TO EVALUATE THE OCCURRENCE, PREVALENCE AND DISTRIBUTION OF VARROA MITE (Varroa *destructor*) AND ASSOCIATED INSECT PESTS OF HONEY BEE (*Apis mellifera*) IN UASIN GISHU COUNTY, KENYA.

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DECLARATION

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ABSTRACT

The loss of *Apis mellifera* colonies in recent years has been in many regions of the world been alarmingly high. No single cause has been identified for these losses, but the interactions between several factors (mostly pathogens and parasites) have been held responsible. The ectoparasitic *Varroa destructor* and other associated bee pathogenic viruses have been identified as a marker of dramatic colony losses in the world.

The study was aimed at evaluating the occurrence, prevalence and distribution of *Varroa destructor* and other insect pest of honey bees in six sub-counties in Uasin-Gishu County during May and June 2014. Purposive and convenience sampling was used to select two apiaries in each sub-county. Four bee hives were randomly selected for inspection, direct observation and experimental setup using the sugar roll technique per 100 bees and 100 brood cells was used to collect data and Interview of farmers. Descriptive statistics was used to estimate the prevalence of *varroa* mite and other insect pest. Chi-square test and odd ratio was performed to find the association between varroa mite infestation and colony strength, Pearson correlation co-efficient was used to test for correlation between the rate of *varroa* mite infestation and elevation. All the tests were significant at $P \leq 0.05$ at 95% confidence interval.

The interview showed a 67.5% hive occupancy in the surveyed apiaries, all farmers were not aware of varroa mites, however 80% and 70% had knowledge on wax moth and small hive beetle respectively.

The study revealed presence of varroa mites in honey bees in all (100%) studied Sub County. Out of 37 inspected honey bee colonies 32 (86.49 %, C.I 95%, 70-93.8%) were found to be infected with *varroa* mites. The average level of varroa mite infestation in adult bees and brood cells was 59.88±31.45 (mean,±SD), with an average varroa mite infestation of 27.22±12.44 (mean,SD) and 32.67±21.92 (mean,±SD) per 100 adult bees and 100 brood cells per apiary respectively, average infestation per colony of 6.88±2.56(mean,±SD), and 7.80±4.94(mean,±SD), in adult bees and brood cells respectively.

The level of varroa mite infestation showed a significant positive correlation with elevation (R^2=0.56, p=0.020), The study revealed that honey bees colonies are not affected by varroa mite yet, in fact, there was a statistical significant association between colony strength and level of varroa mite infestation, ($\chi 2$ = 5.03, df = 1, P= 0.02), with the infestation in strong colonies being 8.1 times higher than in weak colonies (OR = 8.1, C.I 95%, 1.07 – 35.54). The results showed a wide distribution of other associated honey bee pest, small hive beetle (62.16%), wax moth (21.62%), black ants (32.43%) and earwig (13.52%).

Lack of farmers awareness on varroa mite shows that the pest has been introduced recently in the area, therefore, there is a need to further and routine monitoring and surveillance of honey bee colonies so as establish baseline data for initiating control measures

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CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Honey bees of the species (*Apis mellifera*) is one of the world's most beneficial insect given its crucial role both in honey production and as a main pollinator of crops which account for about 35% of global food production (Genersch, 2010), hence they are vital for an economic, sustainable agriculture and food security. In addition, honey bees also pollinate a variety of wild flowers and therefore, contribute to the biodiversity of many ecosystem.

Globally, pollination services amount to USD 212 billion, corresponding to approximately 9.5% of total value of world agriculture production for human consumption in 2005 (Gallai, Salles, Settele and Vaissière, 2009). Honey bees are one of the most important pollinators worldwide, contributing USD 14.6 billion in pollination services to the United States in 2000 (Calderone, 2012) and USD 3.2 billion in the South Africa economy in 1998 (Allsopp, 2004)

According to Food and Agriculture Organization (FAO, 2006), the essential and valuable contribution of honeybees depend upon the healthy population of honey bees. This is mainly associated with the recent emergence of high honeybee colony losses in many parts of the world (vanEngelsdorp and Meixner, 2010) and the vulnerability of honey bees to parasite mites, viruses, bacteria, fungi and other pests. These pathogens and parasites can have detrimental effect on honeybee health and the service they offer which in turn can lead to severe economic losses (Smith, Loh, Rostal, Torrelio, Mendiola, Daszak, 2013) Moreover, modern agriculture increasingly depends on the use of chemical substance to control weeds, fungi and arthropod pests

to ensure high yields, (Rosenkranz, Aumeier and Ziegelmann 2010), this has lead honey bees to be frequently exposed to environmental chemicals as a consequence of their foraging activity (Fazier *et al.,* 2010)

It has been reported that several biological and environmental factors acting alone or in combination have a potential to cause premature colony mortality (Vanbergen, Baude, Biesmeijer and Brown, 2013). The synergistic factors may include several honey bee pathogens especially *Varroa destructor* and *Nosema ceranae* ((Cox-Foster *et al.,* 2007), and environmental factor including pesticides (Fazier *et al.,* 2010), climate change and bee keeping management (Rosenkranz *et al.,* 2010).

Varroa destructor has become the most significant economic threat to agriculture in the world (Pirk *et al.,* 2015) and is an OIE notifiable infestation. *Varroa destructor* is an obligatory ectoparasite of *A. mellifera* that feeds on the heamolymph of both adult and immature honey bees and reproduces in combs containing the brood. *Varroa destructor* originated in Asia, where it parasitized Asian honey bee *Apis cerena,* the jump from the host *A. cerena* to *A. mellifera* occurred several decades ago when Western honey bees was introduced into Asia (Rosenkranz *et al.,* 2010). Currently the mite is has a worldwide distribution due to the global trade of honey bee and honey products.

V. destructor feeds on the haeymolymph of honey bees (all stages) and reproduction takes place inside capped cells. By feeding on the heamolymph, the mite causeses a variety of physical and physiological effects in both colony and individual bee (Le Conte, Ellis, and Ritter, 2010). In addition, the indirect effects of varroa mite primarily caused by the viral infection vectored by the mite are the most devastating and can lead to severe disease and mortality of the individual or colony level

(Mumoki, Fombong, Muli, Muigai and Masinga 2014). The losses of colonies to varroa mite have been reported in Europe and United States (Cox-Foster *et al.*, 2007), in New York, and Germany (Sammataro, Gerson and Needham, 2000). Moreover, *V. destructor* is known to vector several honey bee viruses such as Deformed Wing Virus (DWV), Acute Bee Paralysis Virus (ABPV) (Joachim. de Miranda, Cordoni, and Budge, 2010), Israel Acute Paralysis Virus (IAPV), Varroa destructor virus 1 (VDV-1) and Sac brood bee Virus (SBV) (Boecking and Genersch, 2008), some of which are claiming honeybee colonies. Owing to the challenges of experimental analysis of the synergistic effect, there is limited information on the effect of varroa. However, the quantification of such effects seems to be a requirement for future research on colony losses (Rosenkranz *et al.,* 2010).

Other than *V. destructor*, there other associated insect pests of honey bees such as Small Hive Beetle (SHB) (*Aethina tumida*) and Wax Moth (*Galleria mellanela* and *Archrola griselia*), small hive beetle is limited to Sub-Saharan Africa (Ellis and Munn, 2005), where it is considered as a minor pest (Pirk *et al.,* 2015). However, infestation with SHB is listed by OIE as a notifiable infestation. Neumann and Elzen, (2004) reported devastating effects when SHB was introduced in Australia and USA. Contrary to Africa beekeepers, the SHB is of significance importance due to its ability to vector honey bee viruses (Schäfer, Ritter, Pettis, and Neumann, 2009). Economic damages occur when adult beetle defecates in the honey introducing yeast that cause fermentation of honey, feeding brood, pollen and honey.

Wax moth has a global distribution, causing serious destruction of combs *(Kwadha et al.,* 2017), the greater wax moth *(Gallezia Mellanealla)* infestation can cause absconding of bees. The larvae feed on pollen, honey and wax. They also create

tunnels in combs and leave masses of webs. In addition, they also create a condition called galleriasis (Rosenkranz *et al.,* 2010). The Wax Moth is also a potential vector of honey bee viruses such as Israel Acute Bee Paralysis and Black Queen Cell Virus (Traiyasut *et al.,* 2016)

Even though the majority of parasite, pathogens and pests have an almost worldwide distribution information on health status of honey bees in Africa is poorly characterized (Mumoki *et al.,* 2014), varroa mite is reported to be found in East Africa (Muli et al., 2014), South Africa (Allsopp, 2004), Nigeria (Akinwande, Badejo, and Ogbogu, 2013), Ethiopia (Godifey, 2015). Moreover, the reported cases of colony collapse disorder (CCD) by OIE in Madagascar pointed out to the undocumented case even in other countries. This is a clear indication that the health status of honeybees in Africa is under threat and therefore urgent and extensive health surveys are needed.

In Kenya, honey bees provide critical pollination services, nutrition and income for small holder farmers and rural families. There is considerable diversity in *Apis mellifera* population each adapted to a specific ecological zones (Raina, 2005). Beekeeping is a valuable enterprise within Kenyan Agricultural Sector, contributing about 4.3 billion shilling from honey production alone (Kiptarus, Asiko, Muriuki and Biwott, 2011). In addition, in Western Kenya alone, honey bees provide USD 3.2 million in ecosystem as a result of pollination services (MoLD, 2009). Furthermore, the honey collected serve as an important source of nutrients and income to families.

Reports from Kenya National Beekeeping Institution indicate that farmers have reported significant decline in the number of hives that are being colonized, reduction in the size of migratory swarms, and decrease in honey production (MoLD, 2007). This reduced performance was thought to be disease related as *Varroa destructor* had

just been reported in Kenya (Fazier *et al.,* 2010). In March of 2009, Fazier, *et al.,* (2010) sampled 38 honey bee colonies in various locations in Central and Eastern Kenya employing the Standard Sampling Technique to determine mite presence/absence that utilize powdered sugar to dislodge mites from 300 adult bees, and they reported 717 ± 45 bee bees per colony with 100% prevalence with an average 26.3 ± 25.9 per colony, (Fazier, *et al.,* 2010). In a similar survey, 125 colonies located in the Eastern, Western and Coastal Region were sampled and presented a prevalence of 87%. In another survey, 19 apiaries were sampled employing the standard method (powdered sugar roll) with approximately 350 bees per colony, the survey reported a prevalence of 89.5% in apiaries, and 83% colony prevalence. Furthermore, this survey reported that the level of varroa mite infestation may be influenced by environmental factors. Also, the presence of *V. destructor* did not seem to strongly impacting on honey bees colonies in those surveyed areas. (Muli *et al.,* 2014)

Despite their role in colony destruction, occurrence and distribution of honeybee pest remain understudied in Western Kenya with investigation restricted to Central, Eastern, Western and Coastal parts of Kenya. Furthermore, data on prevalence, epidemiology, occurrence, distribution in different agro-ecological zones are scarce.

Other pests reported in Kenya include the small hive beetles (SHB) and wax moths, both of these pests are endemic in Africa. In Kenya, the small hive beetles have been reported though the beetles do not seem to pose a significant threat to beekeepers (Fazier, 2014). However, due to their ability to vector some honey bee Virus diseases is a matter of concern (Eyer, Chen, Schafer, Pettis, and Neumann, 2009). The greater and lesser wax moth is common in Africa (Pirk *et al.,* 2015). The greater wax moth

causes the heaviest losses to beekeepers (Kwadha *et al.*, 2017). Infestation with wax moth often leads to colony loss, absconding and reduction in size of migratory bee swarm (Kebede, 2015., FAO, 2006).

Information on the occurrence, prevalence and distribution of varroa mite and other pest in Uasin Gishu County is lacking. No research has been conducted on the pest of bees therefore, this study aims to determine the occurrence, prevalence and distribution of varroa mites and other pests of honey bees in Uasin Gishu County.

1.2 Statement of the Problem

Over the past ten years, the honey bee population has been declining; this is evident in the decline of hives that are being colonized, reduction in the size of migratory swarms, and decrease in honey production (MOLD, 2007). The major factors implicated in the decline are pests and pathogen. The detection of varroa mite in Kenya in 2009 indicates that varroa mite was introduced in Kenya recently.

For effective control and management of honeybee pest, there should be proper information on the prevalence, occurrence, distribution and epidemiology of the pests. The role of honey bee pest in transmission of disease is of concern in beekeeping practices in Kenya, this is because most of the honey bee viral diseases have been reported in Kenya, and therefore, knowledge on the distribution of the pest which can act as vector will help in controlling and managing the disease.

1.3 Justification of the Study

Honey bees are the most important commercial pollinators contributing to the economy, sustainable agriculture and for food security. In addition, honey bees also pollinate a variety of wild flowers, and therefore, contribute to the biodiversity of many ecosystems. The current honey bee colony losses experience in the world is of concern. Honey bee pest and pathogen combined with other factors are the major causes of honey bee colony losses.

