BAHIRDAR UNIVERSITY

COLLEGE OF AGRICULTURE AND ENVIRONMENTAL SCIENCE

GRADUATE PROGRAM

POTENTIAL THREATS TO HONEYBEE HEALTH WITH EMPHASIS ON VARROA MITE IN SOUTH WOLLO AND WAGHIMRA ZONES OF AMHARA REGION, ETHIOPIA

MSc Thesis

BY

ALEMU TSEGAYE

September, 2015

Bahir Dar, Ethiopia

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ALEMU TSEGAYE

Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science (MSc.) in Apiculture

Advisors:

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September, 2015

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THESIS APPROVAL SHEET

As members of the Board of Examiners of the Masters of Sciences (M.Sc.) thesis open defense examination, we have read and evaluated this thesis prepared by **Mr Alemu Tsegaye** entitled: **Potential Threats to Honeybee Health with Emphasis on Varroa mite in South Wollo and Waghimra Zones of Amhara Region, Ethiopia.** We hereby certify that, the thesis is accepted for fulfilling the requirement for the award of the degree of Master of Sciences (M.Sc.) in Apiculture.

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Name of Chairman	Signature	Date

DECLARATION

This is to certify that this thesis entitled "**Potential Threats to Honeybee Health with Emphasis on Varroa mite in South Wollo and Waghimra Zones of Amhara Region, Ethiopia**" submitted in partial fulfillment of the requirements for the award of the degree of Master of Science in Apiculture to the Graduate Program of College of Agriculture and Environmental Sciences, Bahir Dar University by Mr Alemu Tsegaye is an authentic work carried out by him under our guidance. The matter embodied in this project work has not been submitted earlier for award of any degree or diploma to the best of our knowledge and belief.

Name of the student

_____

Signature and date_____

Name of the Supervisors

_____ (Major Supervisor)

Signature and date _____

_____ (Co Supervisor)

Signature and date _____

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DEDICATION

This thesis is dedicated to my wife **Weynshet Berhanu** and our children **Fikir**, **Samuel**, **Bezawit** and **Kirubel Alemu** for their unlimited sacrifice, understandings and supports they provided to me during my study.

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ABBREVIATIONS

AFB	American Foul Brood
ANRS	Amhara National Regional State
BOA	Bureau of Agriculture
CSA	Central Statistics Agency
EARO	Ethiopian Agricultural Research Organization
EFB	European Foul Brood
EFSA	European Food Safety Authority
EFSA	European Food Safety Association
ETB	Ethiopian Birr
FAO	Food and Agriculture Organization
GDP	Gross Domestic Product
HBRC	Holleta Bee Research Center
ICIPE	International Centre for Insect Physiology and Entomology
MOARD	Ministry of Agriculture and Rural Development
NGOs	Non-Governmental Organizations
OIE	Organization International Epizootics
SHB	Small hive beetle
SPSS	Statistical Package for Social Sciences.
USA	United States of America
USD	United States Dollar

"Potential threats to honeybee health with emphasis on *Varroa Mite* in South Wollo and Waghimra zones of Amhara region, Ethiopia"

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Abstract

This study was conducted in selected six districts of south wollo and Wghimra zone of Eastern Amhara Region targeting to examine the effect of major honeybee pests with emphasis on varroa mite, diseases and poisoning factors to local honeybees and bee products. This research project was conducted in two main components: The cross sectional study and seasonal monitoring of varroa mite. Laboratory diagnosis was conducted according to the OIE and BEEBOOK standard protocols. The cross sectional study results have indicated that the major pests and predators were ants, wax moths, bee eating birds, varroa mites, wasps, lizards, spiders, bee lice, death head hawks moth and hamagot in order of their importance. From a total of 384 colonies examined for the presence of varroa mite, the laboratory diagnosis confirmed that 330 (85.9%) were found positive. Along with this, the infestation rate of varroa mite was found to be 4.57±0.25 and 7.74±0.66 mites per hundred bees in phoretic and reproductive phases respectively. Furthermore, out of the 330 colonies positive for varroa mite 98 (29.9%) of the colonies were infested with >5% infestation level which has corresponded to an economic thresholds level of western honeybees. The explanatory variables that fit the logistic model: types of management, hive types, altitude and rainfall were associated risk factors for the prevalence of varroa mites as hypothesized. Seasonal monitoring of varroa positive colonies indicated that varroa infestation level reached its peak starting from November to March. The overall average dead brood removal percentage of the local honeybees was found to be 90.83%, indicating the bees are more hygienic. Regarding the use of agro-chemical, 82.4% of the respondents were using agro-chemicals for different purposes (78.9% for crop pest control, 57.6% for weed control and 40.4% to use as anti malaria). About 40.51% of the respondents have applied the chemicals during bees' active foraging times. As a result of this, 60.2% of the total respondents lost their colonies due to unwise use of agro-chemicals on different crops. In this study, Euphorobia spps, Parthenium hysterophorus, Helianthus annuus, Euphorbia tirucalli, Guizotia scarba, Aloea spps, Agave spps, Azadirachata indica, Lanthana camara and Acacia saligna, Digita, Kalkalda and Kuliza belonging to 8 different families were identified as major poisonous plants by the respondents in the area. Finally, it could be concluded that the occurrence of pests and diseases, application of agrochemicals and availability of poisonous plants were a potential threats to the local honeybees which needs an urge to devise and implement an appropriate control and prevention measures.

Key words: *Agro-chemical, Honeybees, Cross sectional, Prevalence, Threats, Varroa mite*

Chapter 1: INTRODCTION

1.1. Background and Justification

Beekeeping is an important component of agriculture and rural development program in many countries. It plays a role in providing nutritional, economic, and ecological security. Directly, it contributes to the values of the outputs produced including honey, bee wax, and other products such as pollen, royal jelly, bee venom, and propolis (MoARD, 2007). Honeybees are valuable insects, known for their importance as pollinators and honey producers (Admassu Addi and Nuru Adgaba, 2000; FAO,2009).

Like all living animals, honeybees are infected with different diseases and attacked by parasites and pests. These diseases and pests of honeybees impose serious problems on honeybee production and productivity. Recently honeybee health has been considered as one of the most important topics in apicultural research agendas (Genersch, 2010) primarily due to the recent emergency of higher honeybee colony losses in many parts of the world (Le Conte *et al*., 2010) and the vulnerability of honeybees to parasitic mites, fungi, viruses and bacteria (Dietemann *et al*., 2012). These pathogens and parasites can have harmful effects on honeybee health and the services they offer (Genersch, 2010). However, the health status of honeybees in Africa is poorly characterized (Dietemann *et al*., 2009).

During the past four decades, the invasive ectoparasitic mite and *Varroa destructor* has become the largest threats to apiculture and honeybee health world-wide (Todd *et al.*, 2007). No other pathogen has had such a large impact on beekeeping or honeybee research throughout the history of apiculture. Mite infestation of bees is known to cause immune-suppression, weight loss, decreased flight performance, and reduction in lifespan (Amdam *et al.*, 2004; Kralj and Fuchs, 2006; Yang and Cox-Foster, 2007). The mite is also serving as a vector for some honeybee viruses (Boecking and Genersch, 2008).

Moreover, even if the global market demands healthy, safe, good quality & organic products, and medication is a must to suppress varroa's damage, it has been very difficult to present hive products to consumers as natural or organic products. Thus, this situation seriously affects the international market accreditation processes (FAO, 2009).

There were some reports of colony losses just after the initial invasion of *Varroa* mites into South Africa (Allsopp, 2006) as well as tested positive for Black queen cell virus (BQCV), Acute bee paralysis virus (ABPV) and two unnamed viruses (Swart *et al*., 2001). Recently, it has been reported that several new parasites and pathogens (*Varroa*, *Nosema*, Deformed wing virus (DWV), BQCV, and ABPV) have invaded the honeybee population in the Eastern Africa particularly in Kenya (Muli *et al.*, 2014). First, in 2009, scientists from International Centre for Insect Physiology and Entomology (ICIPE) in Kenya, together with Centre for Chemical Ecology (USA) and Bee Research Laboratory (USA) reported that they found this mite in honeybee colonies in Kenya, Tanzania, Uganda and Ghana (Sammatro *et al.*, 2011).

1.2. Statement of the Problem

Ethiopian environment is not only favorable to bees, but also for different kinds of honeybee pests and predators that are interacting with the life of honeybees (Desalegn Begna, 2001). The existence of pests and predators are irritants to the honeybees and beekeepers in the country which causes devastating damage on honeybee colonies.

