MAKERERE UNIVERSITY

INFESTATION LEVELS AND CONTROL OF THE VARROA MITE (*Varroa destructor)* IN MANAGED HONEY BEE COLONIES FROM SELECTED AGRO ECOLOGICAL ZONES OF UGANDA

BY

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DECLARATION

I, Namayanja Diana, hereby declare that this work is original and has not been published or submitted for any award of Makerere University or any other institution.

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DEDICATION

This dissertation is dedicated to my parents, benefactors, brothers, sisters, children and dear husband Michael for their incredible love, support and sacrifice all of which have made me what I am today.

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ACRONYMS

A.I:	Active Ingredient
AEZ :	Agro Ecological Zone
ANOVA:	Analysis of Variance
FAO:	Food and Agriculture Organisation
FERA:	The Food and Environment Research Agency
GPS:	Global Positioning System
LVC:	Lake Victoria Crescent
MAAIF:	Ministry of Agriculture Animal Industry and Fisheries
Masl:	Meters above sea level
NARO:	National Agricultural Research Organisation
rmANOVA:	Repeated measures Analysis of Variance
RUFORUM:	Regional Universities Forum
UNMA:	Uganda National Meteorological Authority
USA:	United States of America

ABSTRACT

The Varroa mite, *Varroa destructor* (Arachnida: Acari: Varroidae) is an ectoparasitic mite of the honey bee *(Apis mellifera).* It was first detected in East Africa in 2009. Currently, no control strategies have been put in place to manage the pest in Uganda. A study was conducted to establish the extent of spread of the Varroa mite, determine its seasonal population dynamics and also evaluate some control options against the mite using Varroacides. A survey of nine districts representing four Agro ecological zones of Uganda in November and December 2015 showed that Varroa was present in all. Severity of infestations calculated as Varroa load (number of mites per 100 bees) was below the threshold of 5 mites per 100 bees. An average of 4±0.69 mites were recovered per colony sampled. Altitude, seasons (dry and wet), AEZ had no effect on Varroa mite populations. There were significant differences in the comparative efficacies of two varroacides. Fluvalinate had a higher efficacy (59.65%) than Thymol (38.79%) though both values were generally lower than those that have been reported elsewhere. Effects of the treatments on some colony performance parameters (flight activity and number of combs with honey) were found to be insignificant while they were significant for brood characteristics (brood pattern and number of brood combs). This is an indicator that further studies should be done to establish the effect of these treatments on brood and also the queens within hives. Also, an integrated management approach for the mite should be developed to avoid dependence on chemicals which can result into development of resistance of the mite.

Key words: efficacy, incidence, honey bee, population, severity, *Varroa*, varroacides

CHAPTER ONE
1.0 INTRODUCTION

1.1 Background to the study

The honey bee, *Apis mellifera* L., is a well adapted insect with great economic importance and exists in different ecological conditions of the world (Ivanova et al., 2010). It is a valuable insect, known for its importance as a pollinator and honey producer (Strauss et al., 2013). One third of the total human diet comes directly or indirectly from bee pollinated crops (Fakhimzadeh, 2001). However, majority of beekeepers do not receive any payment for their pollination service from the crop farmers.

The beekeeping industry is an important component of agriculture and economic enterprise worldwide, generating employment and increasing family income in the rural areas. In addition to the products produced from apiculture such as honey and beeswax. The most important aspect of honey bees is the vital role they play in the environment by pollinating both wild flowers and many agricultural crops as they forage for nectar and pollen. By doing so, honey bees contribute to food security and biodiversity conservation (Chemurot et al., 2016). It is therefore important that Uganda maintains honey bee populations that are strong enough to sustain productivity and essential pollination services (Kajobe et al., 2010).

The essential and valuable activities of honey bees are possible only with healthy colonies. Several factors have been shown to negatively impact the health and longevity of honey bee colonies; pests, parasites, pathogens, pesticide exposure, poor nutrition, reduced genetic diversity and poor management practices (FAO, 2006; Muli et al., 2014). Among these factors, the parasitic mites are one of the most debilitating.

More than 100 species of mites are associated with the honey bee, *A. mellifera.* Several species of pollen feeding mites are occasionally found in hives or attached to foragers (FAO, 2006). These phoretic mites are mostly innocuous to beekeeping. There are however three species of parasitic mites; *Varroa destructor* (Varroa mite), *Acarapis woodi* (Tracheal mite) and the *Tropilaelaps spp* mite, that are capable of producing devastating effects on honey bee colonies.

Varroa destructor, Anderson and Trueman (Acari:Varroidae) is considered the most serious pest mainly threatening the development of the beekeeping industry world over (Begna, 2015). The excellent microclimate provided by honey bee colonies protects *V. destructor* from all external limiting factors thereby enabling it survive in a wide range of climatic zones and exist in every continent except Australia (Fakhimzadeh, 2001).

Relatively harmless to its natural host, the Eastern honey bee *Apis cerana*, the Varroa mite has recently crossed onto the Western honey bee *Apis mellifera* and spread from its Asian origins to most of the world (Allsopp, 2006). Colonies infested by *V. destructor* will eventually suffer debilitating effects. Feeding by mites on bee pupae can reduce the emerging adult bees' body weight by 6.3-25%, suppress their immune systems, and reduce their life spans (Fakhimzadeh, 2001; Lee et al., 2010). Mites also can transmit viruses during feeding, which can have devastating effects on colony health (Lee et al. 2010).

In regions of the world where the *Varroa* mite is well established, such as Europe and the USA, wild honey bee populations have disappeared as a result of *Varroa* mortality and commercial beekeeping is only possible with the liberal use of anti-*Varroa* pesticides (Allsop, 2006).

Varroa mites in honey bee colonies in East Africa were identified for the first time in 2009 (Muli et al., 2014). In Uganda, several reports have confirmed *V. destructor* presence, infestation rates, pathology and impacts on productivity of honey bees (Chemurot et al., 2016; Kasangaki et al, 2016). The purpose of this study therefore was to establish the incidence and severity of *Varroa* mites in selected districts in Uganda, *Varroa* seasonal variations and also come up with appropriate control strategies for their effective management.

1.2 Statement of the problem

Varroa destructor is believed to be largely responsible for honey bee colony losses worldwide particularly in the USA and Europe. There is however little information on its prevalence, severity and any underlying factors that may influence its persistence and spread in Uganda. Management measures that are presently unknown among our beekeepers also need to be developed as a way of having a lasting and sustainable solution to the problem.

1.3 Objectives of the study

1.3.1 General objective

The overall objective of this study was to determine the infestation levels, seasonal variation and evaluate chemical control options for *Varroa destructor* infesting hived honey bee colonies in Uganda.

1.3.2 Specific objectives

i. To determine the incidence and severity of *Varroa* mites in hived colonies in selected areas of Uganda.

ii. To determine the seasonal variation of *Varroa* mites' population across wet and dry seasons in two agro ecological zones of Uganda.

iii. To evaluate control options for *Varroa* mites and their effects on associated honey bee colony performance parameters in Uganda.

1.4 Null Hypotheses

i. *Varroa* mite incidence and severity is not variable between agro ecologies

ii. *Varroa* mites' population dynamics are not different between dry and wet seasons, and between the AEZs

iii. The Varroacides have no appreciable effect on level of *Varroa* mite infestations and honey bee colony performance

1.5 Justification of the study

The beekeeping industry in Uganda has registered tremendous growth over the years and is being used as one of the tools for improving rural household incomes. The industry is however faced with a number of challenges among which is the pest and disease burden. Common among the pests is the *Varroa* mite which causes varroasis, a very serious and complex infestation to honey bees. It infests both adults and brood and if not detected early and control measures administered it will continue to be a serious threat to the long term sustainability and prosperity of the Ugandan apiculture industry and to the environment. This will be through the disruption of pollination services and quality and quantity of bee products brought on the market. The overall outcome would be affecting food security negatively and the tangible benefit of direct income from sale of bee products reduced immensely. To combat this, a lasting solution for control and treatment of Varroa infestations was needed and that is what this study addressed.

CHAPTER TWO
2.0 LITERATURE REVIEW

2.1 The honey bee *(Apis mellifera)*

2.1.1 Honey bee products and their uses

The honey bee *(Apis mellifera)* originally described by Linnaeus (1758) is a social insect with the ability to produce large quantities of honey for storage as well as other products of human interest. Two honey bee races *(Apis mellifera adansonii* and *Apis mellifera scutellata)* exist in Uganda whereby *A. m. adansonii* is more widespread in the AEZs in the northern and eastern parts while *A. m. scutellata* is more spread in the southern and western parts of the country (Kasangaki et al., 2017).

Products from the bee hive are quite diverse and they include honey, bees wax, propolis, bee venom, royal jelly as well as bee pollen. Some of these can be eaten raw as primary products or value added to them and utilized in secondary form. Honey is the most important bee product especially from a quantitative point of view though considered the cheapest. Honeys contain a number of sugars from single or varied nectar sources and small amounts of minerals, vitamins, proteins and amino acids.

2.1.2 Economic contribution of honey bees to world agriculture

Honey bees worldwide are highly valued for their products and their role in pollination of many agricultural crops and wild plants. By so doing, they contribute to food security and biodiversity conservation. Honey bees pollinate about one sixth of the world's flowering plant species and some 400 of its agricultural plants (Kapwong, 2016). According to FAO (2006), the value of honey bees in pollination exceeds by ten to twenty times their value in the production of honey

and beeswax. Overall, the value of insect pollination to global agriculture is estimated to be approximately $200 billion per year and this is likely to triple in the near future (Kapwong, 2016; Vandervalk, 2013).

Although the chief contribution of honey bees to agriculture is undeniably pollination services, they also produce many highly valued products mainly honey and bees wax. Various other hive products are also sold as natural health products such as propolis; a substance bees collect from tree resins with uses in traditional medicine, pollen; a protein source frequently used as a dietary supplement and royal jelly; the substance fed to larval queens (Vandervalk, 2013).

In Israel, beekeeping yields 3200 metric tonnes of honey (a value of $12 million) and 100,000 cycles of pollination services to different crops (an augmented income of $250 million) while in Canada an estimated 35.4 million kilograms of honey valued at approximately $151 million were produced in 2011 (Soroker et al., 2010 ;Vandervalk, 2013). This clearly shows the value of honey bees and beekeeping on the global scene.