Detection and assessment of honey bee pest (Varroa mite, small hive beetle and wax moth) in honey bee colonies are important for successful beekeeping. Knowledge and information on occurrence distribution, level of infestation and epidemiology of honey pest will extensively help in deciding on their control and management. Information on the level of colony infestation can be used as baseline information for future research on pest and disease vectored by these pests. Honey bees pest especially the *V. destructor* is known to be a vector of many viruses, therefore knowledge on the distribution of varroa mite infestation can be used to determine distribution of viral infection in honey bee colonies.

The occurrence of *V.destructor* does not seem to be impacting on honey bee colonies in Kenya yet. However, since they may be a time-lag before newly introduced parasites and pathogens cause substantial negative effects, there is need for continuous assessment of honey bee colonies to evaluate the long term interaction of host and pests.

1.4 Objective of the Study

1.4.1 Broad Objective

The main objective of this study was to evaluate the occurrence, prevalence and distribution of varroa mite (*V. destructor)* and insect pests (Small hive beetle and wax moth) of honey bee (*Apis mellifera)* in Uasin Gishu sub - counties.

1.4.2 Specific Objectives

1. To evaluate the prevalence and infestation level of Varroa mite (*V. destructor*) and their impact on honey bee colonies.
2. To assess the distribution of *Varroa* mite (*V. destructor*) in different apiaries.
3. To assess the level of infestation of insect pest of honey bees.

1.5 Hypothesis

1. H_o: *Varroa destructor* is not prevalent and has no impact on the honey bee colonies in Uasin Gishu County.

   H_1: *Varroa destructor* is prevalent and has an impact on honey bee colonies in Uasin Gishu County

2. Ho: There is no correlation between level of *V. destructor* infestation in honey bee colonies and Elevation (height above sea level).

   H_1: There is a correlation between *V. destructor* infestation level in honey bees colonies and elevation (height above sea level).

3. Ho: There is no infestation of honeybee colonies with insect pests.

   H1: There is infestation of honeybee colonies with insect pests.

CHAPTER TWO

LITERATURE REVIEW

2.1 Global overview of Honey bees *(Apis mellifera)*

The honey bees as pollinators are of major importance in global nutrition and food security. Globally, the honey bee pollination services amount to an estimated $200 billion of total value of world agriculture production with $17 billion in United States alone (Calderone, 2012). Honey bees are also important in maintaining biodiversity because they pollinate numerous plant species that require an obligatory pollinator for fertilization. The evolution history of *A. mellifera* is quite complex due to the contribution of both natural and anthropic factors (Moritz, Härtel and Neumann, 2005), indeed this species has been managed by man for centuries. Molecular studies indicate that *A. mellifera* appeared in Africa two million years ago, and later spread throughout Europe and Middle East, resulting in generation of distinct evolutionary lineages through successive colonization (Whitfield *et al.,* 2006)

Despite their importance to agriculture and their products the numbers of managed honey bees colonies have been declining, for instance in United States, Canada and Europe, in United states VanEngelsdorp and Meixner, (2010) reported a 17-20% to 32 % of colony losses increase during the winter of 2006/07 with some bee keepers losing up to 90% of their colonies. There has also been reports indicating losses of colonies in United states and Middle East (Ellis and Munn, 2005).

The African continent honey bees have not been spared either, there has been claims of honey been population decline on the continent (Kluser, Neumann, Chauzat, and Pettis, 2011) Egyptians beekeepers based along the Nile have also reported honey bee

colony losses (Hussein, 2000), in Nigeria Oyerinde, and Ande, (2009). In East Africa, particularly in Kenya, the National bee keeping station have reported a significant decline in honey bee colonies for the last 5 years (Muli *et al.,* 2014).

Although the causes of the honey bee colonies decline remains undetermined most scientists agree that it is likely due to a combination of several factors ranging from pathogens, parasites including bacteria, viruses, protozoa, mites, pesticide exposure and poor nutrition (Bromenshenk, Henderson, Wick, Stanford, 2010), management practice and reduced genetic diversity (VanEngelsdorp, *et al.,* 2012) . In East Africa the cause of honey bee population decline has not been determined. However the prominent suspects are deforestation, agriculture, drought and use of agrochemicals. The pesticides cause toxicity and death of bees whereas deforestation and drought destroy the natural bee habitats, reducing forage and plant diversity thus starvation of bees (Muli *et al.,* 2014).

In recent past, the loss of honey bee colonies worldwide has increased public awareness and concern about the future of honeybees, therefore devoting time and resources in research to identify the causes (Cox-Foster *et al.,* 2007)

2.2 Bee keeping in Kenya and its economic importance.

Bee keeping has traditionally been practiced in the country for decades. Many communities kept honey bee colonies in either baskets, pots, gourds, logs and rock crevices as beehives (MOLD, 2009).

Since introduction of modern technology there has been progressive growth in production of honey and beeswax estimated at 14,653 and 140 metric tonnes respectively (MOLD, 2007), valued at Ksh 4.43 billion per annum, with a potential of

about 100,000 and 10,000metric tones respectively. With this huge potential the country cannot meet local market demand for honey and bee wax which is estimated at about 15,000 metric tons leading to importation of 49,932 metric tons of honey in 2008 (MOLD, 2009).

Work done by the Kenya National Beekeeping Institute reported a significant decline in the number of hives colonized, migratory swarm and honey production in the last 5-7 years (MOLD, 2007). Suresh, (2010) also reported decline in honey bees over the last 5-10 years, this reduction was attributed to pest and diseases which led to the listing of honey bee diseases in OIE Terrestrial Animal Health Code.

2.2.1 Honey bees of Kenya

There is a considerable genetic variability in honey bees from East Africa, this variability could be explained by geographical variability. Morphometric analysis showed that different geographical location have different species of honey bees, in the tropical coastal strip which receives rainfall all the year round is inhabited by the small yellow honey bees *Apis mellifera litorea* spreading along the forage rich low land up to 500m, the large black bee *Apis mellifera monticola* occupies the tropical cool mountains at altitude of 2400- 3100m, with thick forest and with temperatures often below freezing points with sparse flowering but spread all round the year. The bees occupying the acacia-savannah plains are similar to *Apis mellifera scutellata* and differ markedly from *Apis mellifera adansonni*. The bees in the Northern Kenya, cut off by the Koroli -Habasweni desert is more or less the Sudan type that occupy the dry area- rainfall zone (300-1000m)(Raina, 2005).

2.3 Challenges of honey bee colonies

Over the past decades there has been significant colony losses reported from Europe, United States and Middle East (Ellis and Munn, 2005), this dramatic demise of honey bees was coined "Colony Collapse Disorder" (CCD) however the same was not reported in South America, Australia and African continent. Years later, with widespread in colony losses it was concluded that the disease has been due to combination of factors particularly infection with chronic bee paralysis together with poor weather which inhibited foraging and competition for forage (Neumann and Carreck, 2010).

After intensive research it was hypothesized that pest, disease, pesticides and beekeeping practice could be the cause of colony loss, considering that Africa and Africanized honey bees survived without treatment for *V.destractor,* therefore implicating varroa mite for the experienced colony losses. (Ellis and Munn, 2005).

Despite intensive research on the losses with no particular determined risk factor it is believed the losses with no particular interaction potential drivers that are distributed all over the world, With pests such as varroa mites and viruses being the high-profile suspects in collapsing bee colony (Francis, Nielsen, and Kryger, 2013), other factors that interact with pests and pathogens include, habitat degradation (human activities), pollution and other threats, Agricultural activities chemical drifts from spraying and synthetic insecticides (Muli *et al.*, 2014)

The increased demand for food production in developing countries has led to increased crop production and subsequently a greater use of pesticide and agro-chemicals and this in combination with other factors (parasites or pathogens) will negatively impact on the behavior and physiology of honey bees that can directly

influence the overall health of colony (Smith, Loh, Rostal, Torrelio, Mendiola, Daszak, 2013), however there are limited studies on the impact of pesticides on African honey bees, though exposure to pesticides (Imidacloprid) has shown increased lever of trachea mites in bees (Pettis, Vanengelsdorp, Johnson and Dively, 2012).

2.4 Honey bee *(Apis mellifera)* parasites

Numerous parasites (mites and insects) prey upon the honey bees (Pettis *et al.,* 2012). The honey bees health has a great impact on economy and biodiversity worldwide. A large diversity of microorganisms are associated with honey bees, some are commensal, while others are disease causing organisms (Godifey, 2015).

The hive is a suitable habitat for diverse pests (insect and mites) including non-parasitic omnivorous and pollen feeding species and parasites. The mites that parasitize honey bees have become a global problem threatening the survival of managed and feral honey bees. There are many mites species associated with honey bees but the major ones include *Varroa jacobsoni* now known as *Varroa destructor*, *Acaarapis woodi*i and *Tropilaelaps clamae* (Sammataro *et al.,* 2000). Insects pests of honey bees include small hive beetles (*Aethina tumida*) large hive beetles (Oplostomus fulgineus, Oplostomus haroldii), Bee louse (Braula spp) Wax moth (*Galleria mellonella* and *Achnola grisella*) (Whitfield *et al.,* 2006)

2.4.1 Varroa mites

V.destructor belongs to the genus Varroa which in currently represented by at least four species i.e *Varoa jacobsoni Oudemans* which parasitize on Eastern honey bee *Apis cerena*, *Varroa underwoodii*, which parasitize *A. cerena*, *Varroa rinndereri*

parasitizing *A..koschevnikovi*, and *Varroa destractor* which was erroneously classified as *V.jacobsoni* until it turned out to be a separate species (Anderson and Trueman, 2000).

2.4.2 Taxonomy and geographical distribution of *V.destructor*

The mite which is responsible for the clinical symptoms of "varroosis" in *Apis mellifera* belongs to the species *V. destructor* which was erroneously identified as *Varroa jacobsoni* until the year 2000 (Anderson and Trueman, 2000). The genus varroa has at least four species that are of importance in apiculture. *Varroa jacobsoni oudemans* was known as an ectoparasite mite of the Eastern honey bee *A.cerena* in java with a wide distribution on bees throughout Asia and roci *Apis migrocita* in Indonesia (Anderson and Trueman, 2000). The other species *V. underwoodi* parasitizing on *A. cerena* was found in Napel, *V. renderer* was found from *A. koscheviikovi* in Bomeo and finally *V. destructor* which was found in *A.cerena* (original host) and *A.mellifera* (new host) Initially classified erroneously as *V. jacobsoni* (Anderson and Trueman, 2000), with the capacity of *V.destructor* to transfer to another host, other continent have not been spared either, the parasite has been reported in Middle East, Indian sub-continent, America and Europe (Ellis and Munn, 2005). In addition, African continent has also reported incidence of *V. destructor,* in a review by Ellis and Munn 2005, many African countries in North of the Sahara and part of Sub-Sahara have reported presence of *V. destructor*. In East Africa, the mite was just discovered recently (Fazier *et al.*, 2010).

In Kenya, the *V.destructor* was discovered in 2009, following a survey conducted through the Ministry of Livestock due to reported decline of colonized hives *(Fazier*

et al., 2010) In view of the above it's clear that *Varroa destructor* has already acquired a worldwide distribution. (Figure 2.1).

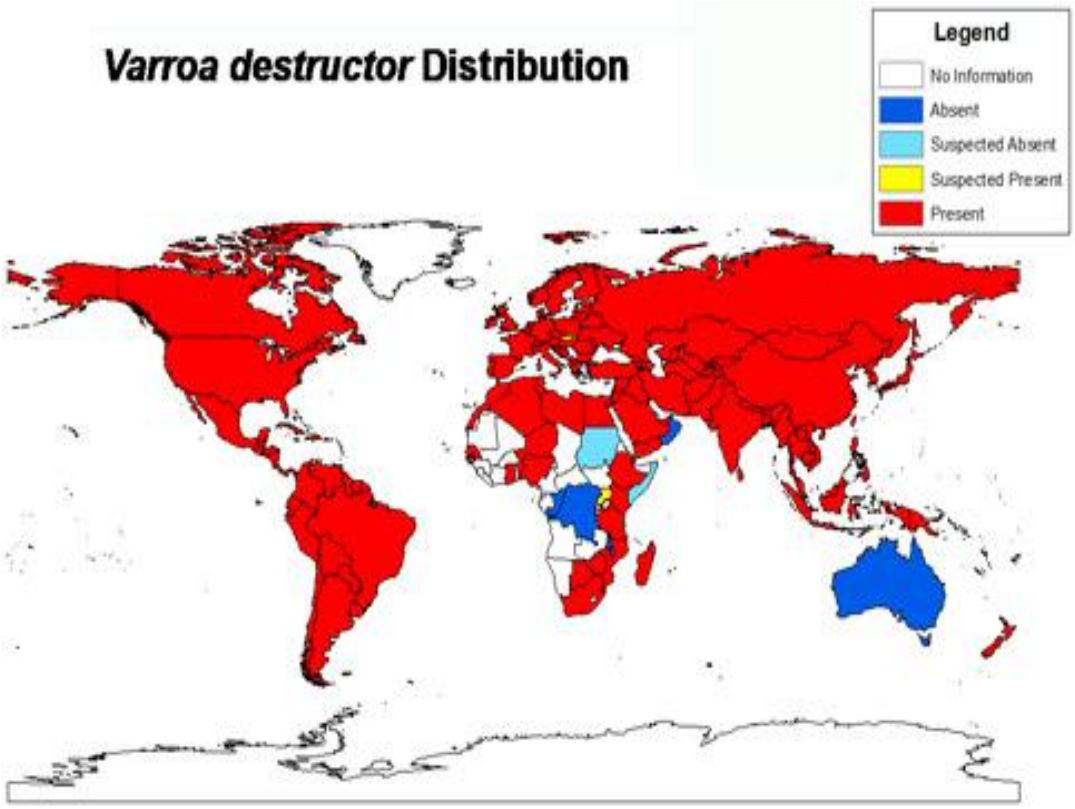

Figure 2. 1: Global distribution of *Varroa destructor*

(Adapted from Ellis and Munn, 2005)

2.4.3 Morphology, Biology and Behavior of *V. destructor*

1. Morphology

The Varroa mite is an external parasite that is visible to naked eyes. The female mite is brown to reddish-brown in color, measuring 1.1 to 1.2 mm in length and 0.7mm to 1.6mm in width (about the size of pinhead), males are smaller about 0.7mm by 0.7mm and light tan in colour (Rosenkranz *et al.*, 2010). The morphology of the mite is an

adaptation to their host (Figure 2. 2). A common feature of male and female mite is the division of the body into two well-defined parts, the idiosoma and gnathosoma, the female mites have a flattened, ellipsoidal idiosoma with greater width than length. The legs of the female are short and show specialized structure the opoteles, for attachment to the host (Samatoro et al., 2000).