The recent honeybee diseases and pests phenomenon in Ethiopia, like: Chalk brood, small hive beetle, and *Varroa* mite has strongly pronounced the importance of assessing the honeybee health and health risks (Desalegn Begna *et al*, 2001 and 2006) in the country. Moreover, honeybees are damaged or destroyed by misuse of pesticides and herbicides that cause to the reduction of honeybee colonies, which in turn result in reduction of bee products and crop yield and thus affecting economic returns of bee products and agricultural crops.

Furthermore, naturally there are certain species of honey plants whose pollens, nectars and honeys are toxic to bees and human. In different countries honey plants which are toxic to honeybees and man are identified and important cautions are exercising. In our country, there are many oral reports about honey plants which are toxic to bees and also honeys which are poisonous to man. Therefore poison plants which have an economic importance on honeybees in our country should be investigated and studied to take the right cautions.

When we come to our emphasis, the first investigation report for the presence of *Varroa* mite, as one of the potential honeybee pests, was published by Desalegn Begna (2014) who reported the overall prevalence of 82% in Tigray Region. Besides, nation-wide diagnostic

survey conducted from 2008-2010 showed that there was a wide scale distribution of *Varroa* mite in most areas of the eastern parts of the Amhara Region (Abebe Jenberie *et al*, 2010, unpublished). Based on this report, except few places, almost all areas starting from Abergele district (Waghimra) up to Kalu district in (South Wollo), *Varroa* mites were observed.

On the other hand, during that time, some areas like Desse Zuria and Legambo districts and some localities in some districts were free from the mites. However, the varroa free areas are not continuous and might not be guarantee to be free from the disease for long. In addition, in recent years, there were some complaints about colony number decline and low productivity in honeybee colonies of these areas. More specifically, these potential threats have incited a great concern in the investigation of the problem in order to come up with a practicable measures and techniques against these potential threats.

In these cases, assessment and identification of the occurrence of harmful honeybee diseases, pests and honeybee poisoning has been recommended as a key step in a plan targeting exploitation of opportunities from the sector (Adeday Gidey *et al*, 2012). Failure to detect and recognize early effects has been witnessed to bring about a decreased productivity and even important colony losses. Regular surveillance is, therefore, very important in prioritizing those important threats and designing appropriate control measures against the hazards.

1.3. Objectives

Thus the general objective of this study is targeted to examine the current status and effect of honeybee diseases, pests with emphasis on *Varroa* mite and poisoning factors to honeybees and bee products condition. The specific objectives are to:

- Identify the types and distribution rates of honeybee diseases and pests
- Detect the possible early stage symptoms and effects of the major honeybee diseases and pests
- Recognize the seasonal dynamicity of *Varroa* mite
- Evaluate the status of pesticide use and their effects on honey production and honeybee's productivity

Chapter 2: LITERATURE REVIEW

2.1. Varroa Mite and its Economic Importance

Varroa mites are ectoparasites that feed on the haemolymph of immature and adult honeybees (Ellis and Nalen, 2010). The genus varroa includes two species, *Varroa destructor* and *Varroa jacobsoni* (OIE, 2008). However, *Varroa destructor* is the only species of economic importance in contrast to *Apis cerana* which can support populations (Ellis and Nalen, 2010).

2.1.1. Taxonomy and Morphology

The taxonomic position of the arachnid *Varroa* is categorized under kingdom Animalia, phylum Arthropoda, class Arachnida, order Mestigmata, family Varroidae, genus Varroa, and species *Varroa destructor* and *Varroa jacobsoni* (IBRA, 2013).

The varroa mite is an external parasite that is visible to the naked eye and its body is divided into two well defined parts, the idiosoma and the gnathosoma. The whole body, including legs and mouthparts, is covered with hairs (MAAREC, 2004).

The female mite is brown to reddish- brown in color, measuring 1.1 to 1.2 mm in length and 1.5 to 1.6 mm in width (about the size of a pinhead). Its dorsal shell covers the entire idiosoma and the mite has indistinct head and four pairs of short and segmented legs, which protrude from one side of this ellipsoid shell (Coffey and Mary, 2007). Its body fits into abdominal folds of the adult bee and is held thereby the shape and arrangement of ventral setae (Sanford *et al.*, 1998). The flattened shape of the female's body makes it easy for the mite to hold onto a bee and move easily into the cells of developing bee brood (MAAREC, 2004).

Males are smaller, about 0.7 mm by 0.7 mm, and light tan in color. Adult males do not feed and are not found outside of brood cells. The male chelicerae are modified for transferring sperm. The legs of the males are longer in relation to the body size than the legs of females (Huang, 2012).

Figure 1 . Dorsal view of adult varroa mite, *Varroa destructor* and the composition of a *''Varroa family''* within a honeybee worker brood cell

(Source: Anderson and Trueman 2000)

2.1.2. Geographical Distribution

The geographical distribution of Varroa mites vary with the type of species. The *Varroa jacobsoni* has a wide distribution throughout Asia whereas *Varroa destructor* is thought to be native to the Far East where it parasitizes the Asiatic honeybee *Apis cerana* and is not invasive, though it has been introduced widely and is now prevalent worldwide, with the exception of New Zealand, Australia and some countries in Central Africa (Sanford *et al.*, 2007).

Varroa mite infestation is influenced by season and climate. It is proposed that varroosis in cold climate is higher than that of warm climate and its rate of incidence is greater in cold seasons (fall and winter) than hot and warm seasons(spring and summer) (Lofti and Shahryar, 2011). There are, obviously, significant differences between the population dynamics in temperate and subtropical/tropical climates with a clear tendency for lower mite population growth under tropical conditions (Rosenkranz et al., 2006). Under temperate conditions, damage at the colony level mainly appears during fall and winter, when the host population declines, the relative parasitization increases and consequently the long-living winter bees are damaged (Amdam *et al.*, 2004).

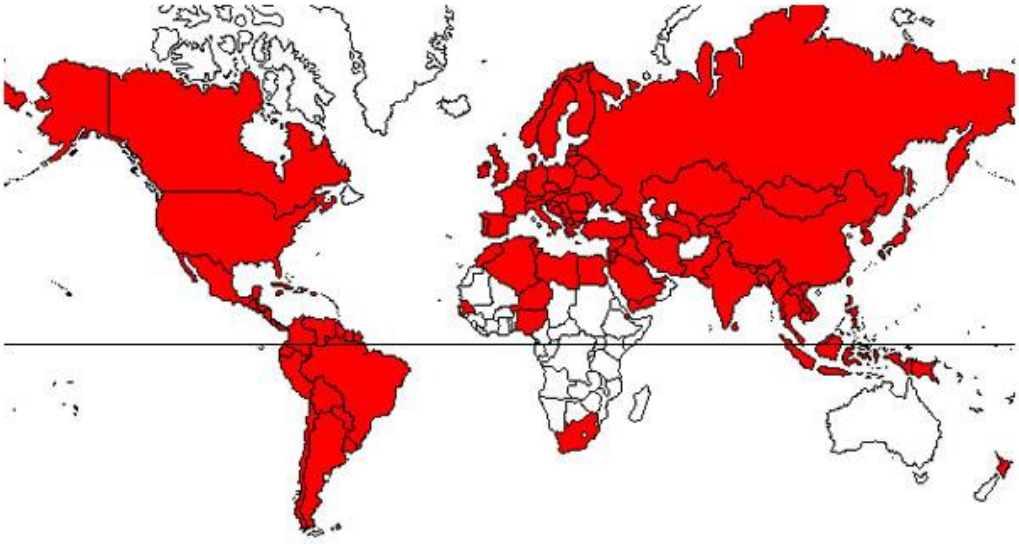


Figure 2. World varroa mite distribution -2010. Red areas indicate establishment of varroa distructor

2.1.3. Transmission

The mites are spread from bee to bee when bees walk past one another in the colony (Ellis and Nalen, 2010). Natural spread between colonies is through the movement of adult bees carrying mites from one colony to another. In apiary, this could be due to natural drifting. Movement between apiaries will occur if there is any robbing. Probably, drones will play a big part in spreading the mites as they are known to move freely between apiaries many miles apart (Bruyn, 1997).

Varroa mites can also be transmitted between colonies as bees from the colonies rob (steal honey) from one another and bee keepers transferring queens, combining colonies, swapping frames of brood between colonies, and transporting inadequately screened hives and boxes of honey (Goodwin and Eaton, 2001). Mites can be introduced to non-infested regions on natural swarms and when beekeepers move infested colonies (Matheson, 2000). The spread of Varroa around the world has been greatly assisted by humans moving honeybees from place to place (Goodwin and Eaton, 2001).

