oils may be attributed to their major constituents and the minor compounds present in the oil (Asawalam *et al*., 2008). They may either act either synergistically or else antagonistically to contribute to some activity of the tested oil. This was evident in our behavioral bioassays with the major compounds that generally elicited repellant activity and others attractants signifying that these compounds contribute to the general repellant activity of Apicure against the small hive beetles. It will, therefore, be interesting to study how these compounds blend to contribute to the general bioactivity of this product. The electrophysiological active compounds identified in this study have been reported to be important in many insects and arthropods especially in host recognition. For instance, camphor which is a repellant to the SHB has also been shown to repel Asian lady beetles (Riddick *et al*., 2000) .In their field experiment, they reported significantly fewer beetles captured in traps containing camphor versus un-baited control traps, this avoidance behavior is similar to that observed in this study. Repellency of p-cymene and camphor in oil of tansy (*Tanacetun vulgare*) against the Colorado potato beetles (*Leptinotarsa decemlineata*) has been documented (Schearer, 1984). Camphor and α-terpineol have been studied individually on toxicity and mosquito repellency and were also detected in this product and elicited repellency to the SHB significantly. Geraniol has been shown to strongly repel ticks (*I. ricinus*) (Tunón *et al.*, 2006) which was also the case with the SHB. Geraniol, highly abundant in nurse bees have been shown to impair the ability of varroa mites to infest nurse bees (Pernal *et al.*, 2005) because of its repellant activity against varroa. Hence this compound can be used to repel varroa and SHB at the colony level. Some monoterpenes such as linalool have been reported to repel mosquitoes (Jaenson *et al*., 2006; Niu *et al.*, 2013). 50 % repellency with geraniol candles was recorded while the use of diffusers provided a repellency rate of 97% (Müller et al., 2009). Geraniol and caryophyllene oxide have been reported to repel *A. gambiae* (Diptera) (Omolo *et al.*, 2004; Odalo *et al*., 2005).

Noteworthy, limonene attracted the SHB through its activity in Apicure is suppressed and this is a compound that can be used in developed traps as an attractant. Small

hive beetles use semiochemicals to locate honey bee colonies. Limonene in this study was found to be an attractant and this could be one of the contributing in-hive semiochemicals that attracts SHB as it has been reported to be present in propolis (Bankova *et al.*, 2014) and we hypothesize that limonene might be among the factors that aid in host recognition by the SHB. Additionally, previous studies have highlighted limonene as an attractant to white pine cone beetle, *Conophthorus coniperda* (Miller, 2009),he reported significant attraction of beetles in traps baited with the host monoterpene limonene .

The interruption of SHB communication is a prospective milestone towards the development of a semiochemical-based tool to manage this invasive honey bee pest. The utilization of plant-based extracts like essential oils and their products, with known effects on insects and arthropods, could be a prospective complementary or alternative strategy to the substantial usage of classical insecticides.

5.2. Conclusion

From this study, it is ascertained that Apicure is a repellant to the small hive beetle. The repellant activity of Apicure seems to be majorly contributed by camphor, which is the main component together with other minor compounds identified from the chemical analysis.

Apicure is a plant-based bio-pesticide that would help in the management of the small hive beetle and other honey bee pests as demonstrated by the results on the influence on small hive beetle behavior. The individual components of Apicure are independently potential semiochemicals that elicited an antennal response with the small hive beetle. This is a milestone towards the development of a tool that is safe to the bees, human and the environment.

5.3 Recommendations

This product can be up scaled and registered with the pesticide control products board for use by beekeepers to manage the invasive SHB and other honey bee pests.
Further research would;

- Evaluate limonene in comparison to reported attractants and its use as a lure in trapping programs.
- Evaluate the efficacy of the Apicure® in a push-pull system in honey bee colonies with high infestation levels
- Similar studies should be done for other honey bee pests like varroa and wax moth to ascertain the possibility of using the same product to manage other pests.
- Further toxicological work with the essential oil and its constituents in future studies.

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