Figure 2. 2: Adult female *Varroa destructor* Anderson and Trueman, anterior view, showing curvature of body

(Adapted from Samatoro, 2000)

The dorsal and ventral shields are highly sclerotized and show reddish brown colouration thin and flexible membrane between the shields enables mites to dilate during feeding and eggs formation. The males body is pear shaped and show only weak sclerotization , which is mainly present in legs and dorsal shield, the males are

smaller than female in all development stages with legs longer in relation to the body size that the legs of females (Rosenkranz *et al.,* 2010).

2. Lifecycle of *V. destructor*

Studies has shown that the life-cycle of *V. destructor* is closely linked to that honeybee, female *V. destructor* has two distinct phases aphoretic phase on adult bee and reproductive phase within the sealed drone and worker brood. The mites are often found on adult bees which allow for dispersal and serves as short term host. The mite prefers young bees to older workers, probably because of the lower titer of the nasonov gland pheromone geraniol, which repels the mites (Rosenkranz *et al., 2010*). Normally the mite pierces the soft intersegment tissue of the bee abdomen or behind the bees head, and feeds on the heamolymph. When on actively reproducing bee colony the mites invade the brood cells with third-stage bee larvae, preferably drone larvae but not limited to workers cells due to high supply of fatty acid esters, in drone cells (Calderone and Kuenen, 2003), one to two day prior to capping the varroa mites enter the pre-pupal cells hiding from the nurse bees by submerging in the remaining liquid brood food, lying upside down. With its modified peritrems protrude out of the fluid surface, allowing for respiration the female remains concealed until the brood cells is capped. To keep from becoming trapped, the female attached to the bee larva as it spins its cocoon. Upon formation of the pre pupa, the mites begins to feed at a site located on the pre-pupas fifth abdominal segment 60 hours after the cell is sealed the mite starts laying eggs. The first laid egg is usually a haploid male, the subsequent female eggs are laid at 30 hours intervals with up to five eggs in worker brood cells and up to six eggs in drone brood cell, Consequently molting into different instars pharate larvae, mobile proto –nymph, pharate deuto- nymph, mobile deuto-nymph, pharate adult and adult (Rosenkranz *et al.,* 2010).

The mother mite creates a hole in the cuticle of the pupa for nymphs to feed through, located near the “fecal acculation site” the mites attains sexual maturity immediately after the last molt the males mature 20 hours before the female mites since mating occurs within the brood cells the males will start mating as soon as the female arrives (Rosenkranz *et al.*, 2010), The male life cycle completes in the blood cells following its death and female emerges to attach to adult honey bees (Figure 2.3).

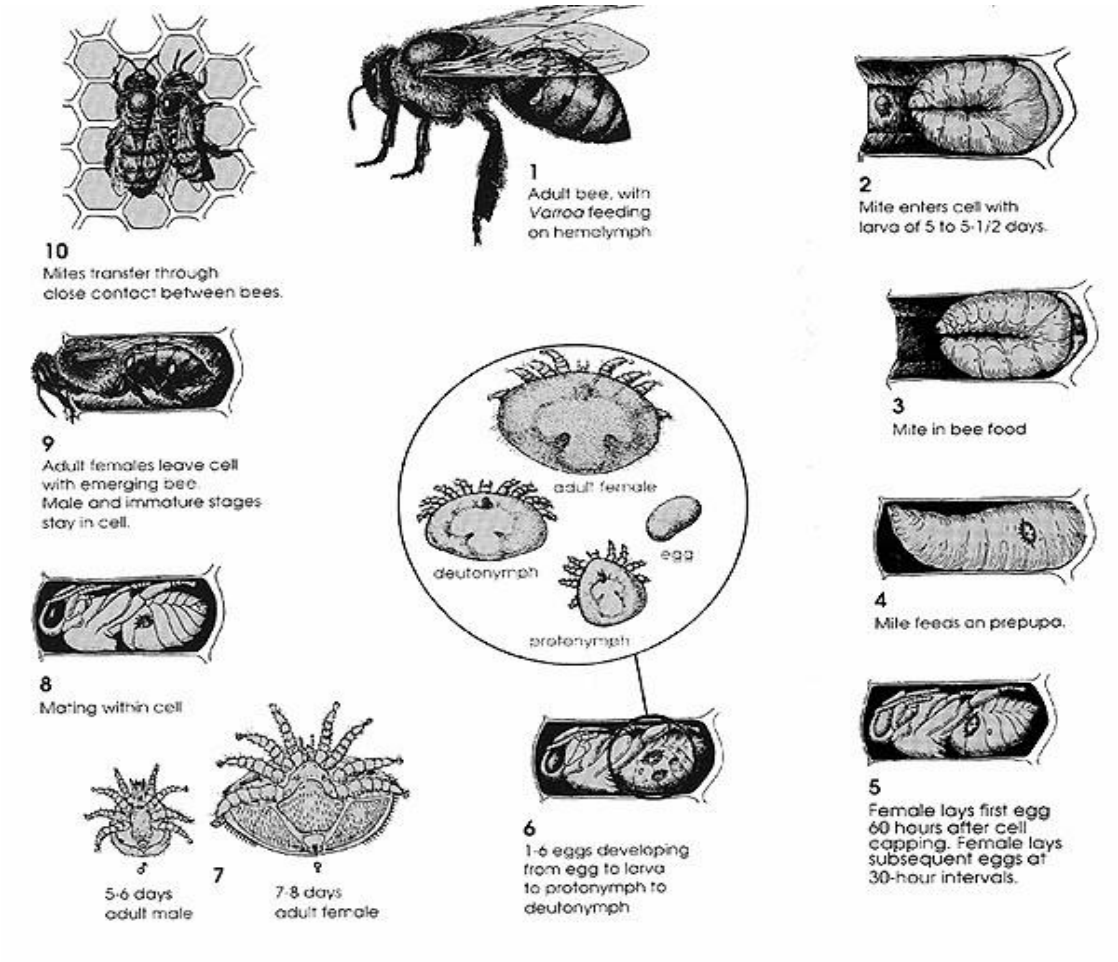

Figure 2. 3: Life cycle of *Varroa destructor*

(Adapted from Sammataro *et al.*, 2000)

3. Reproductive rate of *Varroa destructor*

The reproductive rate of *V.destructor* is determined by a number of factors including mite fertility and fecundity under natural conditions laboratory investigations has shown that Varroa female can perform 7 reproductive cycles, however in natural conditions it can have 2-3 reproductive cycle (Rosenkranz *et al.,* 2010).

In the original host (*A. cerena*) the reproduction of *V. destructor* is limited to drone brood cells this limitation is an important point for the balanced host-parasite relationship in *A .cerena*, however, the reasons are not quiet known, (Anderson, 2000). In the new host (*A. mellifera*) the reproduction can be in both drone and worker brood cells, the variation in the reproduction of varroa mites is partly due to species or sub-species of honey bee and climatic conditions (Navajas *et al.,* 2008)

2.4.4 Factors affecting the population level of Varroa destructor on honey bee colonies

1. Environmental factors

Varroa mites hives at a temperature corresponding to that of the host (honey bee) which is approximately 34 ^{0}C-35 ^{0}C; Laboratory investigation shows that *V. destructor* prefers temperature of approximately 32^{0}C ± 2.9 ^{0}C and temperature preference differ between winter and young summer mites, the mite can discriminate a difference in temperature as low as approximately 1^{o}c (Nazzi and Le Conte, 2016). Studies have shown that temperatures can affect the mite physiology. In an experiment carried out under laboratory conditions mites reproduced at 34.5 ^{0}C whereas not offspring were observed at 31.5 ^{0}C on the other hand when sections of parasitized brood combs were reared at different temperatures the highest

reproductive rate was observed between 32 ^{0} C and 33.4 ^{0} C (Nazzi and Le Conte, 2016). Hygrometric conditions also play an important roles with optimum humidity for reproduction ranging from 55% to 70% and only limited reproduction taking place at higher humidity in sum optimal humidity and temperature values for Varroa mites march quite well with those found within the hive, although temperature in the brood nest can vary from 30.5^{0} C to 35.5^{0}C and humidity is more variable than is usually thought (Nazzi and Le Conte, 2016).

2. Host-Parasite factor

The population growth in a honey bee colony is highly variable and depends on traits of the host and parasite that may influence its reproduction rate and mortality of the mite. Features of the host that influence population growth of the mite includes presence of drone and worker brood cells, swarming level of defense behavior, hygienic behavior among others. In addition, there is an interaction of these factors with climate and environment and nectar flow (Nazzi and Le Conte, 2016). The most significant features being amount of brood and or fertility of the mite, however some host (*A.cerena*) limits the mite to drone brood cells with no access to worker brood cells (Medina-Flores, Guzmn-Novoa, Hamiduzzaman, Archiga-Flores, and Lpez-Carlos, 2014).

3. Host factors

The population of host (honey bee) will determine the strength of bee colony and therefore will significantly influence the population dynamic of Varroa population. The amount of brood throughout the season, the temporal pattern of brood availability, the percentage of drone brood, swarming /migration absconding and brood free period during winter or dry season have an impact on the reproduction of

Varroa mites (DeGrandi-Hoffman, Curry and Acarol, 2004), it is important to note that it is impossible to quantify the multifactorial relationship of the parameters (Fries, Hansen, Imdorf, and Rosenkranz, 2003). Research has shown that the mite per colony population tend to be high in strong colonies (high brood level) than weak colonies (Muli *et al.*, 2014).

4. The geographical location of an area will influence the population growth of Varroa mites due to climatic factors or floral resources (Chemurot *et al.*, 2016), however, there is no sufficient information to support the association between nutrition, climatic and varroa mite load in honey bees (Muli *et al.,* 2014). Different geographical location will have different elevation, vegetation and seasons, these in combination will influence the varroa mite population, (Akinwande *et al.,* (2013) reported a high varroa mite load in the tropics (Nigeria) due to availability of brood cells throughout the year.

2.4.5 Spread of Varroa mites

After the World War II, there was an increase in International travel and commerce, the transportation of bees *A. mellifera* from Europe and Africa-Native area to other areas of the world lead to introduction and establishment of varroa mites in those new areas, thus facilitating the worldwide dispersal of varroa mites (De Jong, De Jong, and Goncalves, 1982). Upon establishment, the varroa mites dispersed by drifting, robbing activities of bees and swarming (migration of feral bees and have even reported on wasps) (Gloria, DeGrandi-Hoffman., Fabian, Ahumada., 2017).

2.5 Detection and Estimation of *varroa destructor* infestations in honeybees colonies

Accurate sampling methods are required to estimate the population size of various mite in honey bee colony. There are several methods used to sample varroa mites, however, the purpose of collection determine the appropriate method. (Branco *et al.,* 2006; Dietemann *et al.,* 2013).The method of varroa detection is by estimating the mite population by sampling immature (drone and worker brood cells) and adult bees, (Dietemann *et al.,* 2013). Estimation of varroa mite on adult bees and brood is the commonly used method, this provides relative estimation of mite population but this method can be used to obtain absolute population estimates if the total number of adult bees is known, the method can be made reliable by including brood sample in adult bee sample (Dietemann *et al.,* 2013).