2.1.4. Life Cycle

V. destructor is closely linked to its honey bee host and lacks a free living stage. There are two distinct phases in the life cycle of V. destructor females: A phoretic phase on adult bees and a repro- ductive phase within the sealed drone and worker brood cells (Sanford *et al.*, 1998; Rosenkranz, et al., 2010). During the phoretic phase, female mites feed on adult bees and are passed from bees to bees when bees walk past one another in the colony. Males and nymphal stages of the mite are short lived and can only be found within the sealed brood cells (Harris *et al.*, 2003).

The life cycle begins after the capping of the brood cell (Lewbart, 2012). When female mites are ready to lay eggs, they move into brood cells containing young larvae just before the cells are capped and they go to the bottom of the brood cells and immerse themselves in the remaining brood food. After the cells are capped and the larvae have finished spinning cocoons, the mites start feeding on the larvae (MAAREC, 2004). The mite is most attracted by drone brood. Shortly thereafter, they begin laying eggs approximately three days after the cell has been capped. Subsequent fertilized eggs are laid by the female mites approximately every 25 to 30 hours and these hatches into female mites (Ellis and Nalen, 2010). The period from egg to adult takes about 6 to 7 days for the female and 5 to 6 days for the male. Mating occurs in the brood cells before the new adult females emerge. The adult males die after copulation since their mouth parts (chelicerae) are modified for sperm transfer rather than feeding (MAAREC, 2004). The female stored the sperm in the spermtheca and will not mate again (Huang, 2012). The old female and the newly-fertilized female offspring remain in the brood cell until the young bee emerges and then exit the brood cell with the newly emerged bees to complete their reproduction cycle again. Female mites produced in the summer live 2 to 3 months, and those produced in the fall live 5 to 8 months. Without bees and brood, the mites can survive no more than 5 days. Mite populations increase rapidly during the heavy brood rearing season (Hood, 2000).

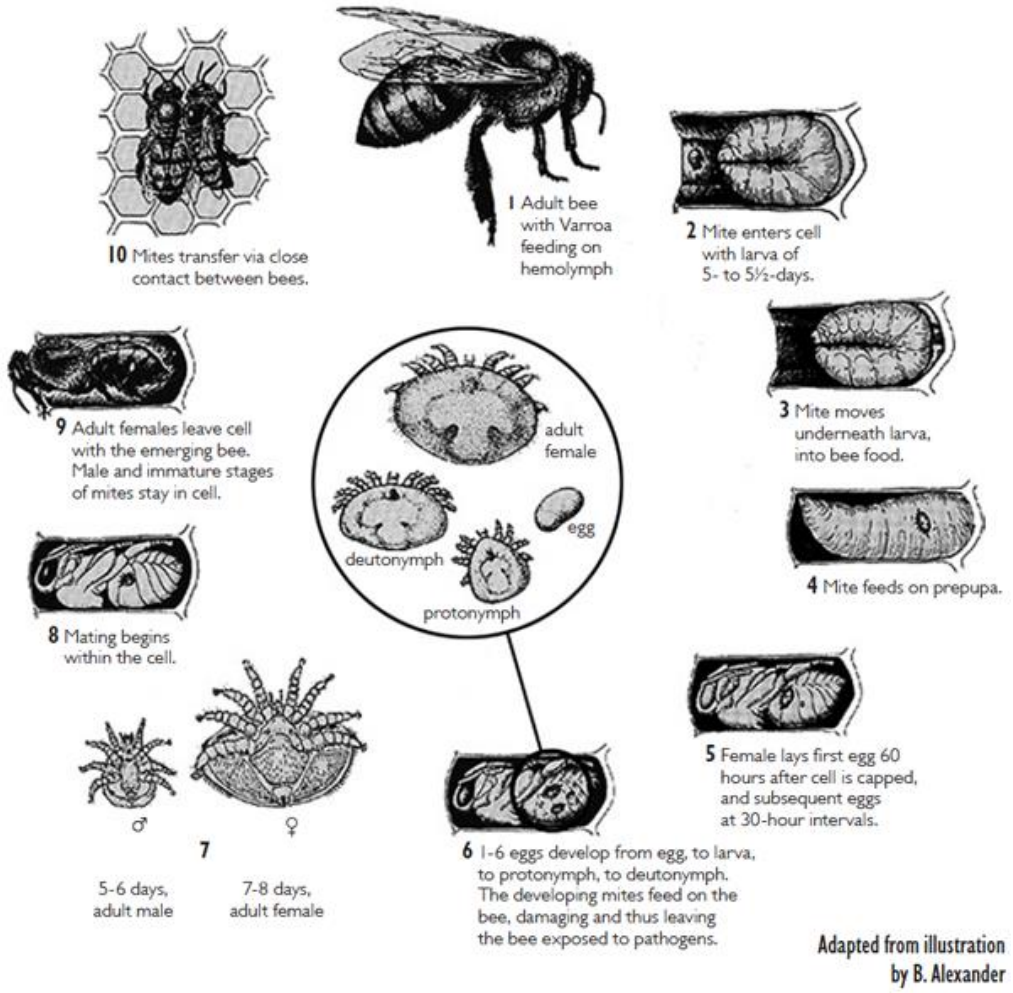


Figure 3. Varroa mite life cycle

2.1.5. Effects and clinical manifestations

Pathological Effects: The damage on honeybees is principally caused by the female mites whereas the male mites cause a little damage since they live only short time in the sealed brood cells of a bee colony (Bruyn, 1997).

Effect on the Individual Honeybees: Initially, Varroa mite infestation is unnoticeable since damage occurs after mite population is built up and this build-up may be over several years or a couple of seasons (Bruyn, 1997). The individual honey bee is damaged in a variety of ways, with the developing larvae and pupae clearly representing the most sen- sitive host stages. First, the loss of hemolymph during the ontogenetic development within the brood cell significantly decreases the weight of the hatching bee. The weight loss depends on the number

of mother mites and the amount of mite reproduction, but even a single infestation results in an average loss of body weight of 7% for the hatching bee (Rosenkranz *et al.*, 2010). The effect of Varroa mites on honeybees come about either directly from the mites feeding on the haemolymph of honeybee adults, larvae and pupae or indirectly as a result of introduction of virus (Goodwin and Eaton, 2001). This has also been proven for parasitized drones, which loose 11–19% of their body weight depending of infestation rate which led to decreased flight performance (Duay et al., 2002). Varroa mites have piercing and sucking mouth parts and feed on haemolymph of honeybee adults, larvae and pupae. Individual developing bees, if infested with one to two adult mites (and offspring), usually emerge without visible damage and are normal in appearance. They may, however, suffer from malnutrition, blood loss, or disease. Individuals those are heavily-infested with more than a few adult mites (which produce as many as 20 nymphs) usually become visibly crippled or die in their cells without emerging. When adult bees are infested with two or more mites, they become restless and fly with difficulty. However, individual developing bees that are heavily-infested with more than two adult mites usually die in their cell without emerging or emerge with misshapen wings, deformed legs, shortened abdomen (Hood, 2000). Their life span is generally shorter than unparasitized bees and they perform tasks poorly. In drones, the spermatogenesis and flight capacity are also affected (Lewbart, 2012).

In addition to the obvious effects of mites feeding on developing and adult bees, the mites can also serves as vectors of several viruses that can kill bees. The secondary infections are facilitated when the mites compromise the bees' immune system and they can cause a condition known as parasitic mite syndrome which can kill colonies within months of infection (Tarpy and Summers, 2007).

Effect on the Colony: On a colony level, the symptoms of a varroa mite infestation depend upon the degree of infestation (FERG, 2005). At low infestation rates clinical symptoms are not visible, and the infestation often remains undetected. Moderate infestation rates 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). Drones which have been parasitized during their development

have a significantly lower chance to mate and infested colonies produce less swarm (Fries et al., 2003). The final break- down of a honey bee colony is associated with the typical ‘‘parasitic mite syndrome” such as scattered brood, crawling or even crippled bees, supersedure of queens and unexplainable reduction of the bee population. The affected colony’s activity and production are reduced. The last stage of the disease is the collapse of the colony (Lewbart, 2012). High mite infestation leads to collapse of the colony (Lewbart, 2012). Varroa has been identified as the cause of significant losses of both managed and feral colonies in a number of areas of the world. However, feral colonies are the most likely to succumb since they are not managed by humans and treated to control mite (Goodwin and Eaton, 2001).