In East Africa, honey bees provide critical pollination services and income for smallholder farmers and rural families (Muli et al., 2014). In Kenya, honey production alone is estimated at 25,000MT and beekeeping generally contributes about $42.5 million annually (Kiptarus et al., 2011) whereas annual export earnings from forest products; honey and beeswax inclusive are valued at around $14 million in Tanzania (Tanzania National Export Strategy 2010-2014). In Uganda, there is insufficient statistical data on the apiculture industry performance regarding sales to both local and export markets although FAOSTAT indicates that 30MT of honey worth $66,000 was exported in 1995 (FIT, 2006). Also, Uganda's annual honey production is estimated

to be about 5,000 tonnes although this is only 1% of an estimated potential of 500,000 tonnes (Horn, 2004). This clearly shows that Uganda produces the least amount of honey in the region.

Various schools of thought could explain the low honey production of bee colonies in Uganda. These include loss of honey bee populations due to increased loss of natural vegetation cover like forests which are natural habitats for tropical bees; low extension services provided to practicing beekeepers especially on how to increase colony numbers and productivity; increased use of insecticides and other agrochemicals that are harmful to bees and increased infections by pests and diseases including a number of invasive pest species in particular the *Varroa* mite.

2.1.3 Health threats to honey bees

Serious health threats to *A. mellifera* include infections by bacteria, microsporidians, viruses, various pests as well as colony disorders. These infections lead to weakened bees and hence non performing colonies. These threats have contributed to the loss of most wild honey bee colonies (Vandervalk, 2013). Reports of heavy colony losses worldwide have raised public concern and awareness for the future of honey bees (Soroker et al., 2010).

Uganda was found to be free of chronic bee paralysis virus, Sac brood virus, Deformed wing virus, Acute bee paralysis virus, Apis iridescent virus and Israeli acute paralysis virus (Kajobe et al., 2010). However, Black queen cell virus (BQCV) was confirmed present in both adults and brood. In South Africa, BQCV has been linked with notably increased honey bee mortality when associated with *Varroa* (Davidson et al., 2003).

It is now evident that *Varroa destructor* is currently widespread in Uganda although farmers together with beekeeping extension workers are not aware about its presence (Kasangaki et al., 2016). Despite having no reports in Sub Saharan Africa about honey bee colony losses due to

Varroa mites, their presence is a potential threat to the beekeeping sector and should be monitored (Chemurot et al., 2016).

2.2 *Varroa* mites: Description, lifecycle and spread

2.2.1 Description

Varroa destructor Anderson & Trueman (Acari: Varroidae) is an ectoparasitic mite that feeds on the haemolymph of immature and adult honey bees (Ellis and Nalen, 2010). The adult female mites are reddish-brown to dark brown and oval in shape (Fig.1) measuring 1.00 to 1.77 mm long and 1.50 to 1.99 mm wide (Ellis and Nalen, 2010). Their curved bodies fit into the abdominal folds of the adult bee and are held there by the shape and arrangement of ventral setae. This protects them from the bee's normal cleaning habits. It attaches itself on the body of the bee and sucks its haemolymph through punctures made in the body wall by their sharp mouth parts (Fig.2). Adult males are yellowish with lightly tanned legs and spherical body shape measuring 0.75mm to 0.98 mm long and 0.70mm to 0.88mm wide. The male chelicerae are modified for transferring sperm.

Figure 1: Structure of *Varroa destructor*

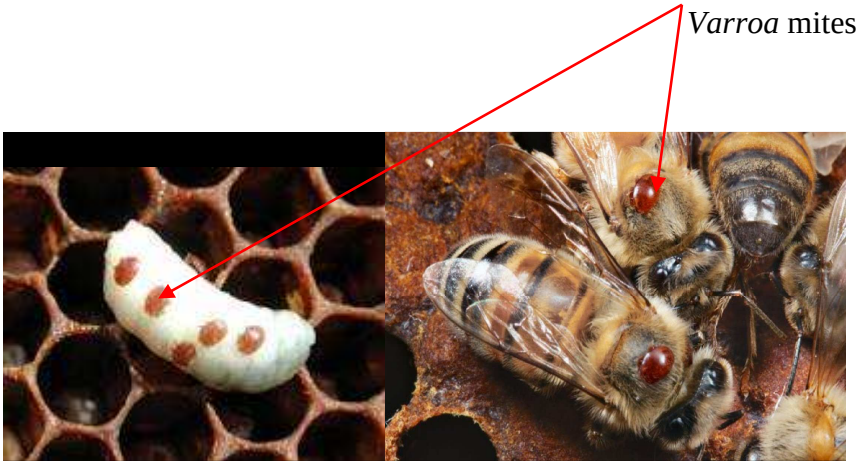

Figure 2: *Varroa destructor* attached on brood and adult honey bees

V. destructor is a native parasite of *Apis cerana* throughout Asia (FAO, 2006). Co-evolution between *A. cerana* and *V. destructor* species resulted in the persistence of low mite populations in colonies at levels that do not endanger their survival (Rosenkranz et al., 2010). However, in the past century *V. destructor* successfully shifted from its original host, the Eastern honey bee *A. cerana* onto the Western honey bee, *A. mellifera* and this has facilitated its spread from its Asian origin to now being almost cosmopolitan (Allsop, 2006; Rosenkranz et al., 2010).

Varroa destructor is a mite as described by Anderson & Trueman, (2000). It is a eukaryote belonging to Kingdom: Animalia; Phylum: Arthropoda; Class: Arachnida, Order: Parasiformes; Suborder: Mesostigmata; Family: Varroidae; Genus: *Varroa* and Species: *Varroa destructor*.

2.2.2 Distribution and Spread

Varroa mites are common nearly everywhere honey bee colonies are found including all the diverse ecological and geographical conditions (Figure 3) with slight variation in mite infestation levels (Mumbi et al., 2014). Today, this parasite is found throughout the world, except for Australia and New Zealand (Allsopp, 2006; FAO, 2006).

Varroa mites are mobile and can easily move from bee to bee within the hive. They are found twice as often on bees in the brood nest as on bees in the honey supers and 10 times as often on brood nest bees as on foragers (Calderone, 2005). However, for their spread across colonies they depend on adult bees for transport through the natural processes of drifting, robbing and swarming. Phoretic *Varroa* mites can be passed between colonies of bees when infested bees drift into another colony. This happens frequently in managed honey bee situations where .individual bee colonies are located within meters of one another. It is common for bees in this situation to return to the wrong colony (drift). Research has shown that bees from colonies heavily infested with *Varroa* tend to drift more than bees from uninfested colonies (Schmid-Hempel, 1998). Also, mites may spread between colonies as bees from the colonies rob (or steal honey) from one another. It is common for strong colonies to rob weaker colonies in periods of nectar scarcity. More so individual colonies may swarm, migrating to a new location and spread *Varroa* simultaneously (Allsopp, 2006). *Varroa* passing within a colony from bee-to-bee and between colonies by drifting and robbing demonstrates that the mites can be transmitted naturally both horizontally and vertically.

Beekeeping activities like transfer of colonies from one area to another for various reasons have also escalated the spread of *Varroa*. First, beekeepers often aid weak colonies by adding bees or brood from a healthier colony and this practice helps spread the mite. Secondly, beekeepers may transport colonies from one area to another, facilitating the spread of *Varroa* regionally. Mites pass easily from bee to bee in these instances. All of these methods have contributed to *Varroa's* global distribution as a honey bee pest.

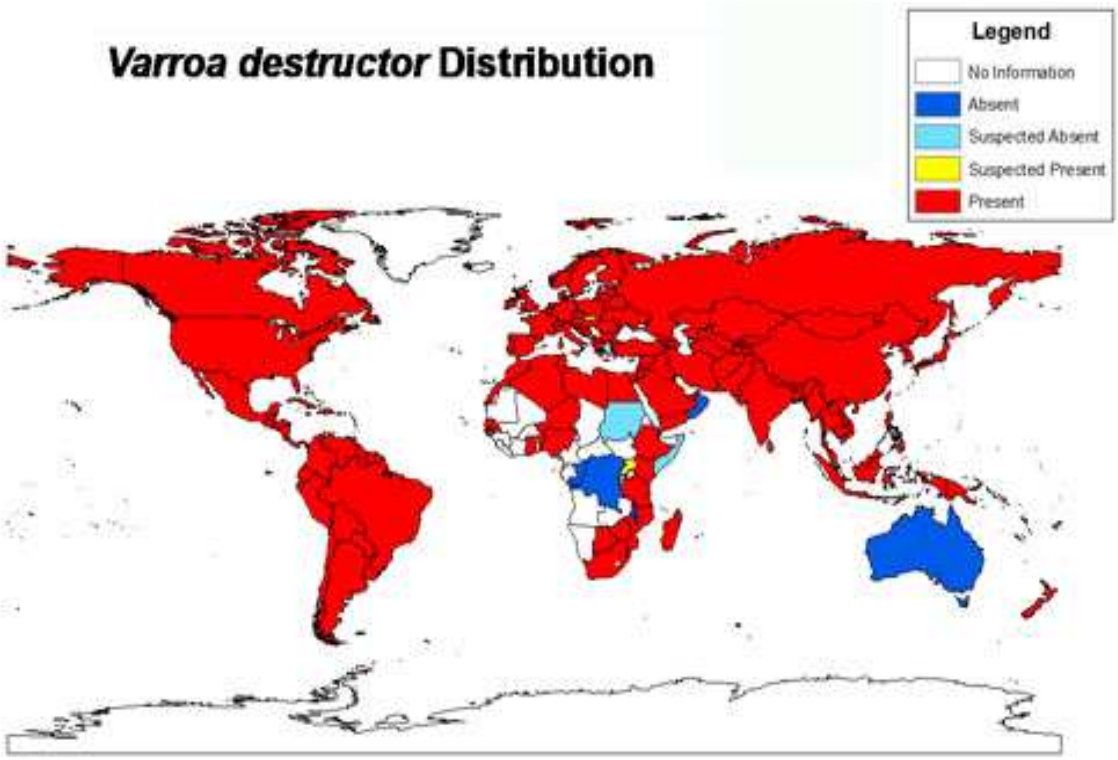

Figure 3: *Varroa destructor* distribution (Ellis and Nalen, 2010)

2.2.3 Life cycle of *Varroa* mites

The entire lifecycle of *Varroa* mites occurs in the bee hive and all stages are obligate ectoparasites feeding on haemolymph (Allsopp, 2006). Varroa mite lifecycle has two stages; these are the reproductive phase and the phoretic phase as illustrated in Figure 4. During the phoretic stage, mites ride on adult workers or drones, at the same time feeding on their haemolymph usually from the intersegment membrane of the abdomen and this lasts about 5-11days (Huang, 2012). The second stage is the reproductive stage and only occurs under the capped brood cell.