The detection of various mite is done using several techniques: 1) Ether wash/Ether roll, 2) alcohol wash, 3) Inert dust roll, 4) Brood examination, 5) Acaricide with sticky boards 6) Examination of hive debris for mites (Dietemann *et al.,* 2013).

One of the most reliable techniques used to estimate *V. destructor* is to kill the mite in a colony using acaricides, then dead mites are collected on floorboard trap and counted. Theoretically, this method gives an absolute population but in reality is not accurate because no chemical guarantee 100% kill (Branco *et al.,* 2006). More so, the mites might develop resistance and the chemical might be toxic to bees or introduce residues to honey (Annand, 2006). In alcohol and Ether wash techniques, 300-350 adult bees are collected in a wide mouth bottle with #8 mesh screen. The bottle is half filled with alcohol or Ethyl, then gently and slowly inverted to empty the alcohol into a glass dish, leaving the mites in the jar. This method dislodge 90-95% of the mites

from the adult bees but it is not bee-friendly as the bees will die it is environmental unfriendly, expensive and destructive (Dietemann *et al.,* 2013).

V. destructor mite population can be estimated by collecting the debris over a set time frame and counting the mites in the debris, debris from uncapping cells and general hive cleaning drops to the bottom of colonies and collect on the bottom board. Grooming by bees also causes phoretic mites to dislodge and fall (Dietemann *et al.,* 2013). This technique can be combined with an acaricide with sticky board so as to kill the mite and prevent the mite from crawling back to the bees. Despite its effectiveness, the technique time consuming (Branco *et al.,* 2006).

The other technique that is preferred and highly used is the sugar roll or sugar shaking technique. This method can be performed in apiary and does not require killing of bees it is practical, low cost, non-destructive and environmental friendly (Macedo and Ellis, 2002). The technique can use different dust (powder sugar, fine sugar, wheat flour, talcum powder, corn starch and baking sugar) to dislodge the mites in jar or colony (Dietemann *et al.,* 2013). A wide jar or container with #8 or 2 mm hardware mesh that can take approximately 300 bee, 7 grams (table spoon) of powdered sugar is added through the mesh. The container is rolled to cover all the bees with sugar then it is shaken to release the mites with the powdered sugar. This technique is fast and allows for several hundreds of mites to be collected it is also practical, low cost, non-destructive and environmental friendly. Research conducted to determine the preferred dust showed a high mite recovery rate. 92.9 ± 5.5% obtained with powdered sugar and talcum powder 84.0 ± 5.6% (Macedo and Ellis, 2002). The results of sugar roll can be easily integrated, in the Middle West during spring and fall. In fall, colonies with more than 0.12 mites per bee when brood is not present will have

increased winter mortality if treatment is not initiated while colonies with more than 0.25 mites per adult bee will almost perish in winter. When brood is present in fall, 0.03 or more mites per bee indicate that treatment to reduce various mite should be done immediately (Macedo *at el.*, 2001).

Brood examination can be done by cutting 5 x 5 cm brood comb areas from drone and/or worker broods. About 100 brood cell are removed from their cells using forceps and checked for the presence of various mites on the worker and/or drone cells (Dietemann *et al.,* 2013).

2.6 Effects of V. destructor on honey bee colonies

The ectoparasite mite *V. destructor* is a serious worldwide pest of the honey bee *A. mellifera* (Rosenkranz *et al.,* 2010) and has been linked with the death of millions of colonies. For the last few decades, there has been mysterious die-offs of honey bee *(Apis mellifera)* colonies that have occurred in many countries around the world. The phenomenon has been named colony collapse disorder (CCD) (Neumann and Carreck, 2010). Many factors are suspected to be involved, either alone or in combination. Parasites and pathogens are considered as principal actors in particular the ectoparasite mite *V. destructor* and associated viruses (McMenamin and Genersch, 2015). Most of the pathogens and parasites affecting global honey bee colony health are present in Africa (Mumoki et al., 2014). *V. destructor* has been reported in many African countries; i.e. South Africa, Tanzania, Angola, Egypt, Morocco, Senegal, Niger, Nigeria, Uganda and Kenya (Ellis and Munn, 2005; Mumoki *et al.*, 2014).

2.6.1 Effects at the individual level.

The pathology of *V. destructor* is determined by the feeding activity of the mite (i.e. injuring the cuticle of pupae and adult, sucking substantial amounts of heamolymph) and vectored viruses. The individual bee is damaged in a variety of ways, with the developing larvae and pupae clearly representing the most sensitive host states. First, the loss of heamolymph during the honey bee pupae development significantly reduce the size and the weight of the hatching bee (Duay, De Jong, and Engels, 2003). The weight loss depends on the number of mother mites and amount of mite reproduction but even a simple infestation result in an average loss of body weight of 7% for hatching bee (De Jong *et al.,* 1982).

For drones, Duay *et al.,* (2003) has demonstrated an 11-19% body weight loss depending on infestation rate. Such a reduced weight leads to decreased flight performance and sperm production. Worker bees which are parasitized during their development, start earlier with foraging and have a significantly reduced life span (De Jong *et al.,* 1982). Parasitized foragers showed a reduced capability of non-associative learning, and their orientation and homing ability was impaired, i.e. infested bees needed longer time to return or even did not return at all to the colony (Kralj, Brockmann, Fuchs, and Tautz, 2007).

Several studies have revealed that mite infestation during pupal development might also have an effect on the immune capacity of the parasitized pupae and even on the adult bees. Therefore, presence *V. destructor* in bees have impact on the bee immune response and thereby, most likely on the susceptibility of honey bee towards various pathogens, though there is no clear understanding on the interplay between the parasite and their host immune system (Gregorc, Evans, Scharf, and Ellis, 2012).

2.6.2 Effects of colony level.

The reproductive capacity, and therefore, the fitness of a varroa infested honey bee colony as a "supeorganism" is reduced in two ways, even if the infestation is at moderate levels in drones which have been parasitized during their development have a significant lower chance to mate and infested colonies produce less swarms (Duay *et al.,* 2003).

From the beekeeping point of view, there exist certain threshold for economic damage and irreversible colony damage. At low infestation rates, clinical symptoms are not visible, and the infestation often remains undetected. Moderate infestation rate may reduce the growth of the honey bee population and therefore, the honey yield, but clinical symptoms may still not be evident. However, the steps to irreversible colony damage are small, especially if during fall, the mite population still increases while the host population is decreasing (Fries *et al.,* 2003). The final breakdown of the colony is associated with the typical "parasite mite syndrome" such as scattered broad, crawling or even crippled bee. The damage threshold is not correlated with fixed number of the bee and broad population and the presence of bee viruses. Studies done in Germany under experimental condition reported that infestation rate during winter of more than 7% could lead to colony collapse (Rosenkranz *et al.,* 2010). Delaplane and Hood, (1999) reported a significantly higher economic threshold for the Southern USA of 3000-4000 mites per colony, compared to Fries *et al.,* (2003) found that untreated colonies which exceeded an infestation rate about 30% in adult bee during the summer do not have a chance to survive the following winter.

2.6.3 *Varroa destructor* and honey bee viruses

In addition to the direct effects of *V. destructor* on performance and health of individual bee there are also indirect effects caused by viruses' vectored through the mite. *V. destructor* vectors various honey bee viruses, to date, about 18-23 different viruses have been isolated from honey bee (McMenamin and Genersch, 2015), and many of them can be vectored by varroa mites. This has been proven for Kashmir Bee Virus (KBV) Sac-Brood Virus (SBV), Acute Bee Paralysis (AVPV), Israeli Acute Paralysis virus (IAPV) and Deformed Wing Viruses (DWV) (Boecking and Genersch, 2008). Initially these viruses were considered a minor problem to honey bee until the arrival of *V. destructor* mite that changed the whole picture (Bowen-Walker, Martin, and Gunn, 1999).

There is a general consensus that the mites association with a range of honey bee RNA viruses is a contributing factor in the global collapse of honey bee colonies (Mumoki *et al.,* 2014) because the spread of the mite has facilitated the spread of virus by acting as a viral reservoir and incubator (McMenamin and Genersch, 2015).

Soon after introduction of *V. destructor* in the *A. mellifera* population of Western world, emerging bees with deformed or atrophied wings were increasingly observed, as the occurrence of these deformed wings were clearly related to mite infestation in the developing pupae. These deformities were first considered a consequence of the heamolymph deprivation by the parasitizing mite (De Jong *et al.,* 1982). Deformed Wing Virus (DWV) is rather benign virus mainly causing covert, symptomless infection (Highfield *et al.,* 2009). DWV is transmitted vertically (through drones and queens) or horizontally (through larvae food). Vertical transmission of DWV to pupae through *V. destructor* is a pre-requisite for manifestation of overt DWV infections

characterized by deformed wings, shortened and bloated abdomen and muscolouring (Yang and Cox-Foster, 2007). Hence, overt infection induced by the mite acting virus can cause considerable damage to colonies. The extent of damage is related to the proportion of overtly DWV-infected and, hence, non-viable bees. In addition, recent studies have shown that replication of the virus in the mites prior to transmission and a higher enough DWV titer in the mites are necessary preconditions for the induction of an overt DWV infection in the developing bee (Martin *et al.*, 2012). These results indicate that *V. destructor* can act not only as mechanical but also a biological vector for DWV and it is the latter function which is related to overt DWV infections. Therefore the more the mite in a colony transmitting the virus and the more of these mites support replication of the virus prior to transmission, the higher the chance that developing pupae will develop a fatal DWV infection and that the colony will eventually collapse (Pirk *et al.,* 2015). Recent studies suggested that *V. destructor* might actively contribute to the activation of endogenous DWV infection by immunosuppressing the host (Navajas *et al.*, 2008).

Association between *V. destructor* and DWV can be considered an emerging viral disease of honey bee with detrimental effects not only for individual bees but also for entire colonies (Wilfert *et al.*, 2016). For instance, a 4-year monitoring of about 1150 honey bee colonies in Germany revealed a significant correlation of colony winter loss with (i) Varroa infestation and (ii) with the prevalence of DWV (Genersch *et al.*, 2010). In Hawaii, Martin *et al.,* (2012) reported an increase prevalence of a single viral species (DWV) from 10-100% which was related to introduction of *V. destructor,* in addition to million fold increase in viral titer and a massive reduction in DWV diversity.

Acute Bee Paralysis virus (ABPV) causes acute bee paralysis and affects both brood and adult honeybees in a colony. Acute bee paralysis disease is present on all continents except Australia (de Miranda, Cordoni, and Budge, 2010). In Africa, it has been reported in South Africa (Allsopp, 2004) and in East Africa (Muli *et al.,* 2014).

ABPV is closely related to two other bee viruses namely Israel Acute Paralysis Virus (IAPV) and Kashmir Bee Virus (KBV) and all the three are sometimes considered to be a complex of related viral species (Yan Ping Chen and Siede, 2007). The virus is spread among the honey bees via salivary glands secretions exchange during trophillaxic feeding between workers, brood and queen. Recent studies have shown that the mite *V. destructor* can vector APBV (de Miranda *et al.,* 2010), supposedly acting as an activator for the virus as well. The apparent harmlessness of ABPV infection dramatically changed with the advent of *V. destructor*. Considering the extreme virulence of ABPV when injected into the bee heamolymph, it is not surprising that the virus started to cause problems when *V. destructor* entered the stage and became established as ABPV vector and began to inject the viruses into pupae and adult bees, (Gauthier *et al.,* 2007).

Varroa destructor virus I (VDV-I) is closely related to DWV and it has been suggested that VDV-1 can cause wing deformity in honey bees. This virus affects larvae, pupae and adult and transmission occurs through various pathways (Levin, Sela, and Chejanovsky, 2016).The presence of the virus in Africa is of concern due to its known association with *V. destructor.*

Kashmir bee virus (KBV) is found nearly everywhere bees are found, the route of transmission is suggested to be trans-ovarian transmission with presence of KBV and Sac-brood Bee Virus (SBV) in both queen and eggs. Additionally, horizontal

transmission can occur among adult bees and from adults, workers to larvae through contaminated food sources (brood food, honey, pollen and royal jelly). Furthermore, it was demonstrated that mites are another route of horizontal transmission, as both viruses were detected in mites and their saliva (Shen, Cui, Ostiguy, and Cox-Foster, 2005).

Sac-brood disease belongs to Iflavirus, the disease easily detected in the field due to the characteristic sac-like appearance of diseased larvae. Infected larvae do not pupate, as the outer larvae skin sags, becomes sac-like and later accumulate fluids (FAO, 2006), possible routes of transmission of this virus include horizontal spread via vectoring by *V. destructor* and *Aethna tumida* (Eyer *et al.,* 2009) and spread from foragers to other colony nest mates through shared food resources such as pollen, honey and royal jelly.

Black queen cell disease is caused by the Black Queen Virus. It was first isolated from field collected samples of queen larvae and pre-pupae found in darkness rearing cells. The virus mostly affects the pre-adult stage of honeybee's development with early stages of infection similar to those of sac-brood disease, however it has been detected in adults and workers pupae (Yan Ping Chen and Siede, 2007).