2.1.7. Diagnosis

Effective mite control is depends on frequent and reliable mite detection. In heavily infested area, individual colony infestations can grow from being undetectable to life-threatening levels within a few months. It is important to monitor mite levels by sampling all or most colonies on a regular basis (Tarpy and Summers, 2007). When sampling for Varroa mite, remember that the number and location of mites in a colony vary according to time of the year. The number of mites is lowest in spring, increasing during the summer, and is highest in the fall. During spring and summer, most Varroas are found on the brood. In late fall and winter, most mites are attached to adult worker honeybees (Hood, 2000). There are different Varroa mite examination methods: Debris examination, brood and adult honeybee examination and laboratory diagnosis (OIE, 2008).

Debris Examination: It is the analysis of the debris collected from the bottom of a hive and examined for the presence of the fallen Varroa mites. It is carried out with the use of sticky sheet on the hive bottom for retaining the mites fallen from the body of bees. This method is sparing for bees because it does not require disruption of the colony while detection of mite infestation (Parkman *et al.*, 2002). However, the method can be considered reliable only if there is an adequate amount of brood and on the early stages of the infestation (Branco *et al.*, 2006).

Brood Examination: Varroa mites spend most of their life cycle inside sealed bee brood cells; therefore, uncapping and checking brood (pupae) for mites is a reliable detection method. To look for mites on brood, the pupae (preferably drone) are examined and mites can

be easily seen against the white surface of worker or drone pupae after they are removed from their cells. It is suggested that a minimum of 100 pupae per colony be examined. The pupae can be removed from their cells by inserting a capping scratcher at an angle through the capping and lifting the brood and capping upward (Hood, 2000). Examination of preimaginal bee stages (larvae and pupae of workers and drones) in newly capped brood combs for the presence of mites can be carried out by looking through a strong light. However, brood examination is a protracted labor-consuming procedure and can be implemented only during the presence of brood in a hive (Calderone, 2005).

Direct Observation of Adult Honeybees: When the mites are moving about on a bee, they are fairly easy to detect; but once they attach themselves between segments, they are difficult to find (Bienefeld and Zautke, 2007).

Laboratory Diagnosis: Accurate and easy methods of predicting mite levels in colonies can be carried out by using various sampling techniques. The most important methods are: Alcohol wash method, ether roll and sugar shake method (Tarpy and Summers, 2007).

Alcohol Wash Method: This method is simple, quick and quite accurate when applied to a larger number of colonies in the apiary. Ether roll test is simple but less accurate than the alcohol wash method because it is more difficult to obtain an accurate count of the number of mites in the sample. Sugar shake method can be used instead of ether roll where all the bees are killed (Fakhimzadeh, 2001).

2.1.8. Treatment

Nowadays, different chemicals are available for the treatment of Varroa mite infestation, even though some of them are ineffective and others have and limitation due to their effect on the bees and beekeepers. These chemicals can be organic varroacides like essential oils and organic acids, and synthetic varroacides including fluvalinate, flumethrin and coumphous (Goodwin and Eaton, 2001). Varroacides (specific miticides) are applied in feed, directly onto the adult bees, as fumigants, using contact strips or by evaporation (Fera, 2010). The challenges of treating varroosis are that the mites have developed resistance to many of the synthetic varroacides used and the wide spread use of chemical treatments lead to the presence of drug residue in honey, beeswax and other honeybee products. Re-invasion of

mites in to treated colonies from untreated colonies is also a major problem in varroasis treatment (Mutinelli and Baggio, 2004).

Table 1. Varroa mite treatment Chemicals

Chemicals	Mode of application	Dosage	Treatment efficacy
Fluvalinate	Plastic strips hung between brood combs	8.8g/strip	95-99%
Amitraz	Plastic strips hung between brood combs	500mg/strip	90-99%
Flumethrin	Plastic strips hung between brood combs	3.6mg/strip	85-99%
Coumphous	Solution tricked over bees	32 mg/application	85-99%
Formic acid	Evaporator kits	15ml/application	61-98%
Lactic acid	Spray over combs of bees	15ml/comb face	41-99%
Oxalic acid	Spray over combs of bees	3-4 ml/comb face	82-99%

Source: MAF, 2001

Timing of treatment is crucially important for successful Varroa control. Late treatment application can result in treatment failure which will lead to colony loss. In practice, treatment is applied at the end of the bee rearing season. Another treatment can be given at the beginning of the rearing season in order to evaluate and control mite infestation (Lewbart, 2012).

2.1.8. Socio-Economic Impact of Varroa Mite

These mites have affected the apiculture industry negatively in every country that it has been introduced. Accurate estimates of the effect of Varroa on the apiculture industry is hard to find, but it is safe to assume that the mites have killed hundreds of thousands of colonies worldwide, resulting in billions of dollars of economic loss (Ellis and Nalen, 2010). Apiculture is severely affected by the activities of Varroa destructor, either by direct parasitism or indirectly by facilitating the spread of bee viruses and diseases. If left unchecked, mites can infest hives beyond an economic threshold and lead to colony collapse within a two years period (Fera, 2010). This is necessitates very careful management from beekeepers perspective to detect and treat mites as and when their population increases to critical levels. There is significant cost in materials and labor involved in Varroa management (Rosenkranz *et al.*, 2010).

Thus, a loss in numbers of *Apis mellifera* due to infestation by *Varroa destructor* could lead to substantial negative but indirect impacts from lower crop yields due a lack of adequate pollinators (Cunningham *et al.*, 2002).Collapse of colonies due to Varroa mite can also have a serious effect on peoples who rely on beekeeping for their livelihoods. Regular treatment of varroosis can cause chemical residues in honeybee products which result a great effect on the consumer (Allsopp, 2004).

2.1.9. Prevention and Control

Varroa mites cannot be eliminated from bee colonies, but beekeepers can monitor its presence and still maintain productive bees, and control methods can be used to keep mites at a manageable level (Fera, 2010). Prevention and control of this mite can be carried out using different methods. These include biotechnical, biological, and chemical methods. However, they are only moderately effective when they are used alone, so that an integrated prevention and control approach is best (Hood, 2000).

Biotechnical Methods: Biotechnical methods involve beekeeping management techniques specifically designed to reduce mite levels in a colony. Biotechnical methods are generally not used as a complete means of Varroa control. However, they are often incorporated into

integrated pest management systems, whether with synthetic chemicals, or more generally with organic control substances (Goodwin and Eaton, 2001).

Commonly used biotechnical methods are: drone brood removal and trapping, artificial swarm, open mesh floors, and dowda method (Fera, 2010). Open-screen floors in hives may interfere with mite population growth by decreasing the rate at which mites invade brood cells, yields leading to fewer mites, a lower percentage of mites in brood cells and more cells of capped brood compared with hives with wooden floors (Harbo and Harris, 2004). A high proportion of Varroa mites can be removed from bee colonies by creating an artificial swarm. This involves moving the parent colony approximately 4 m from the original colony site. A second hive containing newly drawn combs and the queen is placed on the original site, causing foragers to return to this hive, creating an artificial swarm (Fera, 2010).

Brood removal and trapping for control of Varroa is treatment based on the understanding that mites are confined in honey brood cells once the cells are capped. The mites can consumer therefore easily be removed from the colony without the mites being able to escape back onto the adult bees. Probably the most well-known biotechnical control eliminated method for Varroa is drone brood removal and trapping. Drone brood is generally used for this purpose because Varroa mites show eight to ten times greater preference for drone brood than for worker brood. Removal of worker brood can also reduce mite levels, but it greatly affects colony productivity and is labor intensive (Goodwin and Eaton, 2001).

Dowda Method: It involves sprinkling of fine dust particles, such as powdered sugar or certain pollen substitutes on adult honeybees in a colony. The powder does not harm the bees, but interfere with the mite's ability to maintain its hold on the bees and it is also believed to increase the bees grooming behavior. This causes a certain percentage of mites to become dislodged. Powdered sugar works best as an amplifier of the effect of a screened bottom board (Tarpy, and Summers, 2007).

Chemical Methods: The chemical methods of mite control involve various methods of application and ways of dispersal of acaricides, which are determined by the nature of the chemicals being used. Varroacides (specific miticides) are applied in feed, directly onto the adult bees, as fumigants, using contact strips or by evaporation (Fera, 2010). Various

chemicals have been demonstrated an ability to control Varroa in honeybee colonies. These chemicals can be divided into organic and synthetic. The three most common synthetic chemicals which are used to control Varroa are fluvalinate (apistan), flumethrin and coumphous. Essential oils and organic acids are the two organic mite control substances (Goodwin and Eaton, 2001).