Adult female *Varroa* mites enter honey bee brood cells (especially drone brood) at the pre-capping stage and lay two to five eggs after the brood cell is capped. Eggs are 0.5 mm long and are laid on the bottom of the cells, the walls and sometimes directly on the larvae. The first egg

laid is a male and subsequent eggs are female. After hatching, *Varroa* mites pass through two larval stages these are protonymph and deutonymph before developing into an adult. It takes about 5–6 days for male *Varroa* mites to develop and 7–8 days for female mites to develop. Mating occurs in the brood cell. The male *Varroa* mite dies inside the cell shortly afterwards. Young female *Varroa* mites and the mother *Varroa* mites emerge from the brood cell with the emerging honey bee. The daughter *Varroa* mites will lay eggs in other brood cells after 2 weeks. Adult female *Varroa* mites usually live for 2 months, but can overwinter between the sclerites (the hardened plates of the exoskeleton) of adult honey bees.

After the first infestation of a new honey bee colony, *Varroa* mites are able to build up huge populations within a few years. The population growth is highly variable and depends on all of the traits of the host and the parasite that may influence the reproductive rate and the mortality of the mite (Rosenkranz et al., 2010). Features of the parasite that influence population growth are the reproductive capacity during the mite's lifetime and the lifespan. On the other hand, features of the host are brood availability especially drone brood, swarming and level of defense behavior, among others. Some of the host features that influence mite population growth are additionally triggered by ambient factors such as climate and nectar flow (Currie and Tahmasbi, 2008).

Swarming does not prevent the buildup of detrimental population levels of *V. destructor* (Fries et al., 2003). However, as mite levels increase to detrimental levels in the colonies, mite population growth is negatively affected and may also deter colonies from swarming by the negative effects on the bee population.

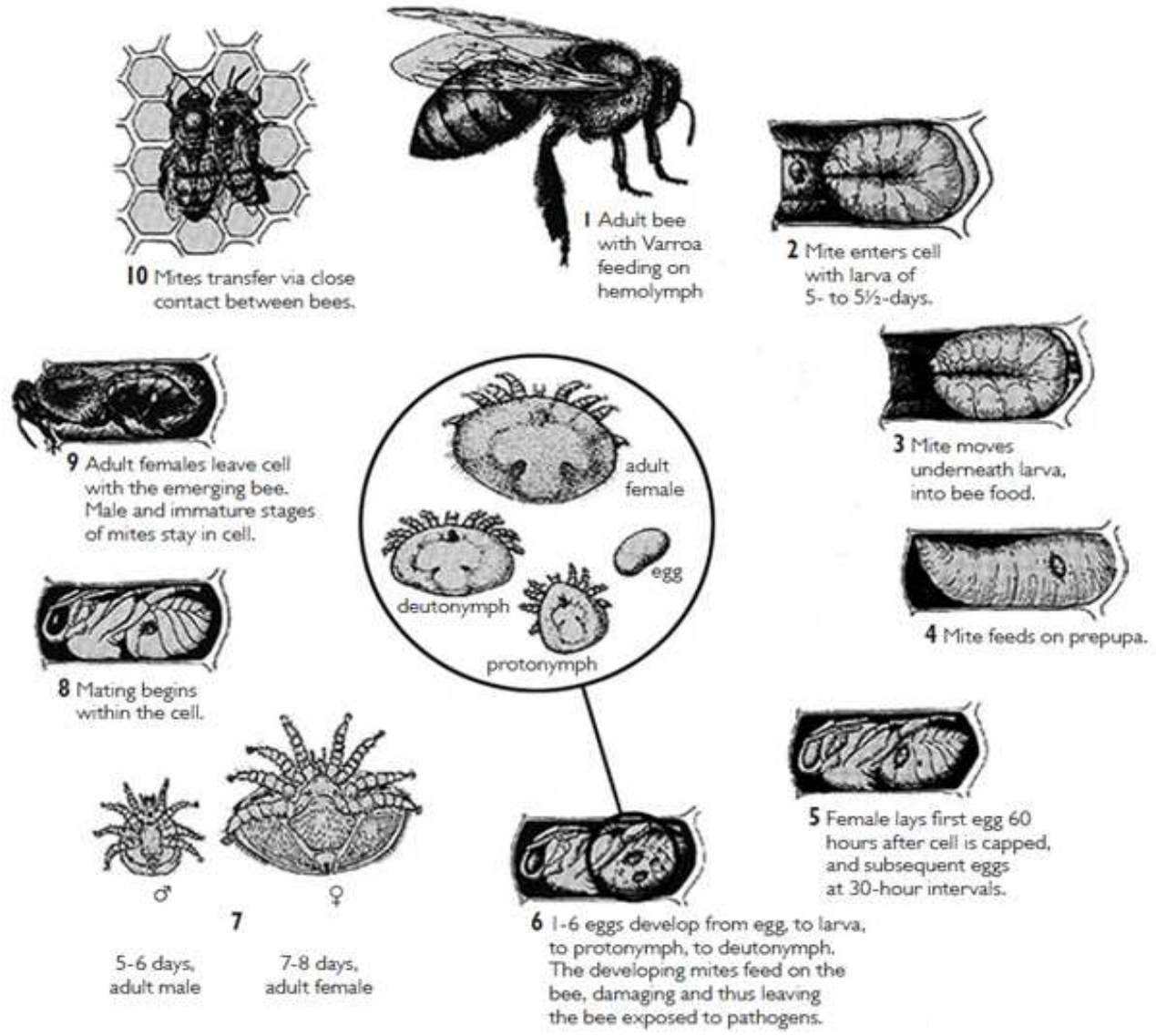

Figure 4: Lifecycle of *Varroa destructor* (Huang, 2012)

Adaptations like bees' behaviour and physiology limit *Varroa* mites' population growth in *Apis cerana* although this is lacking in *A.mellifera* (Martin, 1998). This allows the mite population in *A.mellifera* colonies to increase freely. If no action is taken by the beekeeper, then the colony collapses (Martin, 1998). However, in regions with a high population density of honey bee colonies, Varroa population dynamics are influenced by a permanent exchange of mites when foragers or drones enter foreign colonies or by robbing (Goodwin et al., 2006).

2.3 Potential impact of *Varroa* mites on honey bees and beekeeping

Varroa destructor is the most serious pest mainly threatening the beekeeping industry all over the globe and has affected the apiculture industry negatively in every country it has been introduced (Ellis and Nalen, 2010; Begna, 2015). Effects of *Varroa* on adult bees come about either from the mite feeding directly on the haemolymph of the adult bee or as a result of feeding by the mother mite and her offspring on bee larvae and pupae in the cell and this subsequently affects the development of pupae into adults (Goodwin and Eaton, 2001).

The eventual outcome is to weaken the honey bee colonies and thus decrease honey production, often seriously, a condition known as varroatosis (Begna, 2015). Occasionally in *A. mellifera* and more frequently in *A. cerana*, heavy infestation may cause absconding. The mite can also act as a vector and can directly inject virus particles into the insect haemolymph and this has been linked to the detection of several bee viruses in Varroa mites (Tentcheva et al., 2004). Examples of such viruses include Deformed Wing Virus (DWV), Black Queen Cell Virus (BQCV), Acute Bee Paralysis Virus (ABPV) among others (Tentcheva et al., 2004). Accurate estimates of the effect of *Varroa* on the apiculture industry are hard to find, but it is safe to assume that the mites have killed hundreds of thousands of colonies worldwide, resulting in billions of dollars of economic loss. *Varroa* have also caused beekeeping production costs to rise, thus lowering the profit margin of the beekeeper (Ellis and Nalen, 2010).

During the winter of 1995-1996 in the United States of America, the bleakest period, colony losses ranged from 40% in Delaware to 53% in Pennsylvania to 80% in Maine (Doebler, 2000). However, despite the *Varroa* mite being present in East Africa for more than five years, heavy losses as experienced elsewhere have not occurred even without treatment (Ritter and Ritter,

2016). This could be an indication that probably the East African bees are more adapted to withstanding attacks by *Varroa* mite infestations or the mite has not yet multiplied to numbers big enough to cause serious damage to bee colonies as seen elsewhere.

Honey bees parasitized by *V. destructor* exhibit a variety of physiological, behavioural and morphological symptoms (Vandervalk, 2013). It is a highly destructive pest responsible for reduced honey and brood production, precocious foraging, increased drift, suppresses the immunity of honey bees, impact bee wing shape, can impact virgin queen's acceptance and mating success, higher bee mortality in winter and considerable colony mortality (Downey and Winston, 2001; Abou-Shaara, 2014).

At the colony level, parasitized foragers display a decreased capability of non-associated learning, prolonged absences from the colony and a lower rate of return to the colony (Rosenkranz et al., 2010). This is very dangerous to the colony as the ability to have enough food reserves (pollen and honey) is greatly reduced and in the long run overall colony performance goes down. Without periodic treatment, most of the honey bee colonies in temperate climates would collapse within a 2–3 year period (Rosenkranz et al., 2010).

2.4 Incidence and severity of *Varroa* mites

Among the various races of *A. mellifera* bees, Africanized bees show greater tolerance for the *Varroa* than bees of European races. Reproductive ability of female mites is known to be lower among Africanized bees than that of European bees (Camazine, 1986). Moretto *et al*., (1991) obtained a 40% rate of Africanized bees that were able to rid themselves of the parasite among 20 colonies of artificially infested Africanized worker bees. When European and Africanized

bees are reared together in the same hive, European bees are found to be more heavily infested (Gúzman-Novoa *et al*., 1999; Moretto and Mello, 1999).