Israel acute paralysis disease is caused by Israel Acute Paralysis Virus (IAPV). It was recently identified when homogenate of a single dead bee collected in the course of studies related to several colony mortality in Israel was involved into healthy-looking bee larvae (Maori *et al.,* 2007). IAPV, KBV and ABPV form a complex of genetically and biologically related viruses (de Miranda, 2010). IAPV is extremely virulent when injected into pupae or adults bees (Pirk *et al.,* 2015). Hence, it can be assumed that *V. destructor* plays a role in the virulence of IAPV as it does for DWV and ABPV. So

far, little is known about the transmission and pathomechanism of IAPV as it came into focus of bee virologist only quite recently in the context of colony collapse disorder (VanEngelsdorp, Caron, Hayes, Underwood and Henson, 2012). The potential virulence of IAPV for bees and colons is unquestionable as it has been identified as a marker or secondary agent of CCD (Cox-Foster *et al.*, 2007).

Pathogens, parasites and the negative effects thereof on honey bee population remain an issue of public concern and the subject of active research. Africa with its high genetic diversity of honey bee sub-species is also exposed to various factors responsible for colony losses in other parts of the world. Apart from the current American foulbrood epidemic in the Western Cape of South African, (Mumoki *et al.*, 2014), no large-scale colony losses have been reported elsewhere on the continent (Pirk *et al.,* 2015). However, the introduction of honey bee viruses in Africa poses a threat to the honeybee race, therefore there is need to take precautionary measures to protect and conserve them and their habitats.

Most of the pathogens and parasites affecting global honey bee colony health are present throughout Africa. However, African bees appear to tolerate the mites more effectively than European bees (Mumoki *et al*., 2014). Despite this, African honey bees are still at threat due to the introduction of honey bee viruses and other diseases (Pirk *et al.,* 2015).

2.7 Occurrence of V. destructor in Kenya

V. destructor can be found worldwide whenever *A. mellifera* colonies are kept, and it's hardly possible to find a mite-free colony any longer. The only possible exception is Australia, which still consider *V. destructor* an exotic bee mite since it has not been established (Ellis and Munn, 2005).

Recent studies have shown that *V. destructor* is present in parts of Kenya. In survey conducted in seven location of Central and Eastern Kenya to determine presence or absence of varroa mite using the sugar roll method to dislodge mites from adult bees in 38 different colonies, the results showed that varroa mite was present in all the 38 (100%) colonies. In a further similar survey in Eastern, Western and Coastal Region of Kenya showed varied prevalence. This was the first time that *V. destructor* was discovered and reported in Kenya (Fazier *et al.,* 2010).

In another survey, the presence of varroa mite (*V.destructor*) was assessed using a standard sugar roll assay, using approximately 350 bees, out of the 19 apiaries distributed (Coastal, Western, Eastern and Central), 17 (89.5%) were infested. The only apiaries that did not have varroa mites were in North-Eastern Kenya. In a total of 66 colonies assessed for varroa mites and 55 (83%) were infested. The level of varroa was highly varied across colonies and apiaries (Muli *et al.,* 2014). Despite the presence of *V. destructor* in Kenya, the honey bee colonies did not seem to be affected (Muli *et al.,* 2014). However, the discovery of honey bee viruses that are vectored by *V. destructor* in the country coupled with climate changes could change things. Bearing in mind that varroa mite was just discovered in Kenya recently in 2009, there may be time lag before newly introduced parasite and pathogens cause substantial negative effects (Fazier *et al.,* 2010). Continuous monitoring of these population should be conducted to evaluate the long-term dynamic of these host-pathogen interaction.

2.8 Control and Management of Varroa destructor

(i) Chemical Treatment

Chemicals such as checkmite, Amitraz, Apitol etc. are fed or applied via fumigation, trickling or permanent contact in impregnated plastic strips. They are acting systemically or via contact. The substance mostly lipophilic (except cymiazole) and persistent with high risk to create residue in bee products (especially non polar substance which are applied in strips), thus boosting resistance in mites (Rosenkranz *et al.*, 2010).

(ii) Organic acids and essential oils

Organic acids and essential oils, namely, formic acid, oxalic acid, lactic acids and thymol represents the frame of natural compounds used for the control of varroasis. Numerous studies have been conducted regarding the detail of application under different climate and bee keeping conditions, i.e. concentration, time and number of treatment, the methods of application include ; powdering, feeding, evaporating, fumigating, trickling or spraying (Rosenkranz *et al.*, 2010) The advantage of using organic acids and essential oil is that it able to kill mites inside brood, low risks of residues and accumulation in bee products and low probability of eliciting resistance (Floris, Satta, Cabras, Garau and Angioni, 2004).

(iii)Biotechnical intervention

The most widely practical biotechnical intervention is drone brood removal. It is based mainly on two facts; (i) drone brood is approximately 10-12 times more attractive to female varroa mite than worker brood (Rosenkranz *et al., 2010*), (ii) Its

development is longer than that of workers allowing production of more female mite per cell. If the removal of the trap comb is timed correctly, mite numbers can be kept low by this approach (Calis, Boot and Beetsma, 1999).

The selective breeding of various tolerant bees is considered to be the only long-term solution to the varroa problem e.g. the introduction of the "Russian (Primorski) bee" about 10 years ago and the subsequent selection with multifunctional approach of 18 tolerant strains (De Guzman *et al.*, 2007). Various reports have confirmed at least a partial tolerance of these breeding line expressed by a significantly lower increase of varroa mite population (Ward, Danka and Ward, 2008).

2.9 Associated honey bee pests

2.9.1 Coleoptera (beetle)

Small hive beetles (SHB) (*Aethina tunida)* is limited to Sub-Sahara Africa where they are considered to be a negligible pest. (Cuthbertson *et al.*, 2013). However, infestation with SHB is listed by the OIE as a notifiable infestation. Adult beetles and larvae reside within the honeybee colony and cause damage to brood, pollen and honey which they feed upon (Ellis and Hepburn, 2006), in North Africa the introduction of SHB into Egypt did not have significant negative impact and SHB population remained low or absent (Rosenkranz *et al.*, 2010). Devastating effects have been reported in countries outside Africa (Australia and USA) where the beetles have been introduced (Neumann and Pirk, 2004). From an African perspective, the beetle does not seem to pose significant threat to bee keeping. However, the fact that they act as vectors of honeybee viruses and bacteria should be of concern (Schäfer *et al.*, 2009). A minor infestation is difficult to recognize because the beetles immediately hide in the dark and the most notable sign is the beetle larvae.

Economic damages occurs when adult beetles defecates in the honey introducing yeast that cause the honey to ferment and run out of the cell. In this case, the queen may cease laying and the entire colony may abscond. Weak colonies are particularly vulnerable to attack, but even strong colonies can be overwhelmed by large populations of beetle (Hood, 2004). Considering its ability to fly, the beetle can effectively transmit viral honeybee disease in different colonies and apiaries.

2.9.2 Lepidoptera (moth)

Wax *Moth (Galleria mellonella* and *Achrola Grisella) Gallezia mellanella* (greater wax moth) and *Archrola grisella* (lesser wax moth) are common in honeybee colonies throughout Africa. With greater wax moth being more prevalent and damaging (Pirk *et al.,* 2015). The wax moths were first reported in honey bee colonies of Asian honeybee (*A.cerena)* but later spread to Northern Africa, Great Britain, parts of Europe, North America and New Zealand (Calkins and Faust, 2003). Currently the moth is ubiquitously distributed everywhere beekeeping is practiced (Pirk *et al.,* 2015). Even though there are some religion currently free of the wax moth, a recent case study in Kenya using futuristic scenario models has predicted the potential distribution of honeybee pest including the wax moth in ecological zone currently considered unsuitable for the pest (Makori *et al.,* 2017).

Wax moth occurs naturally or has been introduced by man in almost all region of the world, both the lesser wax moth and greater wax moth are serious destructive pests causing considerable damage to both normal and abandoned combs of bees and brings considerable loss to bee keeping industry (Kwadha, Ong'amo, Ndegwa, Suresh and Ayuka, 2017). The greater wax moth causes the heaviest loss to beekeepers throughout the world. However, the lesser wax moth is generally more common, and

can also cause significant damage. Wax moth larvae are considered to be “hive cleaners” since they consume all the remaining combs and stores once the honeybee have absconded (Kwadha *et al.,* 2017). In addition to the physical damage caused, the larvae feeds on pollen, honey, wax, cast-off honey bees pupae skin and brood creates tunnels in combs and leave masses of webs on the frame (Kwadha *et al.,* 2017). Damage occurs as the wax moth larva creates silk-lined tunnels through the hexagonal cell call over the comb surface, the tunnels and borings made on the cell caps make holes through which honey leaks out. The silken thread entangles emergent bees, which as a result, die of starvation. Phenomena described as galleriasis (Neumann *et al.,* 2013), Moreover, large scale infestation of colonies by larvae of greater wax moth often lead to colony loss, absconding and reduction in size of migratory bee swarms (Kebede, 2015). Wax moth damage is more pronounced in weak or stressed colonies therefore, good management practice are essential to minimize damage and colony losses (Kwadha *et al.,* 2017)

The lesser and greater wax moth have been earmarked as potential vectors of pathogen (Pirk *et al.,* 2015). For instance, fecal pellets of the larvae were found to contain spores of *paenibacillus* (Pirk *et al.,* 2015). Recently, Israel Acute Paralysis Virus (IAPV) and Black queen cell virus (BQCV) have been detected in larvae of moths (Traiyasut *et al.,* 2016)

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site

The study was conducted in Uasin Gishu county, Kenya, located in the formerly Rift valley province. Located on a plateau and has a cool and temperate climate. The study area has six sub counties namely Kapsaret, Moiben, Turbo, Ainabkoi, Soy and Kesses. With a human population of 894,179 according to 2009 national census. The main economic activity includes livestock keeping (indigenous and exotic), beekeeping, wheat and maize farming. Its headquarters are in Eldoret 330km to the North Kenya's capital Nairobi at an altitude of 2,085 m above sea level, between 34^0 55'33''W and 36^0 38'58"E and between 00° 0^0 2' 44" S and 0^o 55'56'' N, with a mean annual rainfall ranging between 1100 and 1500 mm with two peak in May and October and a dry spell in January to March and cool dry season in July to September (Gok, 2002), the mean annual temperature is 23^0c (Mulei, Otieno, and Onkware, 2014).

All the Sub-counties (Soy, Moiben, Kesses, Kapsaret, Turbo and Ainabkoi) in Uasin-Gishu were selected for the survey, (**Figure 3.1**).The global positioning for the sampled apiaries were: Merewet, N. 00:713820, 035.319980.Torochmoi, N. 00.725050, 035.341940. Chebulet, N. 00.616380, 035.416480. Rurigi, N. 00.6163800, 035.4164800.Ndugulu, N. 00.199030, 035.391910. Mois Bridge, N. 00.1989900, 035.391820. Soy/Chemoset, N. 00.711380, 035.197320. Jua kali, N. 00.607740, 035.153590 and Kapsaret, N. 00.431990, 035.200050.

Figure 3. 1: Map showing the study area and GPS referenced points showing the sampled areas

3.2 Study Design

Convenience and purposive samplings was used to select the studied apiaries, two apiaries were surveyed in every sub county. However, in most of the Sub-County only one apiary was sampled due to acceptability, nature of hives and safety of humans and animals near the apiary. A maximum of five beehives (colonies) were randomly selected and examined for Varroa mite. All the locations were monitored, geographically located and referenced for their position and altitude using Global Positioning System (GPS)

3.3 Sampling design

3.3.1. Interviews

Beekeeper/owners of the apiaries were interviewed by use of checklist of questions on different aspects such as number of hives per apiary, number of hives occupied and honeybee pests being encountered. (Appendix 1)

3.3.2 Direct Observation to measure colony strength

The colony strength was measured by visual estimation by two observers (experts). These two general modes in measuring colony strength; an objective mode which uses empirical measures such as weight (mg, g or kg) or area (cm^2) and subjective mode that relies on visual estimates by one or more observers.

The objective mode is more accurate of the two, but it is also invasive and disruptive to the bees, constituting in some cases the complete deconstruction and re-assembly of colonies with disruption to any social cohesion formerly intact. For this reason, the study considered the subjective mode more appropriate for collecting responsive variable (Delaplane, Steen, and Guzman-novoa, 2013).

The observers visually estimated the surface area of a comb covered by bees, brood, honey and pollen. A colony was opened and combs of bees subsequently removed with each observer looking at one side of the comb. For African bees, the hive should be maintained intact as much as possible, working downward, removing the lid. To determine a weak colony, there was small numbers of worker bees, colony not strong enough to defend from enemies and small number of brood on comb (less than 2 sealed brood combs). A strong colony was characterized by its number of worker bees, ability to strongly defend their enemies and have more than 5 combs filed with brood (Delaplane *et al.,* 2013).