However, Varroa mites have a demonstrated ability to become quickly resistant to these chemicals. This has made many acaricides useless in areas where Varroa resistance to chemicals has been developed. Many of these substances are not easy to apply and they are dangerous both to the colony and humans. The effects of chemical treatments on honeybees include reduce longevity of queen bees, reduced sperm loads in and longevity of drones, brood death, and reduced queen egg laying patterns (Ellis and Nalen, 2010).

Biological Methods: The biological Varroa control methods involve the use of the bee's biology, perhaps its natural resistance against mites. The desirable features of bees that can be selected to establish a resistant colony include higher hygienic and grooming activities, shorter post capping periods, low attractiveness of brood to mites, and low mite fecundity factors. The selection and establishment of resistant colonies is the best and cheapest method of control of varroosis since the bees themselves deal with Varroa mites. Achievement of this control method is, however, taking longer time and short term solutions, such as biotechnical or chemical methods have to be used in the meantime to stop colony death (Tarpy, and Summers, 2007).

2.2. Major honeybee diseases reported in Ethiopia

2.2.1. Nosema

Nosema disease of honeybee is caused by protozoan known as *Nosema apis.* The disesae has detrimental effects on honeybees, colony development, queen performance, honey production and deaths of the bees several days earlier than the normal one. It is a microsporidian fungal disease that infects the intestinal tract of adult bees. In Ethiopia nosema was reported in 1989 with low infestation rate in the survey conducted by the initiation of FAO through preliminary laboratory diagnosis on 38 bee colonies at Holleta bee research center (HBRC). Diagnosis made on 152 colony bees in field and laboratory at Addis Abeba reported prevalence of

53.3% (Desalegn Begna and Yosef Kebede, 2005). In Ethiopia Nosema was also reported from different regions with varying prevalence such as 58% in Oromia, 60% Benishangul-Gumuz and 47% in Amhara regions (Aster yohanis *et al*., 2007). Similar survey conducted in 58 districts of Oromia, Amhara, Southern Nations and Nationality and Peoples (SNNP), Tigray, Gambella, Benishangul–gumuz, Somale Regional State of Ethiopia, *Nosema apis* was identified as the species causing nosematosis with 37.3% of infection rate (Amsalu Bezabih and Desalegn Begna, 2005). In the central highlands of Ethiopia, highest infestation level of *Nosema apis* and spore number per individual honeybees was reported in the month of August and September (Amsalu Bezabih and Desalegn Begna, 1998).The study also found positive correlation between Nosema infestation rate, number of Nosema spore per individual honeybee and humidity. After establishing the widely distributions of Nosema disease in most parts of the country (Desalegn Begna *et al*, 2005; Amsalu Bezabih *et al*., 2009; Amsalu Bezabih *et al*., 2010), control experiment was set to investigate its side effects on the life and products of local honeybees and found no considerable effects (Amsalu Bezabih and Desalegn Begna, 2005).

In north western Amhara an epidemiological study on Nosemosis in three Agro-Climatic zones were conducted by Gulima and Awoke between October 2003 and August 2004 which revealed that the overall prevalence of *Nosema apis* during the study was 13.9% and the effect of *Nosema apis* on the honey yield of colonies was found to be significant (Darsema Gulima and Awoke Berhanu, 2006).

2.2.2. Amoeba

Amoeba is diseases of honeybee caused by a single celled parasite called *malpighamoeba mellificae.* The parasite affects malpigian tubules of honeybees and shortens the life cycle of bees. The diseases were reported in Ethiopia in 1989 together with Nosema with low infestation level in the survey made in the country initiated by FAO (Desalegn Begna and Amsalu Amsalu Bezabih, 2005). Moreover, nation-wide diagnostic survey from 2008-2010 to identify and locate amoeba disease of honeybee in most of the places in the country (Amsalu Bezabih *et al.*, 2009; Amsalu Bezabih *et al*, 2010). Survey conducted in the year of 2000, Amoeba was reported in South and South parts of the country (Desalegn Begna and Amsalu Bezabih, 2001). Diagnosis made on honeybees in field and laboratory at Addis Ababa

reported a prevalence of 73% of amoeba infestation (Desalegn Begna and Yosef Kebede, 2005). The diseases was also reported with high prevalence in different regional state of Ethiopia such as; Oromia region with prevalence of (88%), Amhara Region (95%) and 60 % in Benishangul- Gumuz (Aster Yohanis *et al*., 2007). Study on annual cycle and seasonal dynamics of amoeba from the Holeta research center apiary (Amsalu Bezabih and Desalegn Begna, 1998) reported, amoeba cysts were reported throughout the year regardless of hive type. The study also reported that highest cyst number (disease intensity) in the months of April and August and lowest intensity in the month of January (Amsalu Bezabih and Desalegn Begna, 1998). This study helps to understanding the seasonal dynamics of the diseases in the area and to undertake seasonal management of colony honeybees.

2.2.3. Chalk Brood

Chalk brood is an infectious disease of honeybee larvae caused by a fungus *Ascosphaera apis*, which causes death and mummification of sealed brood of honeybee with consequent weakness of the colony (Splitoir, 1995; Splitoir and Olive, 1995). In Ethiopia the survey on chalkbrood diseases was started in the year of 2000 (Desalegn Begna, 2000). Subsequently, further diagnostic survey was conducted to determine its magnitude of distributions from October-January 2001 (Desalegn Begna, 2001). From the 13 randomly selected apiaries and 276 bee colonies diagnosed for the disease, eight apiaries and 48 bee colonies were found positive to chalk brood (Desalegn Begna, 2006). Survey on chalk brood disease in Shoa and Arsi zones, reported an overall prevalence of 56.49%, with higher in west Shoa (24.5%) followed by Arsi (13.74) and lowest in East Shoa (7.63%) and North Shoa (9.92%). A study conducted in some apiary sites in Addis Ababa (Desalegn Begna and Yosef Kebede, 2005) has also reported the disease in 43% of the colonies. Also another large scale and successive two studies which were conducted from March 2004 to November 2006 detected chalk brood disease widely distributed in many localities of Oromia, Amhara, Benshangul-Gumuz National Regional States of the country (Aster Yohanis *et al.*, 2007; Aster yohanis *et al.*, 2010). In addition to these two studies documented September to November as the highest infestation (43.6%) period of the disease followed by March to May (34.6%) incurring annual honey production lose estimated to 64% in dry alpine weather conditioned areas.

In Ethiopia the geographical distribution of chalkbrood diseases in honeybee were recorded (Aster Yohanis *et al*., 2010). The study reported an infection rate of 37.12%, 19.89%, 17.93% and distribution rate of 87.50%, 56.56% and 33.33% in Amhara, Oromia and Benshangul-gumuz. In the study a number of bioclimatic variables such as mean annual temperature, annual rainfall, annual range in temperature and precipitation were used to establish the ecology of the diseases. The finding shows that moist *'Dega'*, moist *'weina-dega'* and wet – '*weynadega'* were identified as suitable ecological zones (Aster Yohanis *et al*, 2007; Aster Yohanis *et al*, 2010). However, the dry alpine, dry '*bereha*' and moist '*bereha'* areas are not suitable for the diseases at all (Aster Yohanis *et al*, 2010). The country wide diagnostic survey from 2008-2010 showed wide scale distribution of chalk brood disease (Amsalu Bezabih *et al*., 2009; Amsalu Bezabih *et al*., 2010).

In Ethiopia the infestation and distribution rate of the chalkbrood diseases is unequal. Aster Yohanis *et al*., 2010 reported distribution rate of (100%) of in West Gojam and 95.42% in Jimma out of 33 districts sampled for the study of the diseases. Distribution of the diseases is associated with delivery of contaminated apiary equipment's such as wax foundation sheets contaminated with *Ascosphaera apis* (Flores *et al*., 2005). The highest distribution rate of in West Gojam and Jimma zone is the results of the introduction of contaminated bee equipment's through the extension service in these areas (Aster Yohanis *et al*., 2010).The disease causes loss of colony of bees and reduction in the productively in infected colonies.Aster Yohanis *et al*., 2010 reported the mean yield of honey in colony infected with chalkbrood diseases (45kg) is lower than the mean yield (80kg) in uninfected bee colony.