Varroa infestation rates differ among different *Apis mellifera* subspecies. Mortensen et al., (2015) found that *A. m. scutellata* colonies had significantly higher *Varroa* infestations than did *A. m. capensis* colonies. Furthermore, hybridized colonies of the two subspecies had *Varroa* infestations intermediate to those of *A. m. scutellata* and *A. m. capensis*. An earlier study by Martin and Kryger (2001) comparing *Varroa* mite infestation rates among colonies of *A.m. capensis, A.m. scutellata,* European honey bees (EHB) and the Africanised honey bees (AHB), it was observed that mite populations in *A. m. scutellata* and EHB colonies increased at similar rates and even the very short worker development period of *A. m. capensis* could not prevent the growth of the mite population. However, AHB, which has a similar developmental time to EHB & *A. m. scutellata* bees had the lowest rate of mite increase and it's due to the low number of viable *Varroa* females being produced in worker cells. However, a similar study needs to be done with other African *A. mellifera* sub species to see whether similar results would be obtained.

In East Africa, *Varroa* mite incidence has been found to be high across the region. This is in comparison with a series of independent studies that have been done in the different countries across the region and have revealed almost similar results. A study done by Muli et al., (2014) in Kenya put *Varroa* mite incidence at 83% for all the colonies sampled, another in Uganda found 100% for all the 16 districts sampled in eight (8) agro ecological zones (Kasangaki et al., 2016) while in Tanzania out of the 25 districts sampled for *Varroa*, 23(92%) districts tested positive and only 2(8%) districts were negative (Mumbi et al., 2014). However, the severity or

infestation levels for Varroa mites in East Africa are still low as compared to those in Europe and America. In Tanzania for instance it has been recorded to be averagely three (3) mites per colony (Mumbi et al., 2014) while in the UK, lower thresholds can be at 1000 mites per colony (FERA, 2010). Also severity has been found to be positively correlated with elevation and colony size (Muli et al., 2014; Mumbi et al., 2014; Chemurot et al., 2016)

From the incidence levels mentioned above, it is very evident that the *Varroa* problem is no longer a localized one and as such calls for collaborative efforts between neighboring countries or regional blocks to have coordinated approaches towards management of the Varroa mite problem.

2.5 Control options for *Varroa* mites

Regardless of other threats to honey bees and the fact that before the ‘‘*Varroa* era” heavy colony losses were also reported, *Varroa* mites seem to be the crucial driver for these periodic losses. It is also assumed that *Varroa* mite infestations are responsible for the decline in colony numbers of both feral and hived honey bees and subsequent decline in quantity and quality of hive products. As such an effective control strategy is necessary to help bring down the mite levels where damage is insignificant.

Without any doubt most of the colonies of *A. mellifera* in temperate climates will be damaged or even collapse within a few years if no control or inappropriate control methods are used (Rademacher and Harz, 2006; Boecking and Genersch, 2008). The beekeeping industry requires sustainable management practices that will keep mite populations below the economic injury level and maintain the high quality of hive products and the best way to achieve this is to use a management program that relies on multiple tactics (Calderone, 2005). Various methods of

control have been developed and these fall in two categories; Non chemical control and chemical control.

2.5.1 Non Chemical / Biotechnical Control of *Varroa*

Biotechnical methods are specifically designed to reduce *Varroa* mite levels in a colony but not as a complete means of *Varroa* control. These methods can be highly successful and can remove as many as 95% of the *Varroa* in a colony and are favoured in many countries (Allsop, 2006). They are often incorporated into integrated pest management systems whether with synthetic chemicals or more generally with organic control substances. Reasons for biotechnical control are to avoid chemical residues in hive products, fear of chemical resistances and also to avoid high cost of chemical control substances (Goodwin and Eaton, 2001). The various non chemical control methods are described below;

2.5.1.1 Brood removal and trapping

Brood removal for control of *Varroa* is based on the understanding that mites are confined in brood cells once the cells are capped hence they can easily be removed from the colony without escaping back onto the adult bees (Goodwin and Eaton, 2001). It involves removal of worker brood as well as using drone brood to trap and remove *Varroa* mites. When capped drone brood is removed from an infected colony, a disproportionately large number of mites are removed without affecting the worker population, and it is mites with the highest fecundity that are removed (Calderone, 2005). The main problem with worker brood removal is that it requires a large number of frames to be removed to achieve good control, and the removal of frames will obviously affect the population and productivity of the colony. However, drone brood trapping reduces mite populations and doesn't affect colony productivity (Goodwin and Eaton, 2001).

2.5.1.2 Using genetically tolerant strains of *Apis mellifera*

Tolerance to *Varroa* is the ability of a honey bee colony to co-exist with an infestation of the mite without it causing any harm. It involves rearing colonies or raising queens from colonies that have shown an ability to tolerate *Varroa* without any harm, displayed a high level *Varroa* sensitive hygiene or self grooming behaviour. Self-grooming in honey bees is heritable behaviour by which a bee removes a mite from her body and gives it a lethal bite (Delaplane, 2007). This behaviour is useful against brood diseases as well as *Varroa* and has been shown to delay significantly the onset of treatment threshold.

2.5.1.3 Isolation

Increasing the distance between apiaries reduces the chances of re-infestation from nearby collapsing colonies. A separation of three miles will provide some protection while a separation of five miles is better. However, isolation is not practical where colony density is high and cannot guarantee that your bees will not be re-infested because there may be wild colonies in the area. Nevertheless, this method should not be overlooked when selecting apiary sites (Calderone, 2005).

2.5.1.4 Screen bottom boards

Varroa mites usually fall off the bees even when no chemical treatment has been used. Many of these mites are still alive and can be suppressed by removal from the colony before they manage to reacquire a host (Calderone, 2005)**.** The screen bottom board allows the mites that fall off from the bees to fall out of the hive and as such cannot move back into the hive. If this is practiced on a daily basis, a beekeeper is able to get rid of a considerable number of mites from

his colonies. This approach also helps the beekeeper to monitor Varroa mite populations among his colonies.

2.5.1.5 Sunny apiary locations

There is evidence that by simply placing an apiary in a sunny instead of a shaded location, a beekeeper can expect significantly reduced rates of *Varroa* mite population growth (Delaplane, 2007). However, not much research has been done to prove this especially in the tropics where it shines almost throughout the year. Could it be one of the reasons why *Varroa* mite infestations rates are still low despite having a high incidence?

2.5.2 Chemical control of *Varroa* mites

Nowadays, beekeepers utilize a wide range of different chemical substances, application techniques and methods to keep mite populations under control (Rosenkranz et al., 2010). Various chemical products have been developed to help manage the *Varroa* mite problem. An overview of the various chemical products on market is as follows;

2.5.2.1 Organophosphates

These come under brand names like Checkmite, Asuntol and Perizin. They come as a solution which is trickled over bees and as such are contact/ systemic pesticides whose active ingredient is Coumaphos. Organophosphates are temperature dependent in their action and have an efficacy of 80-90% (two treatments) though highly variable. Coumaphos is an acetylcholinesterase inhibitor that interferes with nerve signaling and function and through that it is able to kill mites in sealed brood cells (Rosenkranz et al., 2010; FERA, 2010). However, organophosphates are highly corrosive and if misused brood and queen loss can happen (FERA, 2010).

2.5.2.2 Pyrethroids

The active ingredients here are Tau-fluvalinate (with brands like Apistan®, Klartan® and Mavrik®) and Flumethrin (with brands like Bayvarol). Tau-fluvalinate acts at the voltage gated sodium channels (Rosenkranz et al., 2010). Both tau-fluvalinate and flumethrin come in form of plastic strips that are administered by hanging in between the brood combs. They are contact pesticides and their efficacy has been put at 95% (FERA, 2010). Apistan can be effective for killing the mites without harming the bees (Hunt, 2010). However, in many places of Europe and the USA, pyrethroid resistance has been reported and confirmed.

2.5.2.3 Organic acids and essential oils

These represent the frame of natural compounds that are being used in the control of *Varroa* mites. Rosenkranz et al., (2010) indentified the following as advantages of using organic acid and essential oils for Varroa control;

i. Sufficient efficacy against *V.destructor,* with formic acid as the only acaricide which is able to kill mites within the sealed brood cells.
ii. Low risk of residues and accumulation in bee products. Most of these substances are water soluble and/or volatile and furthermore, natural ingredients of honey. Therefore, contamination which jeopardize the quality of honey or beeswax is unlikely.
iii. Low probability of eliciting resistance after repeated treatments.

Examples of such substances are Formic acid, Oxalic acid, Lactic acid, Thymol and various oils from plant extracts. They can be used singly or in combination. They come with various brand names for example Thymol is branded as Apiguard formulated as a slow release gel.

CHAPTER THREE
3.0 MATERIALS AND METHODS

3.1 Description of the study area

Uganda is one of the countries in East Africa and it is landlocked. Agriculture is the main economic activity in Uganda of which beekeeping is part. Nearly 80% of Uganda's 34 million people are engaged in agricultural production and/or trade. To better manage its agricultural potential, the country was divided into ten different agro-ecological zones (AEZs) on the basis of distinct vegetation type, elevation and climatic conditions as follows; Eastern; Lake Albert Crescent; Lake Victoria Crescent; Mid North; South East; Southern Dry lands; Southern Highlands; West Nile; Western Highlands and Karamoja Drylands. It is important to note however that beekeeping is practiced throughout the country in all the AEZs. For purposes of this study, only four of the ten AEZs were selected for sampling that is; Eastern, Lake Victoria Crescent, Mid North and Western Highlands (Fig.5).

The Eastern AEZ receives bimodal rainfall ranging from 900 mm to over 2,100 mm per year. It has a minimum temperature of about 7.5^0C and an altitudinal range of 1,000 to 4,000 m above sea level. The vegetation ranges from montane forest to high open moorland and the main farming system here is intensive banana and coffee production. In this zone Mbale and Kapchorwa districts were sampled.