3.3.3. Observation of Small Hive Beetle and Wax Moth and other Pests

The presence of small hive beetle infestation (*Aethian timida*) was identified through its adult larvae or pupae and colony examination methods as described by (Neumann *et al.,* 2013), the larvae of SHB has pairs of prominent brownish dorsal spines on each segment with 3 pairs of anterior prologs only. The presence of Wax Moth was determined by inspection of the beehives for the larvae or combs created in the hive.

3.3.4. Estimation of *V. destructor* on Adult Bees.

The powdered Icing Sugar (shake) Roll method was adopted during this study as described by (Dietemann *et al.,* 2013; Macedo and Ellis, 2002), this method is considered superior over the other methods or Roll (e.g. Ether roll) since it separates up to 90% - 95% of the mites from bees. It is practical in the field, low cost, non-destructive (the bee can be reintroduced in the colony and will be cleaned by their nest mates) and environmental friendly (Dietemann *et al.,* 2013).

3.3.5. Procedure for *Varroa destructor* sampling

Armed with protective clothing (protection from bee sting), hive tool to open the hive, bee brush to manipulate the bees and a lidded jar, preferably plastic approximately 500 grams size in which approximately 300 bees can fit with a lid containing # 8 or 3-5 mm holes (gauze) to allow sugar and parasites to be tipped out leaving the bees behind. According to Lee, Moon, Burkness, Hutchison, and Spivak, (2010), three hundred bees can occupy a volume of 100 ml water. The volume of water is filled in the container and a line marked at the water surface. Given that bee size changes with race, this study used a pre-calibrated container from the National Beekeeping Institute.

An occupied hive was opened using the hive tool **(Plate 1)**. Holding the frame at approximately 10 degrees from the vertical on the upward facing side **(Plate 2)**, the graduated container is slide downwards on the back of the bees so that they tumble in it, making sure the queen is not included. The container was tapped on a hard surface so as to move the bees at the bottom and within the marked like-bees were removed or added accordingly.

Every colony was sampled 3 times (3 x 300) so as to have an average estimate. To account for variation among frames, bees were sampled from three different frames (Branco *et al.,* 2006). After collecting approximately 300 bees, the lid was closed and a one heaping table spoon (approximately 7gms) of powdered sugar was added through the mesh. The jar was rolled so as all the bees are covered with sugar then it was let to stand for one minute. The container was turned upside down over a white surface (size A4 printing paper on a white tray) and the bees were shaken for 1-2 minutes. The fallen mite and sugar was placed in a zip lock back and labeled

according to site of sampling, hive number and the dates of collection and the status of the hive (strong/weak) and kept in a cooler box to be transported to the laboratory.

In the laboratory, the mites were sorted from the sugar using a camel brush, then they were counted. The numbers of varroa mites were expressed as per 100 adult bees.

Plate 1: Showing opening of the hive. Source, Author (2014)

Plate 2: Assessing of the colony strength and sampling of bees. Source, Author (2014)

Plate 3: dislodging mite from adult bees using the sugar roll technique Source, Author (2014)

3.4 Estimation of Varroa mite in brood cells

The rate of infestation on brood cells was determined using a standard procedure (Dietemann et al., 2013). This method is considered the less damaging collection method for the mites. To estimate the infestation rate of *V. destructor* in brood cell, 10 x 10 brood cells were randomly selected and cut (This can be achieved by cutting 5 x 5 cm brood comb) to account for the spatially irregular infestation by Varroa. Three different brood combs per hive were randomly sampled to obtain an average.

The 5 x 5 cm comb cutting were placed in a zip lock bag and labeled according to the site of collection, apiary, hive number, status of the colony (strong/weak) and date of collection using a cooler box the sample was transported to the veterinary research and investigation laboratory for examination. In the laboratory the brood cells were placed on a tray, the cells were uncapped using a fine forceps or scalpel, the cell walls pushed away to free the developing larvae or pupa (Plate 4). Using a soft forceps, the larvae or pupa was pulled out. Careful examination was done on the larvae or pupa and cell wall to determine any presence of mites (Plate 5). The mites collected were counted, the infestation rate was expressed as Varroa mites per 100 brood cells.

Plate 4: showing inspection of brood cells for varroa mites Source, Author (2014)

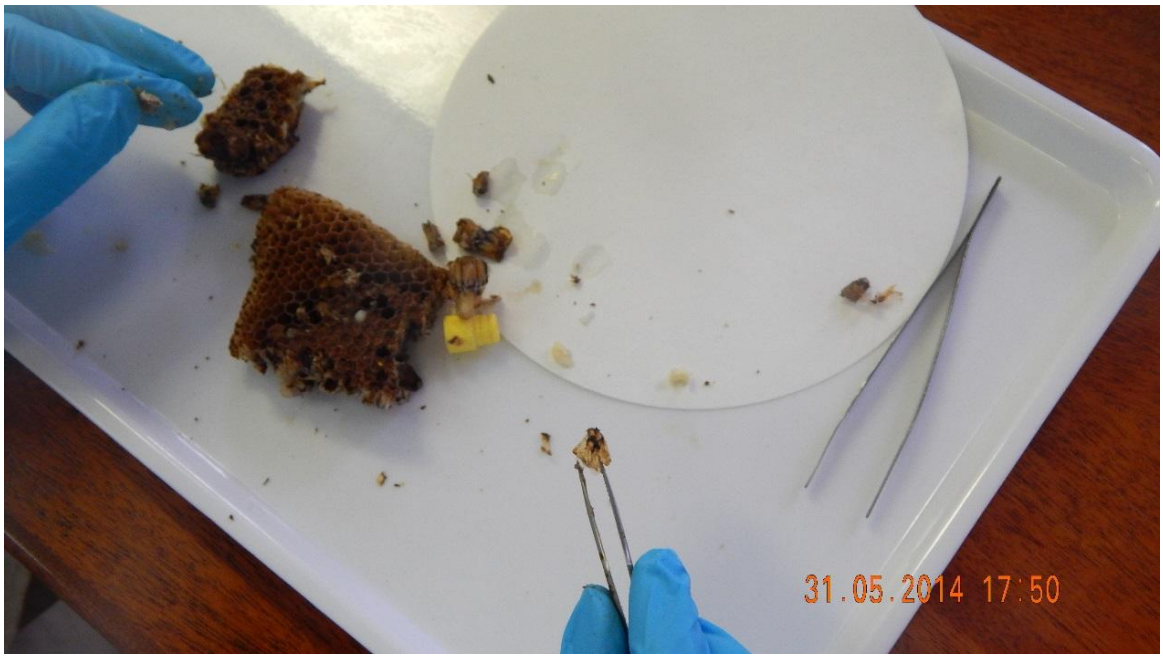

Plate 5: examination of larvae or pupa and cell wall to determine any presence of varroa mites Source, Author (2014)

3.5. Estimating Colony Infestation Rate

The colony infestation rate was estimated by adding up the number of Varroa mites in adult bees and brood cells as recommended by Branco, *et al.*, (2006).

3.6 Data Analysis

The collected data were stored in Microsoft Excel Spreadsheets 2013 program used for data management. Analysis was done using SPSS Software Programs (SPSS @ Version 16). The statistical analysis used in the study varied depending on the type of variable and information obtained. T – test was used to compare the difference in mean varroa mite infestation. Summarized data was presented in the form of table and graphs.

The rate of varroa mite infestations for brood cells, apiary, colony adult per bee and other pests were presented in percentages. A chi-square test was performed to find the association between the level of varroa mite infestation and colony strength (Weak or Strong), a further Odds ratio (O.R) was calculated to determine the strength of association at 95% confidence interval and 0.05 significant level.

Pearson correlation co-efficient was used to test for correlation between the rate of varroa infestation and elevation at $P = 0.05.$ and 95% confidence interval, .the rate of varroa mite counts was converted to logarithms scale so as to normalize the data.

CHAPTER FOUR

RESULTS

4.1 Interviews

In the study area the number of occupied hive were 85(67.5%) out of 165 hives in the in the sampled apiaries. The knowledge of beekeepers on honey bee pest was also assessed, it was evident that all the farmers were not aware neither did they had any knowledge on the *varroa* mite. 80% of the farmers were aware of wax moth, 70% confirmed having knowledge on small hive beetle while all the farmers had knowledge on ants and ear wig.

4.2 Infestation level of *Varroa destructor*

In total 37 colonies were sampled to estimate the prevalence of varroa mite in the study area, varroa mites were found in 32(86.49 %, C.I 95% 70-93.8%), colonies ranging between 75- 100%, (Figure 4.1)

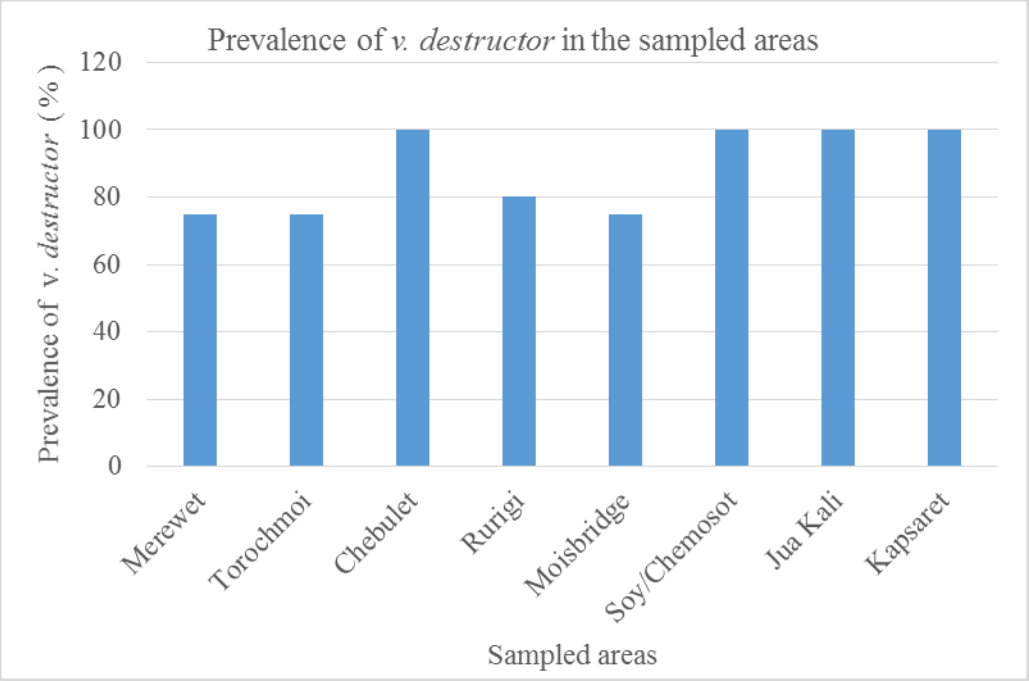

Figure 4. 1: Showing apiary Prevalence of Varroa destructor in the sampled areas.

The level of varroa mite was significantly different across apiaries at P = 0.0001. The average level of varroa mite infestation in apiaries was 59.88 ± 31.45 (Mean and SD), ranging between 15 and 116 varroa mites in Soy/Chemoset and Ndugulu respectively, (Figure 4.2)

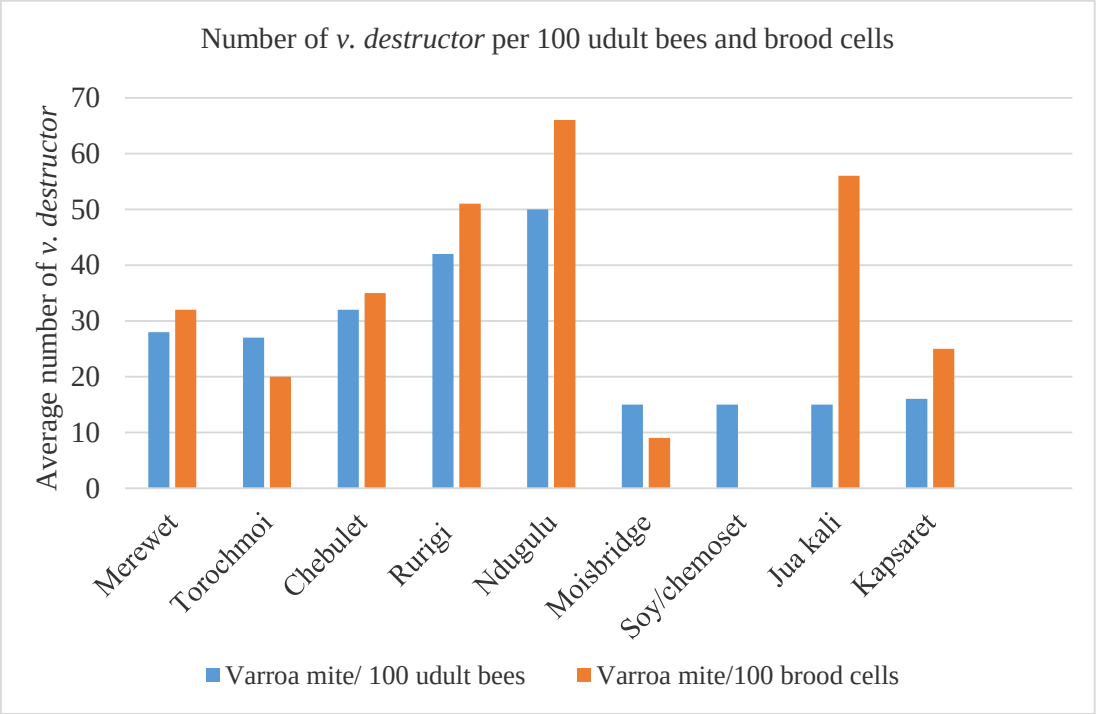

Figure 4. 2: Showing average Level of Varroa destructor infestation per apiary in the study area