2.3. Major Honeybee pests reported in Ethiopia

Practical knowledge on the identifications of honeybee pests that endangers the life and the products of honeybees and developing appropriate control measure is largely a question of success in the beekeeping sector. With this understanding, assessments were conducted in different parts of the country at different times to identify local honeybee pests along with their distribution ranges and kinds of products they interact with. Accordingly, more than 15 honeybee pests were identified and recorded in the country with the bee products types they are affecting. Ants (different types), Wax moth (greater or *Galleria mellonella* and lesser waxmoth or *Achroia grisella*), mice (*Mus musculus)*, birds (different types), honey badger

(*Mellivora capensis*), wasps *(Vespula vulgaris)*, death's head hawks moth (*Acherontia atropos*), bee lice (*Braula coeca*), beetles (different types), lizards (*Lacertilia*), toads/frogs (*Amietophrynus gutturalis)*, prey mants (*Mantis religiosa*), spiders (*Achaearanea tepidariorum*), pseudo scorpions (Chellifer species) were among the honeybee pests locally registered (Desalegn Begna 2001; Desalegn Begna and Amsalu Bezabih 2001; Desalegn Begna *et al.*, 2005; Amsalu Bezabih *et al*., 2009 and Amsalu Bezabih *et al.*, 2010).

In north western Amhara, the hive and apiary inspection reported by Darsema Gulima and Awoke Berhanu (2006) revealed the existence of 10 different types of honeybee pests and enemies. According to their study, the total prevalence of pest/enemy in north western Amhara was 90.1%, Spiders 81.8%, Ants 78.7%, Wax moths 70.4%, Termites 61.6%, Lizards 59.3%, Wasps 43.3%, Beetles 39.1%, Badgers 34%, Birds (bee-eaters) 22.5% and Bee-mites 8.6% (Darsema Gulima and Awoke Berhanu, 2006).

2.3.1. Wax moth

The wax moths are one of the most important pests of honeybee colony with worldwide distributions and it was identified as one of the serious local honeybee pests (Desalegn Begna, 2001).Wax moths cause significance damage in colony of honeybees in several countries such as: Algeria, Egypt, Kenya, Tanzania, Uganda, Sudan, Ghana, Nigeria and Senegal (Moustafa, 2001). The wax moth (smaller and larger) in honeybees were reported in the South and South West parts of Ethiopia in the year 2000 (Amsalu Bezabih and Desalegn Begna, 2001). Understanding the severe problems due to wax moth, research program that aimed at assessing its prevalence and special effects on honeybees and their products has been conducted in selected three zones of the country (Amsalu Bezabih and Desalegn Begna, 2007). The study investigated the prevalence of the wax moth varies from zone to zone and southwest Shewa is with high infestation level (26.6%) followed by West and East Shewa 22.85 and 26.66%, respectively.

Also, the study indicated that about 56-75% of the waxmoth infected honeybee colonies are registered as absconded and the rest dwindled (Amsalu Bezabih and and Desalegn Begna, 2007). The diagnostic survey conducted by Amsalu and his colleagues indicated wider scale distributions of the pest (Amsalu Bezabih *et al*., 2009 and Amsalu Bezabih *et al.*, 2010). In

the considerations of its widely distributions and serious damages, practical prevention experiment designed and identified effective preventive and/or control management practice that is 82.3% effective in restraining the pest from entering the bee hives (Amsalu Bezabih *et al*., 2011). Hence, the study recommended management techniques of strengthening honeybee colonies via feeding; removing unoccupied suppers and combs, combinations of these practices; and trapping adult wax moths before it enter beehives (Amsalu Bezabih *et al*., 2011). Moreover, the study identified Dec-March as high prevalence seasonal of the pest, which obviously overlaps with dearth period (feed scarce season) (Amsalu Bezabih *et al*., 2011).

Similarly wax moths (*Gallera mellonela*) and the night flying sort of wax moth (*Aphomia sociella*) was reported in honeybees in the study carried in Tigray regional state in three district of Atsewonberta, Aheferom and Wukro (Etsay Kebede and Ayalew Kassaye, 2001). In the five regional states of Ethiopia wax moths ranked among the disastrous pests of honeybees by bee keepers (Amsalu Bezabih et al, 2010). Alemu Tsegaye *et al.* (2014) reported that 33.3% of the experimental colonies have absconded due to wax moth infestation at Wag-lasta area of the Amhara region. Based on the current trends in wax moth damage in the country, studies on all possible safe preventive strategies will be a focus of future national research directions. Accordingly, the prevention methods, that they have tested in this study against wax moth attack, have indicated that supplementary feeding to ensure more colony population and bring about vigorous colonies that can safe guard their hives against wax moth by themselves and colony fumigation with tobacco leaf smoke for lower wax moth infestation have to be used as ready-to be-used combined recommendation (Alemu Tsegaye *et al*., 2014).

2.3.2. Ants

Ants are most troublesome to honeybees and bee keeping sector. Among the enemies of honeybees registered in Ethiopia, Ant shares the greatest grievances in causing serious problems on honeybee colonies. It kills bees, robs their products and forces the bee colonies to leave their proper nest which results in reduction of honey production. In Ethiopia ants in colony of honeybees were reported from different regions such as Addis Abeba (Desalegn Begna and Yosef Kebede, 2005), Keffa, Shako and Bench-Maji zone (Awraris Getachew *et al*., 2012), Kombelcha (Tesfaye Kebede and Tesfaye Lema, 2007), highlands of Southeast

Ethiopia (Solomon Bogale, 2009). In Atsbi-Womberta, Ahferom and Wukro districts of Tigray regional state, there was reported on the occurrence of ants as a serious problem in beekeeping (Etsay Kebede and Ayalew Kassaye, 2001) and the problem was also considered to cause a major problem in the adoption of improved beekeeping technologies (Workneh Abebe, 2007). In Tigray, Amhara and SNNP regional states and Gomma district Jimma zone beekeepers ranked ants as the first problematic pest in honeybees (Amsalu Bezabih *et al.*, 2010). Study on ants economical effect assessment was conducted in two potential beekeeping zones (West and South west Shewa) and identified 44.2% of honeybee colonies attacked by ants every year of which 24% absconds and 4.2% dies (Desalegn Begna, 2007).The study also estimated the annual honey yield loss to 29% and the general economic losses both in terms of honeybee colony their products to over 3,839,810 birr (Desalegn Begna, 2007). Understanding this real problem from ant, three ant protection methods (inner tube, smooth iron sheet and tin filled used engine oil) were developed and their effectiveness in subduing ant effects were evaluated (Amsalu Bezabih and Desalegn Begna, 1999). According to the result from the effectiveness evaluation, no considerable differences between the tested methods were reported (Amsalu Bezabih and Desalegn Begna, 1999).

2.3.3. Small Hive Beetle

The small hive beetle, *Aethina tumida* Murray, is a nest parasite of honeybees (*Apis mellifer* L.) colonies native to sub-saharan Africa, where it is considered a minor pest of honeybees. The pest was reported from many African countries such as South Africa (Lundie,1940), Kenya, Namibia and Eritrea (Mustafa and Williams, 2002).The beetles multiply to huge numbers, their larvae tunnel through comb to eat brood, ruin stored honey, and ultimately destroy infested colonies or cause them to abscond. The beetle also defecates in the honey, causing it to ferment and run out of the combs. Until 2006, there was no any report on its occurrences in association with local honeybees (Desalegn Begna and Amsalu Bezabih, 2006). The first hand report from Desalegn Begna and Amsalu Bezabih (2006) indicate that the investigation of this pest in the country was done through external and internal inspections of 427 bee colonies located in six districts/places and 43 bee colonies with its prevalence ranging from 21-66% (Desalegn Begna and Amsalu Bezabih, 2006). Subsequent studies on the distribution of the pest showed its wide distributions in maize and coffee growing areas of

Oromia regional state; Jimma, Illu Abbabora, Horo Guduru, Wollega, East Wollega and Western Showa(Amsalu Bezabih and Desalegn Begna, 2008) and in most part of the country (Amsalu Bezabih *et al* 2009 and Amsalu Bezabih *et al* 2010). According to the study that was concluded in 2008, the pest is known to exist in Jimma, west Shoa and East Wellega zones with the infestation rates of 60, 22.6 and 22.5%, respectively (Amsalu Bezabih and Desalegn Begna, 2008). Similarly the small Hive beetle was reported in six districts; Mega, Moyale, Teltele, Konso, Key-Afer and Segen in the South and Southern parts of Ethiopia with prevalence infection ranges from 21% in Konso to 66% in Teltele (Amsalu Bezabih and Desalegn Begna, 2006).The study speculated that the locality of the pest in region might be that the pest entered to the country from this direction.