The Lake Victoria Crescent AEZ is composed of districts within the central region of Uganda and the most distinct physical feature in this zone is Lake Victoria. The area receives rainfall ranging from 700 mm to over 2,100 mm per annum almost throughout the year with two rainfall peaks. It has a minimum temperature of about 12.5^0C and the altitude varies between 1,100 -

2,400 m above sea level. The vegetation is forest and savanna mosaic characterized by patches of dense tropical rain forests in the south and scattered trees in shrubs and grassland in the north. However, the vegetation cover is gradually reducing due to increased urbanization in this zone. The farming system is mainly banana, coffee and several seasonal crops. In this zone Luweero and Masaka districts were sampled.

The western highland AEZ is a narrow zone along the western boundary of Uganda and include part of the Albertine rift valley famous for wildlife species diversity. Annual rainfall in this zone ranges from 875 to 1800 mm while the altitude ranges from 600-4500 m above mean sea level. Temperature conditions vary widely from cold in the mountains, cool in the highlands to hot on the rift valley floor. In this zone, Bushenyi, Kabarole and Kyenjojo districts were sampled.

The Mid-North AEZ includes a bigger part of the northern region of the country. Annual rainfall ranges from 700-1700 mm while the altitude is between 800-1800 m above sea level. The rainy season mainly occurs between April to October while the dry one extends from November to March. In this zone, Lira and Kitgum districts were sampled.

3.2 Incidence and severity of *Varroa* mites in hived honey bee colonies in Uganda

3.2.1 Sampling procedure

Sampling was carried out in nine study districts that fall within four of the ten different agro-ecological zones of Uganda (Fig.5). The zones and districts were: Eastern (Mbale and Kapchorwa); Lake Victoria Crescent (Luweero and Masaka); Mid Northern (Lira and Kitgum) and Western Highlands (Kabarole, Bushenyi and Kyenjojo).

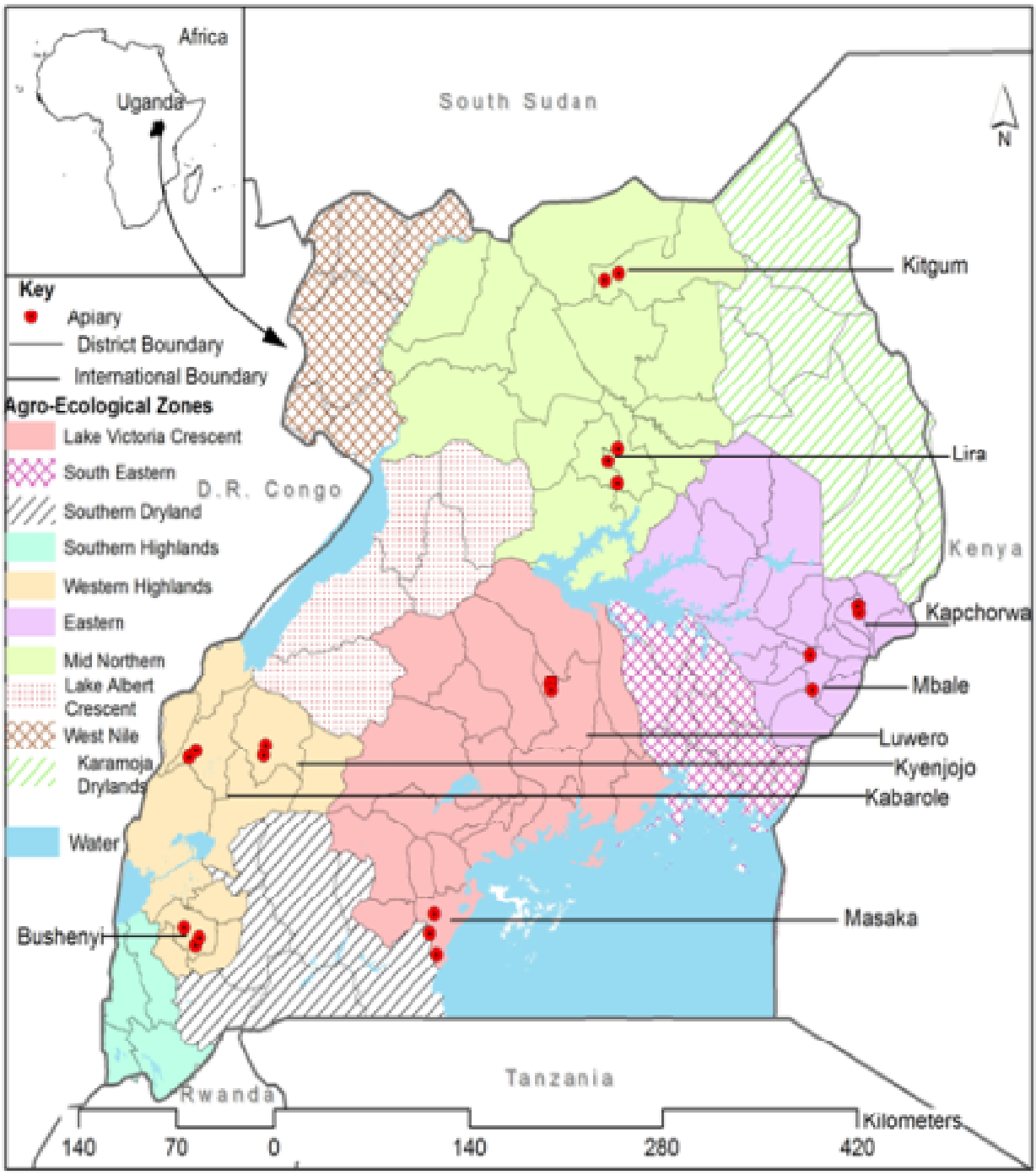

Figure 5: Location of sampled AEZs, districts and sites in Uganda

This study was done in November and December 2015. With the participation of district entomologists, apiaries were selected in each of the nine districts. Simple random sampling was used in the selection of apiaries and honey bee colonies within apiaries for inspection. The apiaries were selected in such a way that they are in different sub counties or more than 3.5 Km away from each other to minimize sampling honey bees foraging within the same area (Chemurot et al., 2016). In each district, three apiaries were randomly selected and from each apiary a maximum of three bee hives were randomly selected and examined for mites' presence using the procedure of Mumbi et al. (2014). All sites from which sample collection was done were geo-referenced using a GPS.

3.2.2 Estimation of *Varroa* mite incidence and severity

Between 100 -200 worker bees were sampled from each hive and placed in 100 -150 ml of 70% ethanol. *Varroa* attached to the bees were washed off into the alcohol and thereafter counted. The number of bees actually sampled was also counted. Percentage incidence in any given district or AEZ was estimated as the ratio of number of colonies infested with *Varroa* mites to the total number of colonies sampled and expressed as a percentage using the following formula;

$$Percentage\ Incidence = \frac{Number\ of\ infested\ colonies}{Total\ number\ of\ colonies\ sampled} * 100\%$$

The severity per colony sampled was calculated as number of *Varroa* mites per 100 bees using the formula below;

$$Colony\ Severity = \frac{Number\ of\ Varroa\ mites\ in\ sample}{Number\ of\ honey\ bees\ in\ sample} * 100$$

3.2 Seasonal variation of *Varroa* mites' population across wet and dry seasons in two AEZs of Uganda

3.2.1 Sampling Procedure

Two districts namely; Bushenyi and Masaka were selected to represent their specific AEZs. For each district, two apiaries with at least six active honey bee colonies were selected. Two colonies were randomly selected per apiary and screened for presence of Varroa mites and severity determined. Altogether, eight colonies were evaluated in this study. The colonies under study were marked for easy identification during every visit and apiary locations georeferenced as 00°30.543'N, 031°40.977'E; 00°23.752'N, 031°42.685'E for Masaka and 00°34.399'N, 030°10.549'E; 00°28.574'N, 030°06.026'E for Bushenyi.

Counts of *Varroa* mites were taken as described in section 3.2.2 (Estimation of Varroa mite incidence and severity) above once every month for a period of 6 months between February and July, 2016 to assess population counts over time and seasons; wet and dry on the assumption that all the colonies under study did not abscond. Those that absconded were replaced with new colonies within the same apiary. To confirm the seasons, information about the times and dates of sampling was synchronized with Uganda National Meteorological Authority Dekadel Agro-meteorological Bulletins (www.unma.go.ug).

3.2.2 Control options for *Varroa* and their effect on the associated honey bee colony performance parameters

3.2.3 Sampling procedure

A completely randomized design was used to evaluate the efficacy and resultant colony performance of two treatments; Fluvalinate and Thymol. This experiment on evaluation of control options for *Varroa* was also done in the districts of Bushenyi and Masaka. For each of

the two districts, two apiaries were selected and from each apiary, six colonies were randomly selected. The selected colonies were marked with specific codes and tags and monitored every 21 days for a period of six months. Each treatment was administered as per the manufacturer's recommendations to two top-bar hives which were infested with *Varroa* (Fig.6). Another two untreated hives were used as a control in each of the four apiaries. A white sticky board was also placed at the bottom within each study hive to monitor the numbers of free falling mites. Colonies were followed up in three phases; Before Treatment, During Treatment and Post Treatment. Data on *Varroa* infestation, flight activity, comb construction and numbers, sealed brood patterns and honey storage and availability was captured on every visit.

a)  b)  c) 

Figure 6: Application of *Varroa destructor* treatments in hives (a) Fluvalinate strip fixed in between top bars (b) Thymol gel placed on top of the top bars (c) A white sticky board littered with *Varroa* mites and other debris from the hive

Pre-treatment involved colony strength assessments and *V. destructor* population assessments prior to assignment into treatment groups. All colonies were checked to ensure queen presence prior to onset of the trial. The pre-treatment colony assessments were performed from 10th February to 2nd April, 2016 at three week intervals. Samples of 100-200 worker bees were also taken during this time to determine *V. destructor* infestation levels using the ethanol wash method.