For the sampled colonies per 100 bees 10 (27.02%) colonies had 5 or few varroa mites, 14(37.84%) colonies had 6-10 varroa mites while 8 (21.62%) colonies had 11-18 mites. In addition the level of varroa mites per 100 drone and/or worker brood cells were 14(35.14%) colonies had 10 and fewer varroa mites, 9(24.32%) colonies had 11-20 varroa mites, 2(5.41%) colonies and 21-30 varroa mites and 1(2.70%)colonies had 31-40 varroa mites. (Figure 4.3)

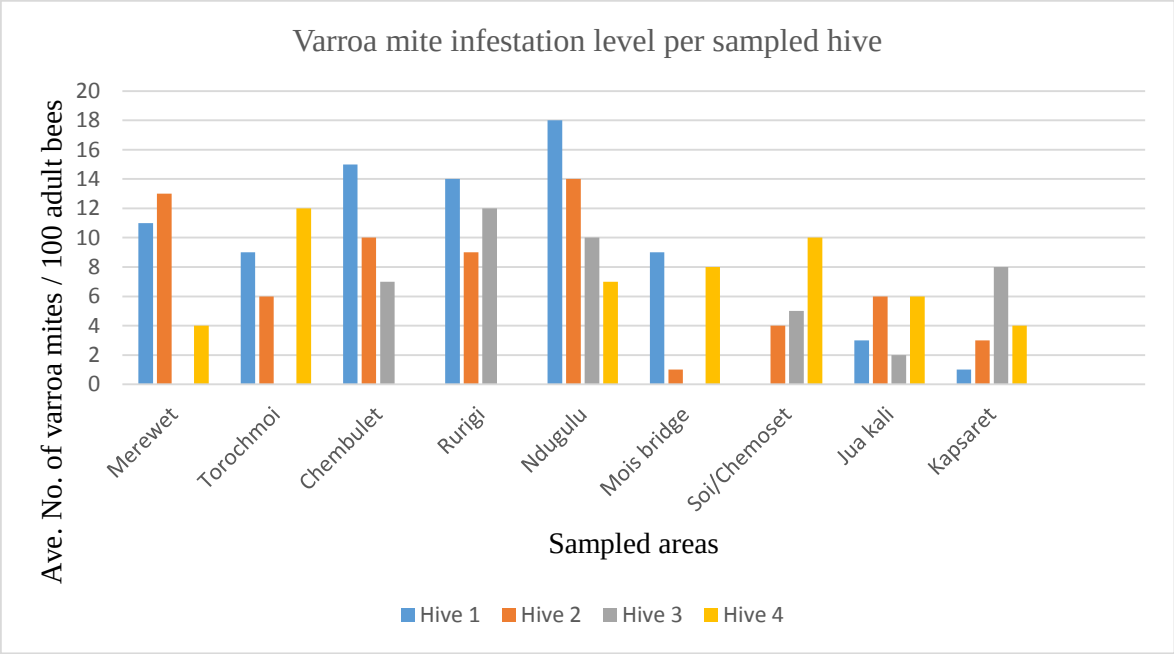

Figure 4. 3: Varroa mite infestation rate per hive

The average colony level of varroa mites infestation was 6.88 ± 2.56 (Mean, SD) varroa mites per 100 bees in the study area, ranging between 3.75 and 10.5 for colonies in JauKali and Rurigi respectively. In addition the average colony level of varroa mite infestation in drone and or worker brood cells was 7.80± 4.94(Mean, SD) varroa mites per 100 brood cells, ranging between 0 and 14 varroa mites in Soy/Chemoset and Jua kali respectively (Table 4.1).

Table 4. 1: Colony level Infestation rate of varroa mite in the study area

Study Area	Average No. of varroa mite /100bees	Average No. of varroa mite/ 100 brood cells
Merewet	7	8
Torochmoi	6.75	5
Chebulet	8	8.75
Rurigi	10.5	12.75
Ndugulu	10	13.2
Moisbridge	5	2.25
Soy/Chemoset	3.75	0
Jua kali	3.75	14
Kapsaret	4	6.25

Considering the number of varroa mites per colony, the average infestation rate per adult bee in the study area was 0.068 ± 0.027(Mean, SD) Varroa mite ranging between 0.04 in Kapsaret, JuaKali, Soy/Chemoset and 0.11 in Rurigi.(Figure 4.4).

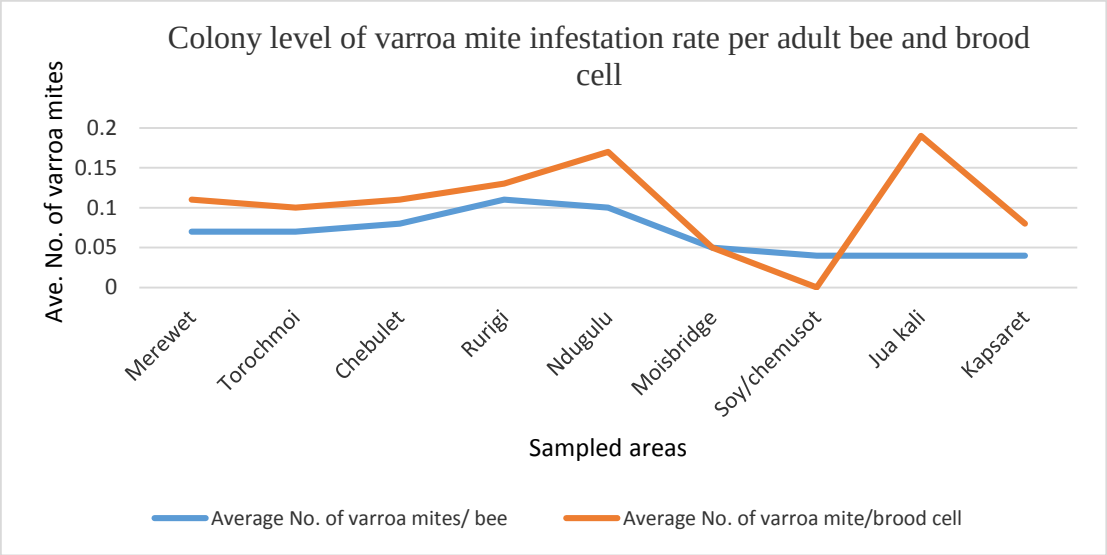

Figure 4. 4: Colony level of varroa mite infestation rate per adult bee and brood cell

The level of varroa mite Infestation in the surveyed area showed a significant positive correlation with elevation (m) (Pearson correlation co-efficient R^2= 0.56, P = 0.020) (Figure 4.2)

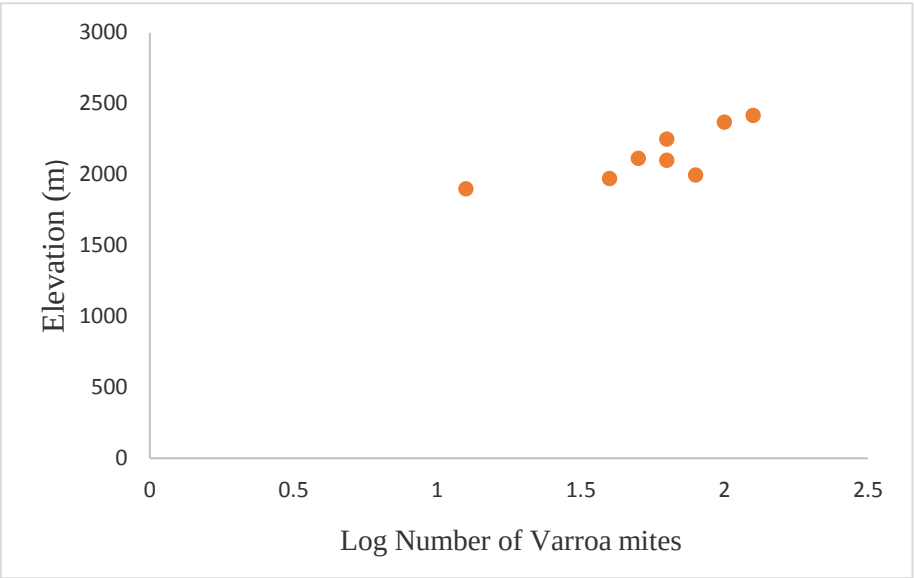

Figure 4. 5: Association of Varroa infestation with elevation

4.3 Effect of varroa mites on Honey bee colony

During the survey all the colonies were assessed on their strength. For the sampled colonies 27 (72.97%) colonies were strong and infested with varroa mites, 2 (5.41%) colonies were strong but not infected with varroa mites and 3 (8.11%) colonies were weak but not infested. Chi-square test showed a statistical significant association between colony strength and level of varroa mite infestation, ($\chi 2$= 5.03, df = 1, P= 0.02). To find the strength of association odds ratio showed that the rate of varroa mite infestation in strong colonies was 8.1 times higher than the rate of infestation in weak colonies, (OR = 8.1, C.I 95%, 1.07 – 35.54)

4.4 Other Insect pest of honey bees

In the 37 hives inspected, Small hive beetle was highly prevalent in 23 (62.16%) hives followed by Black ants in 12 (32.43%) hives, Wax moth in 8(21.62%) hives and lastly the ear wig in 5(13.51%) hives. (Figure 4.3)

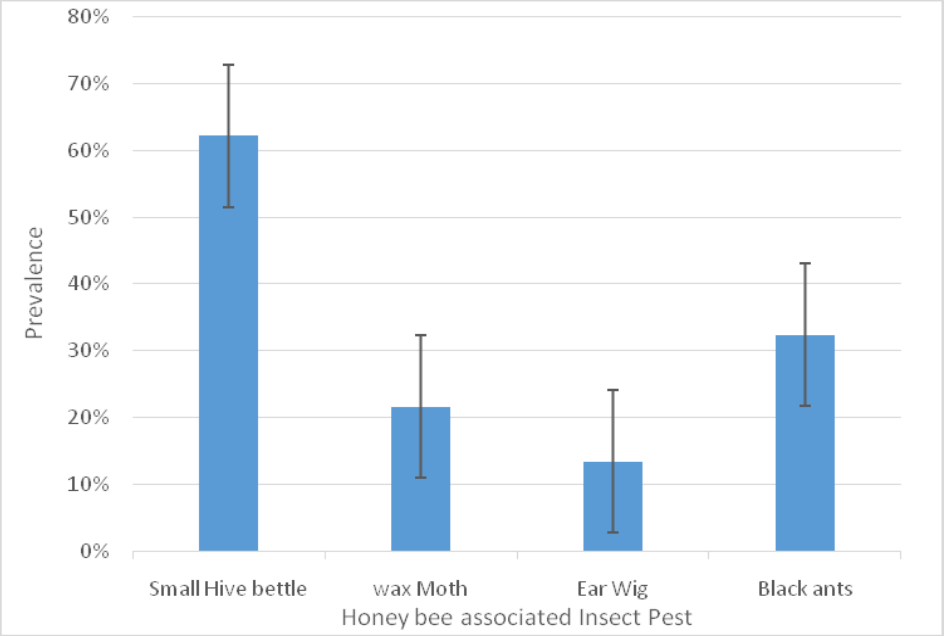

Figure 4. 6: Prevalence of other honey bee associated pests

CHAPTER FIVE

DISCUSSIONS

5.1. Interview

The results from interview conducted showed a decline in the number of hives that are being colonized out of 165 hives in the sampled apiaries only 85 (67.5%) were colonized, which was in agreement with the report from the Kenya National Beekeeping Station (MOLD, 2007), It was speculated that the decline could be disease-related because around that time varroa mite was discovered in Kenya for the first time (Frazier *et al.,* 2010). In addition, all the interviewed beekeepers were neither aware nor had any knowledge on varroa mites. However, most of them had knowledge of other pest of honey bees, 80% of the farmers confirmed to be aware of small hive beetles (SHB) while 70% of the farmers were aware of ants, ear wig, spiders, lizards, this results is in agreement with research conducted in Ethiopia (Godfrey, 2015).