2.3.4. Bee Louse (Braula Coeca)

Bee louse are wingless ectoparasite fly which causes significant damage in colony bees. Bee lice larvae feed on honey and pollen by tunneling under the cell capping (Morse and Nowogrodzki, 1990). The adult lice feed on nectar directly from the mouth of honeybees, this reduce food availability of queen and reduce egg-lying capacity (Somerville, 2007). Bee louse are widely distributed in Africa, Asia, and North America and southern of Africa. A cross sectional study was carried out to determine prevalence of bee lice, and to find out associated risk factors in Holleta and its surroundings, West-Shoa zone of Oromia region. According to this study out of 385 bee colonies examined, an overall prevalence of 42% lice infestation was observed. The study also indicated that the highest prevalence (70.8%) of bee lice was observed in Gemechis, followed by Holleta (50%), while the lowest prevalence (17.1%) was observed in Jaldu. Prevalence of lice observed in bees kept in apiary management system (50.4%) higher than those bees kept in backyard (37.9%). Higher prevalence of bee lice observed in medium altitude areas (50.4%), than that of highland areas (40.4%; Gemechu Gizachewu *et al*, 2013).

Similar study conducted in Tigray regional state reported an overall prevalence of 4% and 5.5% in brood and adult bees in three rural kebeles of Genfel, Adikisandid and Aynalem in Wukro districts (Adeday Gidey *et al*, 2012). The study reported the main lice species affecting honeybees in the area is *Braula coeval*. Similarly bee louse was also identified as major constrains of bee keeping in Addis Ababa (Desalegn Begna and Yosef Kebede, 2005),

in Illu Ababora and Arsi Zone (Aster Yohanis *et al*, 2010) and in Burie District of Amhara (Tessega Bellie, 2009).

2.3.5. Varroa mite

Nation-wide diagnostic survey from 2008-2010 to identify and locate major honeybee disease and pests of honeybee showed wide scale distribution of *Varroa* mite in the largest areas of the eastern parts of the Amhara National Regional State (Abebe Jenberie *et al*., 2010, unpublished). Generally except few places, almost all areas starting from Abergele district (Waghimra Zone) up to Kalu district in (South Wollo) *Varroa m*ites has been observed, on the other hand during that time some areas like Desse Zuria and Legambo districts and some localities in some districts were free from the mites. The study reported 56% and 42.6% prevalence of varroa mite in the sampled districts and sampling districts of the eastern Amhara Region (Abebe Jenberie *et al*, 2010, unpublished). The study also reported the variability of varroa mite infestation from place to place with high degree of infestation of brood observed in South wollo at *Tehuledere* districts at *Jarie* sampling locality(Abebe Jenberie *et al*, 2010, unpublished). Darsema Gulima and Awoke Berhanu (2006) also reported 8.3% prevalence of varroa mites in western Amhara.

The first hand investigation report for the presence of varroa mite was published by Desalegn Begna (2014).. According to his report, all the surveyed areas tested positive to varroa mite with infection levels ranging 37.5% with the overall infestation rate of 82% and high infestation rates (100%) was reported for Alamata, Kilete Awulalo and Ganta Afeshum of Tigray Region.

2.4. Risk of pesticide poisoning to local honeybees of Ethiopia

2.4.1. Status of pesticide Use

Pesticides of various kinds such as organophosphates, carbamates, and to some extent organochlorides have been widely used in Ethiopia for the last four decades. Some pesticides that are restricted and banned in industrialized countries are used in many third-world countries. Pesticide sprayers are regularly engaged in spraying pesticides that are applied at different growing stages of a particular crop (Wessling *et al*., 1997). They are usually applied

as an aerosol produced from knapsacks and from simple hand sprayers. Misuse and overuse of pesticide is very common among farmers of developing economies and Ethiopia is no exception (Mekonnen and Agonafir 2002).

Recently, there has been much debate on the indiscriminate use of agrochemicals that result in environmental pollution and toxicity risk to non-target organisms. Agrochemicals can contaminate soil, water, turf and vegetation. In addition to killing, honeybee it can be toxic to most of other organisms including birds, fish and beneficial insects. Some chemicals no longer effective and are also banned in many countries (Akca *et al*., 2005).

According to Amsalu Bezabih *et al*, 2012 result, all tested agro chemicals, except agro 2, 4-D amin 720 A, caused significant mortality on honeybees when ingested with food (figure 4). The widely used agrochemicals except agro-2, 4-D Amin 720A, were highly toxic to honeybee. This result also indicated that Thionex 35 EC has caused significantly high mortality of honeybees within half an hour of chemical application compared to other tasted chemicals and control. Ethiodemeteren 2.5 % EC, Ethiolathione 50 % EC and Agro-thoate 40% EC had significant toxicity effect for more than a day. The latest chemical which is registered as highly toxic standard chemical in Europe is on use in Ethiopia (Amsalu Bezabih et *al*, 2012). This indicates that agrochemicals imported for crop protection and pest control are highly toxic to honeybees and cause reduction of honeybee colonies, which in turn result in reduction of bee products and crop yield obtained by honeybee pollination (Desalegn Begna, 2015).

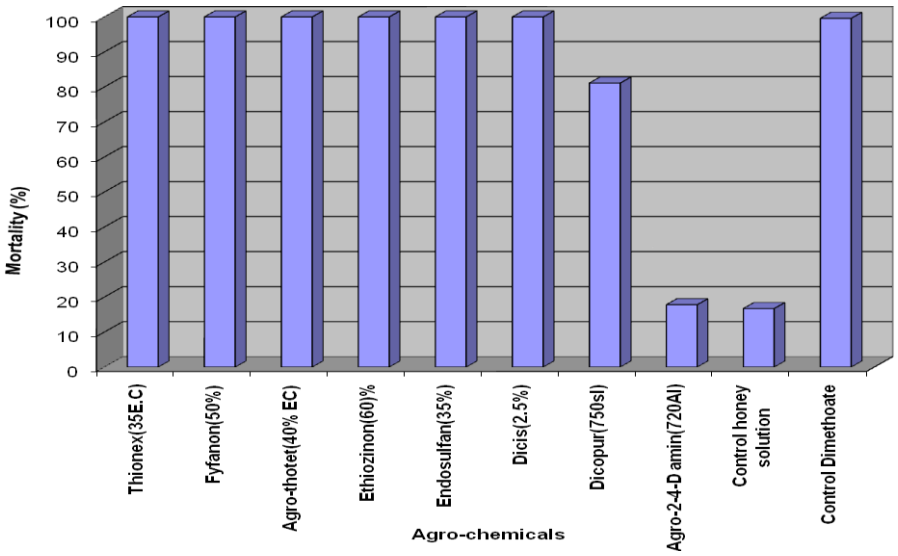


Figure 4 . Mortality of honeybee caused due to agro-chemical ingestion

(Source: Amsalu Bezabih *et al*, 2012)

According to the survey result by Marta Zelalem, 2013 at Mecha district of Amhara region, among the crops through which agrochemicals were applied; teff, barley, wheat, millet and onion are non-attractive to bees while potato and maize are attractive to bees. In addition, Vetch, Orange and mango are highly attractive to honeybees. In the area most of the farmers apply the chemical on mango (92.9%), orange (97.2%), potato (81%) and maize (81%) at blooming and on *teff* after blooming (Marta Zelalem, 2013). This shows that most of the farmers sprayed agrochemicals on crops and fruits that are highly attractive to honeybees at the blooming stage when honeybees are actively foraging (Marta Zelalem, 2013). Direct contacts with foliar spray are the most obvious exposure routes for honeybees.

On the other hand, in wetern Amhara Region at Mecha district the types of pesticides applied are malathion (76%) followed by actalic (methyl parathion) (24%). Malathion is a group I which is highly toxic to honeybees and severe losses may be expected if used when bees are present at treatment time or within a day thereafter (Marta Zelalem, 2013). However, farmers in the area apply the chemicals as liquid spray in the morning (27%) and in the middle of the day (73%; Marta Zelalem, 2013). This indicates that agrochemicals are applied when honeybees are actively foraging and they are exposed to direct spray. Applying agrochemical, having a residual hazard to bees at late evening, after the bees have stopped foraging, and mid

night are the best times to protect honeybees against the effect caused by pesticides and herbicides (Rediel *et al*., 2006).