All treatments were placed on 2nd and 3rd April, 2016 in Masaka and Bushenyi respectively. Apiguard®, a thymol based treatment in form of a gel was applied to the colonies and left for two weeks. It was then removed and replaced with a new dosage that stayed for four more weeks. This was done in respect to the manufacturer's recommendations. On the other hand, Apistan®, an acaricide from the pyrethroid family in form of plastic strips whose active ingredient is Tau-fluvalinate was put in the colonies and left there for six consecutive weeks. For the control colonies, no treatments were put though follow up was also done on them for six consecutive weeks and data collected at a three week interval.

At the end of the application period recommended by the manufacturer, all treatments were removed from the hives. The colonies under trial including the controls were then monitored for another six weeks post treatment. Data on various colony parameters were taken at three week intervals.

3.4 Data analysis

3.4.1 Incidence and severity of *Varroa* mites in hived honey bee colonies in selected areas in Uganda

A non parametric ANOVA (Kruskal-Wallis) test was done using SPSS 16.0 statistical program. This was to compare the relationship between incidence and severity of infestation of *Varroa* mites across Agro-ecological zones. Also, a comparative analysis of *Varroa* incidence against altitude was done. Severity of Varroa mite infestations against altitude was compared using a line graph.

3.4.2 Seasonal variation of *Varroa* mites' population across wet and dry seasons in two agro-ecological zones of Uganda

One way ANOVA test was done to test differences in *Varroa* mite populations across seasons (wet and dry) and across AEZs. Statistical tests were run using SPSS 16.0. Mean *Varroa* load per apiary monitored was also plotted on a bar graph. Box plots were used to explain the monthly *Varroa* mite load.

3.4.3 Control options for *Varroa* and the associated honey bee colony performance (colony strength, absconding rates and honey yield) in Uganda.

The formula of Henderson and Tilton (1955) adopted from El-Ela (2014) was used to determine efficacy of the acaricides used;

$$Percentage\ population\ reduction\ = \left[1 - \frac{(Va \times Cb)}{(Vb \times Ca)}\right] * 100\%$$

Where;

Va = *number of V. destructor after treatment.*

Vb = *number of V. destructor before treatment.*

Ca = *number of V. destructor in the control after treatment*

Cb = *number of V. destructor in the control before treatment*.

This formula was used to calculate the reduction rate among *V. destructor* populations (*Varroa* per 100 bees) after application of the two tested acaricides/ Varroacides.

Comparative analysis of the mean mite fall among the different treatment groups was analysed by ANOVA. A multivariate analysis to test effect of treatments on selected colony parameters

was run using ANOVA, SPSS version 16.0. The same version was used to test variation in *Varroa* mite load in two AEZs with time and treatments as factors using rmANOVA.

CHAPTER FOUR

4.0 RESULTS

4.1 The incidence and severity of *Varroa* mites (*Varroa destructor*) in hived honey bee colonies in Uganda

4.1.1 *Varroa* mite infestation

Varroa mite infestation was above 50% for all districts sampled and thus all the four Agro-ecological zones under study though the distribution was uneven. From the 81 bee colonies sampled, *Varroa* mites were found in 61 colonies (75.3%). An average of 235±21.89 bees per colony were examined and averages of 4±0.69 (range 1-8) Varroa mites were recovered from the colonies. Infestation rates were found to be high for the districts of Masaka and Kitgum which were both at 100%. However, per colony sampled, the average number of *Varroa* mites was highest for Bushenyi and lowest for Luweero districts as summarized in Table 1 below.

Table 1: Sampled districts with percentage of colonies tested positive for *Varroa destructor*

District	Number of sampled sites	Number of sites positive for *Varroa*	Total Number of bee colonies sampled	Number found positive to *Varroa* mites	Percentage Infestation	Average bees sampled per colony	Average *Varroa* recovered per colony
Masaka	3	3	9	9	100	250	5
Bushenyi	3	3	9	8	88.9	347	8
Kabarole	3	3	9	6	66.7	170	3
Kyenjojo	2	2	6	5	83.3	228	3
Luweero	6	5	16	8	50	332	1
Mbale	3	2	8	4	50	169	3
Kapchorwa	3	3	7	5	71.4	192	3
Lira	3	3	9	8	88.9	239	3
Kitgum	3	3	8	8	100	192	6
Total	**29**	**27**	**81**	**61**	**699.2**	**2119**	**35**
Average	**3.2**	**3**	**9**	**6.8**	**77.7**	**235.4**	**3.9**
STDEV				**2**		**66**	**2**
SEM				**0.59**		**21.89**	**0.69**

However, the severity across districts and agro-ecological zones which is indicated as number of *Varroa* mites per 100 bees was generally low across the board (Table 2).

Table 2: Severity of *Varroa destructor* infestations among districts and AEZs

Agro-ecological Zone	District	Severity (Mites/100 bees)
Lake Victoria Crescent	Masaka	2.2
	Luweero	2.4
Eastern	Mbale	1.5
	Kapchorwa	1.2
Mid-North	Kitgum	0.4
	Lira	1.5
Western Highlands	Bushenyi	1.4
	Kabarole	1.2
	Kyenjojo	2.9

There were significant differences at 95% level of confidence among districts sampled based on Kruskal-Wallis rank test (Appendix i) for both incidence (χ^2=16.052, df=8, P=0.042) and severity (χ^2=18.058, df=8, P=0.021)

4.2 Effect of altitude on *Varroa* mite infestation

Varroa mites were found at all altitudes (lowlands and highlands) for all the AEZs surveyed. (Fig.7). Altitudinal range for the areas surveyed was 900-2400 m a.s.l. Severity was highest at altitudes below 1,000 m a.s.l and 1400-1600 m a.s.l. On the other hand, a significant drop was observed for altitudes 1000 -1200 m a.s.l as well as 1600 - above 1800 m a.s.l. Kitgum district

had the lowest altitude while Kapchorwa district had the highest. Peak levels of severity (2.96 mites per 100 bees) were observed at 1400- 1600 m a.s.l while the lowest levels (0.71 mites per 100 bees) were at 1000- 1200m a.s.l.

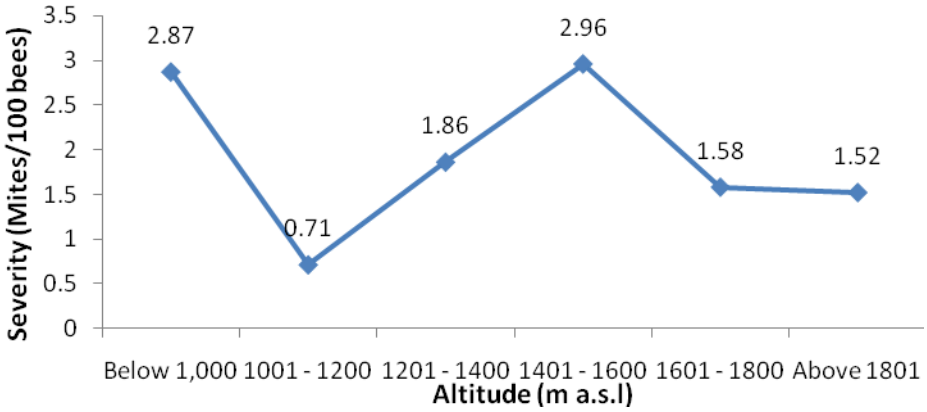

Figure 7: Severity of *Varroa destructor* across a range of altitudes

4.3 Seasonal variation of *Varroa* mites' population across wet and dry seasons in two agro-ecological zones of Uganda

Varroa mite populations were presented as *Varroa* per 100 bees and compared across seasons and AEZs. During sampling, the months of February, May, June and July were recorded as dry while March and April as wet (UNMA Dekadel Agro-meteorological Bulletins). In Fig. 9, increase in *Varroa* mite populations across apiaries was observed between the months of February and March as well as May to July and these were the periods recorded as dry. Reductions in *Varroa* mite numbers were however observed between March and May which were the wet months. There were no significant differences across seasons; dry and wet based on an Analysis of Variance test ($F=3.31$, $P=0.076$, $\alpha = 0.05$, $df=46$) (Appendix ii).

Also per apiary sampled, Apiary 2 (Kyesiiga, Masaka) had the highest recorded numbers of Varroa mite populations and Apiary 4 (Kyamuhunga, Bushenyi) the lowest. Apiary 1 (Buwunga, Masaka) and Apiary 3 (Bumbiire, Bushenyi) had averagely the same *Varroa* mite populations during the period sampled (Fig. 8). Similarly, variation in *Varroa* mite populations across the two AEZs (LVC and Western Highlands) was not significant based on an Analysis of Variance test ($F=0.619$, $P=0.436$, $\alpha = 0.05$, $df=46$) (Appendix iii).