5.2. Prevalence and the rate of *varroa destructor* infestation.

The result from the sampled apiaries confirmed the presence of *V. destructor* in Uasin-Gishu County. Out of 37 colonies, the prevalence of *V. destructor* was 83.8%, this results support the assertion of Muli *et al.,* (2014), Mumoki *et al.,* (2014), that *V. destructor* is widely spread in most parts of Kenya. In addition the result on the varroa mite prevalence agrees with other studies, in Kenya, 83% (Muli *et al.,* 2014), in East Africa (Kenya, Tanzania and Uganda) 87%, (Fazier *et al.,* 2010), 82%, in Ethiopia (Godfrey 2015), and in Nigeria 73.8% (Akinwande *et al.,* 2013), however, the result was slightly higher than in Tanzania with 48% (Mumbi *et al.,* 2014) and Uganda 59% (Chemurot *et al.,* 2016)

The higher level of prevalence indicate that the *V. destructor* spreads very quickly since it was detected in Kenya very in 2009 (Fazier *et al.,* 2010). Moreover, *V. destructor* lives permanently on its host, therefore it can move with the bees from one place to another (Rosenkranz *et al.,* 2010). The Africanized honey bee is well known for its swarming behavior and migration, therefore, it can effectively disperse the mites. Also, the varroa mite can be dispersed through drifting, robbing and sometimes even when honey bees are foraging (Rosenkranz *et al.,* 2010). Moreover, rapid spread of the mite can be facilitated by the passage of varroa mite into feral colonies (Allsopp, 2004).

The infestation level was slightly varied between apiaries and colonies. For apiaries the results showed that all (100%) apiaries were infested with *V. destructor* with an average infestation rate of 59.88 ± 31.45 (mean± SD) ranging between 15 and 116 varroa mites per 100 adult bee and 100 brood cells in Soy/Chemusot and Ndugulu areas respectively. The cause of variation in infestation rates among studied areas might be attributed by different factors such as ecological variability, Seasonal flora of the area and genetic variation in the host (honey bee) (Makori *et al.,* 2017). Other features that may cause variation include reproductive capacity during the mite lifetime and the lifespan, features of the host are brood availability, presence of the drone brood and swarming behavior (Fries *et al.,* 2003). For the colonies the average level of infestation per 100 adult bees was 27.22 ± 12.44 (mean± SD) indicating a lower level of infestation compared with 100 brood cells having 32.67 ± 21.92 (mean± SD). This results agreed with other studies in Tanzania (Mumbi *et al.,* 2014), South Africa (Allsopp, 2004), Nigeria (Akinwande *et al.,* 2013) and Benin (Rasolofoarivao *et al.,* 2013). This study showed tremendous infestation behavior of *V. destructor*. Even though high varroa mite infestation evident in brood

than adult, there is no mechanism explaining why the varroa leaves the adult bee to invade brood cells. It is known that the number of *V. destructor* on brood is related to the season of the year (Nazzi and Le Conte, 2016) and availability of brood in the hive (Mezgabu et al., 2016).

According to Boot, Calis, and Beetsma, (1993) the mite enters a brood cell immediately after abandoning the body of an adult bee. This behavior might be an adaptation of the mite to avoid detection and removal by hygienic behavior of the bee (Rosenkranz *et al.,* 2010). However, for the mite to complete their reproductive cycle, the female must abandon adult bee and enter workers and/or drone caste.

The average level of infestation per colony (100 bees) in the study area was 6.88 ± 2.56 (mean± SD). V. *destructor* mites per colony ranging between 3.75 and 10.5 mites per 100 bees in Jua kali, Soy and Rurigi respectively, while the level of infestation per 100 brood cells was $7.80 \pm 4.94.$ (mean± SD). *V. destructor* mites per 100 brood cells (worker and or/drone cells) per colony ranged between 0 and 13.2 mites in Soy and Rurigi respectively. This translates to 0.068 ± 0.027 (mean± SD) *V. destructor* mites per adult bee and 0.1 ± 0.058 (mean± SD) *V. destructor* mites per brood cell. These findings were in agreement with previous findings in Kenya that reported 26.3 mites per colony (Fazier *et al.,* 2010), in Ethiopia Mezgabu et al., (2016) reported 15.73% of mites in 300 bees, in Nigeria, Akinwande *et al.,* (2013) reported 0.15 mites per adult bee, in Mexico 0.55 mites per adult bee (Medina-Flores *et al.,* 2014), however, our findings varied from records in Brazil 38 mites/100 bees, while in Mexico (Medina, 1998) reported 48 mites in 100 bees.

The study reported low level of *V. destructor* mite per adult been in Uasin Gishu County, however, economic threshold have not been attained with regard to level of infestation in other countries. Delaplane and Hood, (1999) observed colonies in the Midwest USA with more than 0.12 mites per adult bee when brood is not present (in the fall) will have increased mortality if the mite population is not reduced. They further claimed that colonies with more than 0.25 mites per bee will perish in the winter. Similary, Macedo and Ellis, (2002) suggested that the mid-August in USA even when brood is present and infestation is more or equal to 0.03 mites per bee, treatment must be applied as soon as possible. However, the economic threshold for varroa mite is seasonally and regionally specific.

The lower level of infestation of mite per adult bee can be justified by the fact that the African Honey Bee has defensive capacity against this parasite (Aumeier, Rosenkranz, and Gonçalves, 2000). It could be expected that in tropical climate, where worker brood rearing and varroa reproduction takes place all year round the impact would be devastating. However, this is not the case in African honey bee (*Apis mellifera*), the African race appear more resistant to this parasite than European races (Moretto and Leonidas 2003). Several studies have shown that Africanized bee worker were almost eight fold more efficient in getting rid of mites their bodies compared to Italian bee workers (Rosenkranz *et al.,* 2010). Artificially infested Africanized bee reacted to the presence of varroa from the very beginning of infestation. Strong body movement involving the abdomen, legs and mandible were performed by the infested workers, this movement executed by infested worker permitted nearby workers to identify the mite on their body thus attacking the mite using their tongue and mandible (Aumeier *et al.,* 2000). In addition, Africanized bees allow only a significant lower percentage of mites reproduced on worker brood.

Rosenkranz, (1999) reported that, in the tropics, 43.0% of the mite do not reproduce at all in Africanized colonies compared with only 19.0% European colonies. Hygienic behaviour of Africanized bees is related to bees opening up capped brood cells and removing the diseased and parasitized brood (Harbo and Harris, 1999), bees that demonstrate hygiene behavior by fast removal of Freez killed pupae, also remove mites from infested cells. Spivak and Reuter, (2001) reported that colonies selected for hygiene behavior had lower mite levels than non-hygiene ones. Harbo and Harris, (2005) suggested that bees with a suppressed mite reproduction trait (SMRT) removed reproductive mite more often than they removed non-reproductive mites. In a previous study, Vandame *et al.,* (2002) demonstrated that Africanized worker bees removed up to 32.5% infested broods while European honey bee could only remove 8.0% of infested brood. In Africa, Fries and Raina (2003), demonstrated that African bees can remove up to 95% of infested brood, showing a possible mechanism that could contribute to the tolerance of Africanized honey bee. Other traits of African honey bee that reduce the varroa mite population are increased swarming and absconding (Muli *et al.*, 2014).

Despite the presence of varroa mite in the study areas, the varroa mite does not seem to have a significant impact on the honey bee colony population. In fact, there was a positive correlation between varroa mite and colony strength. Strong colonies were eight times more likely to be infested than weak colonies. These results is in agreement with several other studies by Muli *et al.,* (2014), Frazier *et al.,* (2010) and Mumbi *et al.,* (2014) who reported a significant positive correlation between level of *V. destructor* and colony size/colony strength. However, the result did not agree with Chemurot et al., (2016). This fact was supported by results of Rosenkranz *et al.,* (2010), explained that the reproduction of varroa mite is closely synchronized with

brood development of the host. Moreover, varroa mite population dynamic is influenced by its host population dynamics and internal and external factors. Therefore, a weak colony without brood cells will have a very low population of varroa mite. Also, the African honey bee is well known for robbing, normally, workers from strong colony will go robbing honey from weak colonies, the worker bee will carry the mites from the weak colony to their strong colonies (DeGrandi-Hoffman, Ahumada, and Graham, 2017).

The study showed a positive correlation between elevation and varroa mite levels, suggesting that environmental factors (climate and landscape ecology) may play a key role in mediating the host parasite interaction and perhaps honey bees' health is general, the result in this study is in agreement with other studies (Chemurot *et al.,* 2016; Muli *et al.,* 2014; Mumbi *et al.,* 2014).

The occurrence and distribution of varroa mite is influenced by various biotic and abiotic variables like other pests. Varroa mite can survive in certain optimal bio-climate conditions, for instance, the optimal temperature, humidity, precipitation, altitude and biomass/not primary productive ranges. Studies have shown that reproductive ability of honey bees pests can be limited by the prevailing dry conditions and enhanced by hot and humid conditions (Makori *et al.,* 2017). According to Nazzi and Le Conte, (2016) relative humidity is mostly required for brood development. Temperature and relative humidity are the climatic variables that had most significant effect on varroa mite reproduction. Rosenkranz *et al.,* (2010) reviewed that infestation rates of adult Africanized honey bees rose from 4% to 11% when they were moved from warmer to colder climates in Brazil. Based on Nazzi and

Le Conte, (2016) varroa mites are susceptible to dehydration when temperature is very high, hence body weight is lost making difficult to reproduction.

The environmental factors of an area can determine the diversity and abundance of plant, which will determine the population of honey bees. If the honey bees are in an area with plenty of flowering plants, they will always have plenty of resources and brood, therefore, allowing for varroa mite reproduction. However, there is a need to explore in great details the effect of environmental factors.

5.3. Prevalence of other pests of honey bee colonies.

Apart for varroa mite, other pests were reported in the present study. The small hive beetle and the wax moth were reported in nearly all the apiaries investigated, while the ear wig and black ant were not evenly distributed. Other studies that have reported similar results include Akinwande *et al.,* (2013) in Nigeria, Chemurot et al., (2014) in Uganda and Makori *et al.,* (2017) in Kenya. The high distribution of small hive beetle is due to its ability to fly, therefore, it is dispersed from one apiary to another *(*Makori *et al.,* 2017). According to FAO (2006), the small hive beetle and wax moth do not seem to be negatively affecting honey bees. However, high infestation of honey bee colony can lead to swarming and absconding (Kebede, 2015).

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

(i) Depending on the evidence obtained from the study, all the bee keepers were not aware of the presence of *V. destructor*; neither did they have any knowledge on the mite.

(ii) The study demonstrated that varroa mite is present in Uasin Gishu County with a very high prevalence. However the presence of *V. destructor* in the area does not seem to negatively impact on honey colony bee yet. However, the reporting of honey bee viruses by other studies shows that there is need to think of their control since they are known to vector the virus infection. It could be only a matter of time before these newly introduced pest significantly impact on honey bee population, therefore long-term monitoring is necessary.

(iii) The study showed a significant association between the level of varroa mite infestation and altitude (height above sea level).

(iv) Other pests that were reported were small hive beetles, wax moth, ear wig and black ants.

6.2 Recommendations

(i) It is recommended that frequent monitoring and surveillance of honey bee colonies for pest be conducted in the study area to determine the level at which to control the mite.

(ii) It is recommended that further investigation be conducted to assess the effect of environmental factors on varroa mite infestation level.

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APPENDICES

APPENDIX I: QUESTIONNAIRE ON BEE KEEPERS AWARENESS OF PESTS OF HONEYBEES AND THEIR PESTS MANAGEMENT PRACTICES

District _____division_____ location _____

Date _____ Longitude_____ Latitude _____

1. Beekeeper's name _____
2. Sex of respondent(tick in the box)

   ☐ Male ☐ Female
3. How long have you practiced beekeeping?
4. What is your main occupation?

   _____
5. How many lives do you have? _____
6. Which type of lives do you use?
   a) Traditional _____
   b) Langstroth _____
   c) Kenya Top Bar Hive_____
7. How many hives are occupied?
8. When is the harvesting period?_____
9. How much money did you harvest during the last harvest?

   _____
10. Where do you sell your honey?_____
11. In which form do you market your produce?
    i) Crude form _____
    ii) Refined form _____
12. Has honey production increase or decreased per hive over the past year?_____
13. What do you think are the reasons for the change in honey production?
14. Do you carry out hive inspection?
15. Are there period when the honeybee population goes down? Y_____ N_____
    a. How frequent?_____

b. Are flowering trees available around the honeybee colonies? _____

c. Which are the floral trees available in the vicinity for honeybees?

_____

_____

d. What do you do to strengthen your bee colonies

_____

_____

16. Apart from honey what other benefit do you get from beekeeping?

_____

_____

_____

_____

17. Do you get any extension support from government officers?

Y_____ N_____

a. Which form of extension support do you get from the government?

_____

_____

_____

18. Do you get support or device from any sources apart from government

Y_____ N_____

a) If yes list the other sources of support

_____

_____

_____

b) Name the form of support that you get

_____

_____

_____

19. Which challenges to you face in industry?

_____

_____

_____

20. (i) Are you aware of any pests that attack your bees?

Y_____ N_____

(ii) If yes, name the pests

_____

_____

_____

(iii) Is there a specific period in the year when you notice the pests?

(iv) Do the pests affect honey production?

(v) If yes, How?

(vi) Which methods do you use in controlling and managing the bee pests?