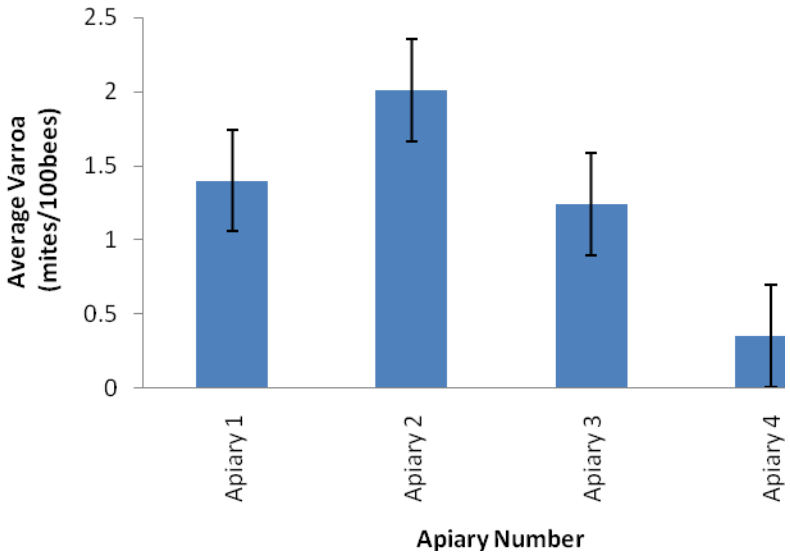

Figure 8: Variation of *Varroa* mite populations among apiaries

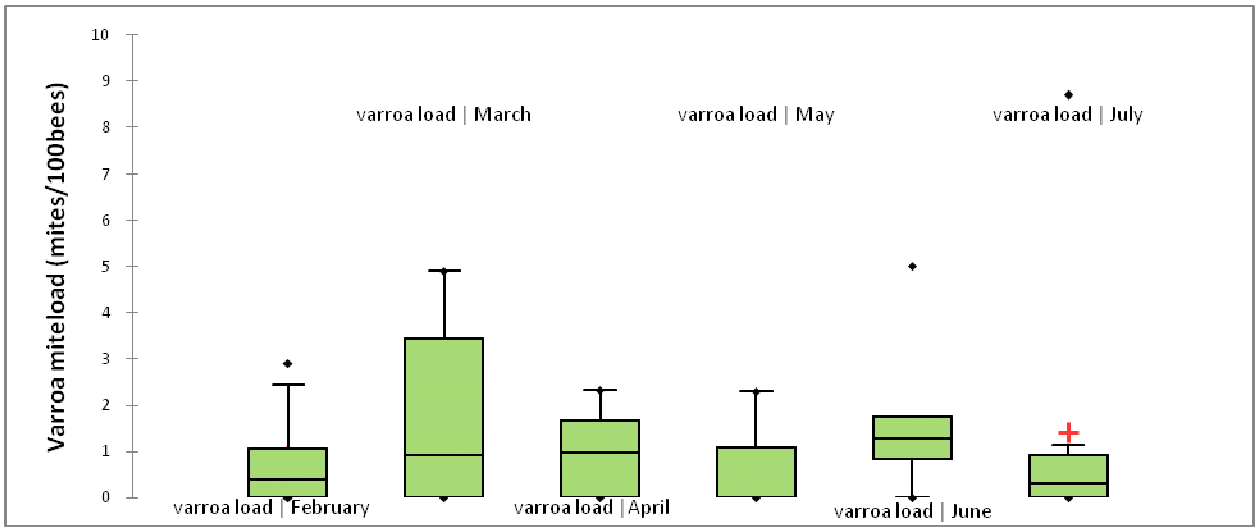

Figure 9: Monthly *Varroa* load between February and July, 2016

From Fig. 9, slight variations were observed in *Varroa* mite load per 100 bees except in March where *Varroa* load exceeded the rest. For all sampling periods, the median *Varroa* mite load did not go beyond 2 mites. A few outliers were however observed in June and July with *Varroa* load going beyond 5 and 9 mites per 100 bees respectively.

4.4 Control options for *Varroa* and their effects on associated honey bee colony performance parameters

The comparative efficacy of the two varroacides in reduction of *Varroa destructor* infesting hived honey bee colonies under field conditions is shown in Table 3. The two varroacides, Thymol and Fluvalinate caused an average reduction in *V. destructor* of 38.8% and 59.7% reduction respectively within 3, 6, 9 and 12 weeks after application of the treatments.

However, highest reduction for Fluvalinate was 63.9% in the 9th week while that of Thymol was 78.9% in the 12th week (Table 3). The overall change caused by Thymol, Fluvalinate and control

was 76%, 51.9% and -10.2% respectively. Therefore, Thymol was more potent than Fluvalinate in reducing the size of mite populations.

Table 3: Efficacy of two varroacides against the *Varroa* mite in honey bee colonies of Uganda

Treatments	Pretreatment Number	Number (N) and percentage reduction (R) of *V. destructor* per 100 bees								Average Reduction (%)	Overall change (%)
		3 weeks		6 weeks		9 weeks		12 weeks			
		N.	R.	N.	R.	N.	R.	N.	R.		
Thymol	1.5	2.2	-5.9	0.5	29.6	2.3	52.6	0.3	78.9	38.8	76
Fluvalinate	1.9	0.9	65.0	0.5	53.3	0.7	64.0	0.9	56.3	59.7	51.9
Control	1.3	1.8	-	0.7	-	1.3	-	1.4	-		-10.2

On comparison, there were some slight fluctuations in activity for Fluvalinate after every three week interval while for Thymol activity was more of exponential with percentage reduction in *Varroa* mites increasing with time over the period of treatment (Fig.10).

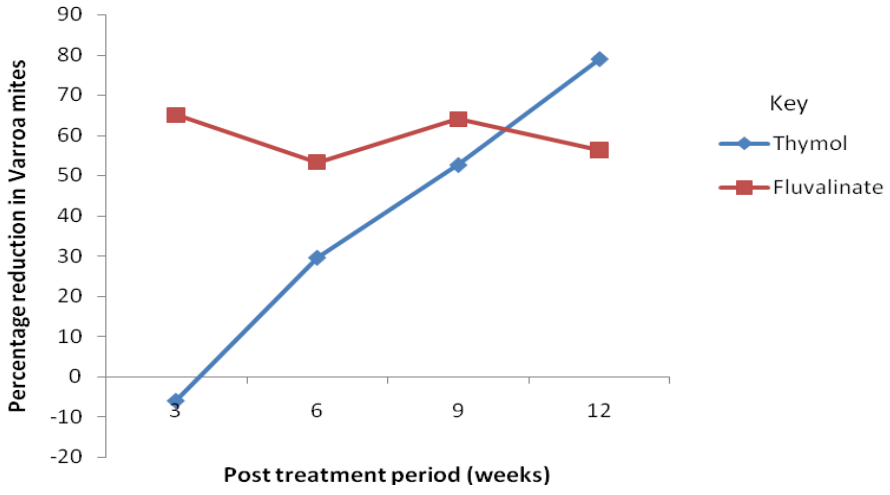

Figure 10: Activity of Thymol and Fluvalinate compared

Varroa mite fall statistics during and post application of treatments are summarized in Table 4 and Table 5 respectively. The mean *Varroa* mite fall for all experimental colonies was found to be 5.55 mites during treatment while for individual treatment groups it was 3.35, 12 and 2.29 for Thymol, Fluvalinate and Control respectively. There were significant differences (P= 0.002, α = 0.05) among treatment group means in terms of *Varroa* mite fall during treatment (Table 4). No significant differences (P= 0.698, α = 0.05) were observed among the treatment groups for *Varroa* mite fall post treatment (Table 5).

Table 4: Average *Varroa* mite fall during treatment

Treatments	N	Mean ± S.E	Range	
Thymol	8	3.35 ± 0.865[a]	0	17
Fluvalinate	8	12.0 ± 3.457[b]	1	48
Control	8	2.29 ± 0.928[c]	0	18

**Different letters indicate significant differences among the means (P<0.05)*

Table 5: Average *Varroa* mite fall post treatment

Treatments	N	Mean ± S.E	Range	
Thymol	8	1.59 ± 0.707	0	10
Fluvalinate	8	1.21 ± 0.604	0	8
Control	8	2.69 ± 1.929	0	30

The average *Varroa* mite fall post treatment was 1.59, 1.21 and 2.69 mites in Thymol, Fluvalinate and Control respectively. This clearly shows that mite fall greatly reduced within colonies after removal of the treatments whereas it remained the same for the control colonies.

A multivariate analysis (Appendix iv) was done to test the effect of treatments used on selected colony performance parameters (flight activity, number of brood combs, percentage of brood pattern and number of honey combs). There were no significant differences for both flight activity ($P=0.115$, $\alpha = 0.05$, $df=2$) and number of combs with honey ($P=0.679$, $\alpha =, 0.05$, $df=2$). However, the treatments had significant differences on number of combs with brood ($P=0.009$, $\alpha = 0.05$, $df=2$) and brood pattern ($P=0.035$, $\alpha = 0.05$, $df= 2$).

Results of rmANOVA on *Varroa* load for the two AEZs with time and treatment as factors (Appendix v) indicate that there were no significant differences between treatments and time of application ($P=0.635$, $F=0.793$, $df=10,93$)

CHAPTER FIVE
5.0 DISCUSSION

5.1 Incidence and severity of *Varroa* mites in hived honey bee colonies in Uganda

Generally, the study revealed that the *Varroa* mite occurs in all the sampled districts. *V. destructor* in Uganda is 100% identical to the South Korean haplotype (Chemurot et al, 2016). The wide occurrence of *Varroa* is an indicator that the mite has spread vastly across the country. Also, it shows that the mites have tremendous infestation behaviour and are not obstructed by variations in vegetation type, climatic conditions and elevation of an area (Mumbi et al. 2014). This is because mites were found among honey bee colonies from the diverse agro-ecologies and geographical locations with slight variation in mite infestation levels. The different agro-ecological zones differ according to climatic conditions, farming systems, vegetation and elevation.

Varroa mite prevalence reported in this study was comparatively lower than that reported in neighbouring countries that is Tanzania (Mumbi et al. 2014), Kenya (Muli et al. 2014) and Ethiopia (Begna, 2015). This could be because these mites are relatively new in Uganda and that their populations are not entirely settled since the process of spread is still ongoing from the east to the west (Chemurot et al. 2016).

Also, the severity (mites/100 bees) was generally low and below the threshold. This is in comparison with the recommended threshold which is 5 mites per 100 bees for an infestation to be considered alarming (Mumbi et al., 2014). This therefore means that Ugandan bees are able to keep mite levels low through natural mechanisms like absconding if heavily infested or having less drone brood since it is most preferred by the mites.

Altitude was not a limitation to *Varroa* spread since the mites were present at all the altitudinal levels sampled. However, there was some variation in levels of severity with some altitudes having lower numbers of mites per 100 bees. Areas in low altitudes are seen to have a relatively higher varroa load as well as higher temperatures than areas in high altitudes. The high temperatures favour high honey bee activity as well as proper brood development. This in a way also facilitates the reproduction and survival of the *Varroa* mites since their reproductive stages take place within brood.

5.2 Seasonal variation of Varroa mites' population across wet and dry seasons in two AEZs of Uganda

There were no significant differences between *Varroa* mite populations across seasons (wet and dry) which is similar to earlier studies in Uganda (Chemurot et al., 2016). This is however different from Salima (2015) whose study in Algeria revealed that the level of infestation of *Varroa* in a colony varies depending on weather conditions (season) but can also be affected by internal conditions of the colony.

The wet season in Uganda is characterised by increased occurrence of rains almost on a daily basis with low sunny intervals as well as reduced daily temperatures. The temperatures however usually do not fall below 20°C in most parts of the country. The dry season on the other hand is characterised by long sunny days with temperatures going beyond 30°C in some parts of the country.

Weather conditions in Uganda do not create a big gap in temperature variation there by keeping the *Varroa* mite natural reproduction rates constant with slight variations almost throughout the year. Honey bees also have a natural mechanism of maintaining hive temperatures within a

certain range despite the prevailing temperature and climatic conditions outside the hive. This in a way keeps the in-hive temperatures constant and favourable for *Varroa* mite reproduction and survival since they have coevolved with their hosts. As such, *Varroa* populations are not so much disturbed.

Further still, brood production among tropical honey bees which is the case here in Uganda is almost throughout the year without particular breaks in the cycle. Since *Varroa* mites naturally prefer to multiply from within drone brood, this facilitates their continuous reproduction without major breaks hence their populations not being affected by seasonal differences.

In addition, there have been changes in seasonal variations due to effects of climate change in such a way that there are no clear variations in wet and dry seasons in Uganda currently.

5.3 Control options for *Varroa* and the associated honey bee colony performance (colony strength and absconding rates)

The results showed that *Varroa* mite fall decreased in treated colonies from 3.35±0.86 during treatment to 1.59±0.7 mites after treatment for Thymol and from 12.0±3.45 during treatment to 1.21±0.60 mites after treatment for Fluvalinate while there was no major difference in mite fall for the control colonies during and after treatment. This clearly shows that the treatments helped in dislodging the mites off the bodies of bees hence the increase in mite fall during the period of treatment for both treatments. Both varroacides reduced the mite population over the experimental period with Fluvalinate causing a bigger reduction than Thymol.

Thymol is the active ingredient in the varroacide, Apiguard® (Watkins, 2012) that is widely used against *Varroa* mites in honey bee colonies. It is an organic near natural treatment. The active ingredient (A.I) is released gradually from a food-grade gel. Fluvalinate on the other hand comes

under the trade name Apistan® whose active ingredient is Tau-fluvalinate, a powerful varroacide from the pyrethroid family. It comes in form of a plastic matrix strip enabling controlled release of the A.I (Watkins, 2012). It is a contact varroacide.

For the average reduction in mites per 100 bees, Thymol registered 38.79% while Fluvalinate was 59.65%. This was generally low for both treatments especially Thymol. These efficacies of the treatments were probably underestimated due to the reproduction of the surviving mites and possible re-invasions during the experimental period (Gregorc and Planinc, 2012). Reported efficacies for gel-based Thymol as was used in this experiment include 76% (Matilla and Otis, 2000), 46% (Gregorc and Planinc, 2005) and 82.33% (Vandervalk, 2013). It should also be noted that an important consideration when applying Thymol based products is the ambient temperature whose range should be 15-20°C for it to be effective (Imdorf et al., 1995). This however could have been the main cause of the low efficacy observed with Thymol since the prevailing temperatures in Uganda and especially the areas where the trial took place were higher than the recommended limit of 20°C there by affecting the efficiency of the treatment.

The analyses of *V. destructor* infestation rates and efficacy data both confirm Fluvalinate provided significantly better management of the *Varroa* mite than both Thymol and the control. The calculated efficacy of Fluvaliante was 59.65% which was also way far below the efficacies reported in similar studies with Fluvalinate based treatments. Some of the reported efficacies include 85% (Loucif Ayad et al., 2010) and 87.7% (Akkaya and Vurusaner, 1996). It should also be noted that the effect of Fluvalinate is only achieved when the honey bees get into contact with the strips and the A.I is then transferred by social interaction in the hive. In this way, the A.I eventually gets to the mites and are killed. However, there is likelihood that during the

experimental period a few *Varroa* mites were able to get in touch with the Fluvalinate strips hence registering a low efficacy as compared to previous reports.

Also, it could have been applied at a time when most of the mites were still hidden in capped brood therefore making them get less exposed to the treatments. Ellis and Baxendale (1994) found that up to 90% of *Varroa* mites present in a honey bee colony are in sealed brood cells. This greatly reduces the efficacy of varroacides that are contact in nature. Therefore, effective performance of Fluvalinate was found to be affected by having most of the mites still covered up in capped brood since it is a contact varroacide.

The results also suggested that there were no significant differences between treatments and some colony parameters (flight activity and number of combs with honey) while there were significant differences between treatments and brood characteristics (number of combs with brood and brood pattern). This suggests that the treatments could have had an effect on the queen laying characteristics and abilities negatively there by affecting the brood pattern as well as the resulting number of combs with brood.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Varroa mites have spread to all the AEZs that were sampled in this study. However, the severity (mites per 100 bees) is still low and below the threshold though the mite populations are still building up. Altitude is not a limiting factor to *Varroa* mite spread though it might have an effect on severity. Also, seasonal variations of wet and dry did not have any effect on *Varroa* mite population growth since they were not causing varied ranges in temperature changes.

Different efficacies between treatments were found with Fluvalinate having a higher efficacy than Thymol. The efficacies for both treatments were however lower than reports of similar studies. Effects of these treatments on other colony parameters were also tested and found not to not have significant effects on flight activity and honey production but a significant effect was observed with brood characteristics like brood pattern and number of combs with brood. This is the first study of its kind in Uganda therefore, more field studies are necessary to assess the efficacies of the treatments over a much longer period of study and in different apiary set ups so as to obtain a better understanding of the effects of the applied treatments on colony performance.

6.2 Recommendations

- ✓ Awareness should be created and raised among beekeepers and extension staff country wide about the invasion of our bee colonies by the *Varroa* mite and appropriate measures put in place to manage the mite.

- ✓ The relevant authorities particularly MAAIF should intervene by financially supporting the management of this honeybee pest and also through policy and long-term political commitments.
- ✓ More *Varroa* mite monitoring should continually be done in the country so that severity levels are kept at the minimum and don't go beyond the threshold.
- ✓ Given the low severity which is even below the threshold, chemical control should not be adopted for *Varroa* control currently in Uganda since it is costly and is bound to create resistance among the mites.
- ✓ Studies should be conducted on other methods of control of *Varroa* mites so that an intergrated management approach of the mite is developed.

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APPENDIX

Appendix i: Kruskal-Wallis test of significance for *Varroa* mite incidence and severity across districts

Ranks

	District	N	Mean Rank
severity (mites/100 bees)	Masaka	9	57.33
	Bushenyi	9	49.78
	Kabarole	9	38.67
	Kyenjojo	6	42.75
	Luweero	16	23.94
	Kapchorwa	7	41.00
	Mbale	8	34.50
	Lira	9	40.28
	Kitgum	8	55.50
	Total	81	
incidence	Masaka	9	31.00
	Bushenyi	9	35.50
	Kabarole	9	44.50
	Kyenjojo	6	37.75
	Luweero	16	51.25
	Kapchorwa	7	42.57
	Mbale	8	51.25
	Lira	9	35.50
	Kitgum	8	31.00
	Total	81	

Severity: Chi-squared = 18.058, df = 8, P = 0.021

Incidence: Chi-squared = 16.052, df = 8, P = 0.042

Test Statistics[a,b]

	severity (mites/100bees)	Incidence
Chi-Square	18.058	16.052
Df	8	8
Asymp. Sig.	.021	.042

a. Kruskal Wallis Test
b. Grouping Variable: district

Appendix ii: *Varroa* mite populations across seasons

ANOVA

Varroa population (mites/100bees)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9.441	1	9.441	3.307	.076
Within Groups	128.471	45	2.855		
Total	137.913	46			

Appendix iii: *Varroa* populations across Agro-ecological Zone

ANOVA

Varroa population (mites/100bees)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.871	1	1.871	.619	.436
Within Groups	136.042	45	3.023		
Total	137.913	46			

Appendix iv: Multivariate analysis for effect of treatments on colony productivity

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Noncent. Parameter	Observed Power[b]
Corrected Model	flight activity	8911.246[a]	2	4455.623	2.187	.115	4.373	.443
	no. of combs with brood	88.394[c]	2	44.197	4.832	.009	9.663	.794
	brood pattern	3124.391[d]	2	1562.195	3.425	.035	6.851	.638
	no. of combs with honey	6.209[e]	2	3.104	.679	.508	1.359	.163
Intercept	flight activity	1587841.409	1	1587841.409	779.247	.000	779.247	1.000
	no. of combs with brood	17874.654	1	17874.654	1.954E3	.000	1954.009	1.000
	brood pattern	391249.062	1	391249.062	857.886	.000	857.886	1.000
	no. of combs with honey	448.263	1	448.263	98.107	.000	98.107	1.000
treatments	flight activity	8911.246	2	4455.623	2.187	.115	4.373	.443
	no. of combs with brood	88.394	2	44.197	4.832	.009	9.663	.794
	brood pattern	3124.391	2	1562.195	3.425	.035	6.851	.638
	no. of combs with honey	6.209	2	3.104	.679	.508	1.359	.163
Error	flight activity	368816.907	181	2037.662				
	no. of combs with brood	1655.731	181	9.148				
	brood pattern	82547.212	181	456.062				
	no. of combs with honey	827.009	181	4.569				
Total	flight activity	1962084.000	184					
	no. of combs with brood	19687.000	184					
	brood pattern	481575.000	184					

	no. of combs with honey	1284.000	184					
Corrected Total	flight activity	377728.152	183					
	no. of combs with brood	1744.125	183					
	brood pattern	85671.603	183					
	no. of combs with honey	833.217	183					

a. R Squared = .024 (Adjusted R Squared = .013)

b. Computed using alpha = .05

c. R Squared = .051 (Adjusted R Squared = .040)

d. R Squared = .036 (Adjusted R Squared = .026)

e. R Squared = .007 (Adjusted R Squared = -.004)

Appendix v: rmANOVA of data on Varroa load for the two zones with time and treatment as factors

Type II tests of fixed effects:

Effects	Num DF	Den DF	F	Pr> F
Treat	2	20	0.013	0.987
Time	5	93	2.023	0.083
treat*time	10	93	0.793	0.635

treat*time| F= 0.793, df = 10,93; P=0.635| no sig effect for time or for treat