2.4.2. Effects of pesticides

The use of chemicals and pesticides for crop pests, weeds, *Tsetse* fly, mosquitoes and household pests control brings in to focus the real possibility of damaging the delicate equilibrium in the honeybee colony, as well as the contamination of hive products. Pesticides can kill bees and are strongly implicated in pollinator decline, the loss of species that pollinate plants, including through the mechanism of Colony Collapse Disorder in which worker bees from a beehive abruptly disappear. Application of pesticides to crops that are in bloom can kill honeybees which act as pollinators (Fox *et al*., 2007).

The USDA estimate that US farmers lose at least $200 million a year from reduced crop pollination because pesticides applied to fields eliminate about a fifth of honeybee colonies in the US and harm an additional 15%. Important losses to beekeepers and to agriculture in general have been caused by certain pesticides appearing on the market during the last few years (Anderson and Atkins, 1958).

Effects of insecticides

Insecticides are a particular class of pesticide specifically designed to kill insect pests of crops and livestock, or in domestic environments. They kill or repel insect pests at high enough doses (lethal), but they also may have unintended (sub-lethal) effects at low doses on non-target insects, including upon the natural enemies of pests and upon pollinators (Desneux et al, 2007). it is now more evident than ever that some insecticides show clear negative effects on the health of pollinators, both individually and at the colony level (Mullin et al, 2010). Some insecticides are systemic, meaning that they do not stay outside when applied to a plant, but enter the plant system and travel through it (EFSA, 2012).The neonicotinoid chemicals become distributed through the plant stems and leaves, and may eventually reach the guttation water (drops of water produced by the seedling at the tip of the young leaves). Consequently, bees feeding on these flowers will potentially be exposed to the chemicals in this way as well. The increased use of neonicotinoids means there is a greater potential for pollinators to be exposed to these chemicals over longer periods, as systemic insecticides can be found in

various places throughout the life cycle of a plant, growing from coated seed, to guttation water, and to the pollen and nectar of plants throughout their blooming period (Ellis, 2010).

Moreover, there are two other circumstances in which bees are killed on plants by chemicals these include insecticides applied to non-crop pests such as mosquitoes and *Tsetse* flies and by herbicides applied to plants on which the bees are foraging. Insecticides have a much more dramatic effect on population of honeybees by jeopardizing the contribution of bees to the production of food and human nourishment (Keralem Ejigu *et al.*, 2009).

Bees Effects of insecticides on pollinators can be described as acute or lethal, when the effects are quick and severe and cause rapid mortality, and sub-acute or sub-lethal, when the effects do not induce mortality in the experimental population, but may provoke more subtle physiological or behavioural effects in the longer term, for example by impacting learning performance, behaviour or other aspects of neurophysiological performance (Desneux et al., 2007).

It is effective against a broad range of insects and can be applied in ways that reduce wind-borne cross-contamination, such as soil treatment and seed dressing. It is frequently used in rice, maize, sunflowers, rape, potatoes, sugar beets, and many vegetables and fruits (Bonmatin *et al*., 2005).It should not be surprising that insecticides are toxic to honeybees even at very low levels of exposure. After all honeybees, are insects and therefore it is unreasonable to assume that we can kill all the unwanted insects and preserve the beneficial ones by slight manipulations of dose and timing. Many researchers have discovered that honeybee brood exposed to low doses of insecticide exhibits a variety of symptoms that may affect colony survival (Tesoriero *et al*., 2003).

Effect of herbicides

Herbicides may affect bees by limiting the food resources available to them and to other pollinators, especially if the large-scale crop monocultures typical of industrial agriculture are also present (Brittain and Potts, 2011). The body size of the pollinator might determine the overall impact, with smaller species being more impacted. Larger bees might be able to fly further foraging for food, but smaller ones might starve (Brittain and Potts, 2011).

Although herbicides are often considered harmless to honeybees, research shows otherwise. For example, Papaefthimiou *et al*. (2002) found cell death in the isolated atria of the honeybee heart (*Apis mellifera macedonica)* after exposure to the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D). Only 1 μM (micro mol) of 2, 4-D is required to reduce the force and frequency of heart contractions by 70% in 20 minutes. The honeybee is much more sensitive to this chemical than other insects tested.

Effect of anti-malaria

The use of DDT for mosquito and other insect control has sometimes resulted in many dead or weakened bee colonies. Losses usually have resulted from daytime air applications of DDT at times when large numbers of bees are flying, failure to provide adequate warning to beekeepers and failure to follow a publicized spray schedule (Atkins, 1982).

Effects of fungicides

Farmers routinely apply fungicides to many bee-pollinated crops during the blooming period when bees are foraging, as they are classified as less toxic to bees, and currently there are few restrictions to this practice. However, some fungicides have exhibited direct toxicity to honey or solitary bees at field use rate (Mullin *et al,* 2010). Equally as worrying, some fungicides have been found to increase the toxicity to honeybees of pyrethroid insecticides (Brittain and Potts, 2011). Mullin *et al.,* (2010) indicates that: frequent coincidence in pollen of high levels of the non-systemic fungicide chlorothalonil with lower levels of systemic pesticides including fungicides is another probable synergistic combination that needs further exploration concerning bee decline.

High levels of fungicides in stored pollen might also inhibit the growth of certain strains of fungus that are necessary to convert pollen in to honeybee bread. The loss of the fungus could reduce the nutritional value of the pollen to honeybees (Oliver *et al.,* 2005). In addition, fungicides may disrupt the lactic acid bacteria. Without the lactic acid beneficial microbes produce, other microorganisms may decompose the honeybee bread which becomes useless or harmful to the honeybees (Vasquez and Olofsson 2009).

2.4.3. Routes of agro chemicals exposure to bees

The risk posed by pesticides to honeybees showed that foraging bees and larvae are the most exposed category of bees via oral exposure. However, nurse bees are also highly exposed when considering simultaneous oral exposures via pollen and nectar. Larvae in contact with wax and foragers, drones, queens and swarms intercepting droplets and vapour in/out field were found to be the most exposed categories of bees via contact and inhalation exposures, respectively (Winston,1997).

Contaminated pollen and nectar

The presence of chemicals in nectar and pollen delivers the active ingredient directly to the bees and other pollinators. Some systematic insecticides can be very persistent, staying in plant tissues for many months or even years, and may build up after repeated applications. Honeybee larvae are primarily fed royal jelly and consume only small amounts of diluted honey and pollen. Maximal exposure to systemic insecticides is expected among honeybees that consume the greatest amounts of contaminated pollen and nectar. Large amounts of pollen are consumed by nurses and to a less extent by larvae, whereas large amounts of nectar are consumed by wax-producing bees, brood attending bees, "winter" bees, and foragers (Jay, 1963).

Neonicotinoids are found in pollen loads brought to hives by honeybees (Winston, 1997). Honeybees can also intoxicate the whole colony by bringing contaminated pollen and nectar back to the hive (Villa *et al*., 2000). However, the risk of any chemical transfer into the hive is greater with systemic insecticides (Waller *et al*., 1984).

Direct spray

Insecticides sprayed on plants can be toxic to foraging honeybees when they are in contact with treated plants and when they fly through the adsorption of contaminated dust particles (Prier *et al*., 2001). Most pesticides sprayed on the surface of the plant have a rapid and residual action of a few hours to a few days, whereas systemic insecticides penetrate into the plant and protect it all through its development from soil invertebrates and in some cases from sucking insects (Elbert *et al*., 1991).

Direct contacts with foliar spray may be the most obvious exposure routes for bees. This may occur when an application is made while bees are actively foraging on flowers or nesting on the ground with in a field or, when pesticides drift on the adjacent habitat. The most serious problems to honeybee colonies occur when hives are directly sprayed with an insecticide or are covered by spray drift. However, bees commonly forage within a radius of 1-3 kilometres from the hive and can extend this distance to 11 km when food is scarce (Jennifer *et al*., 2012).

Spray applications on flowering crops or honey dew cause a contamination of nectar and pollen. Residues may also be translocated in nectar and pollen from spray applications and systemic seed and soil treatments (SSST). For residues in nectar, pollen and honey, data is only available for a limited number of substances. The concentration in nectar and pollen can be used to predict exposure to both foraging bees and bees of other casts in the hive, including larvae (EFSA, 2012). Imidacloprid is used as a foliar spray or a seed treatment on a variety of crops that includes corn, canola, blueberries, and sunflowers. Usage labels require the bees to be protected from the foliar spray, even though it has been found to be more toxic to honeybees when ingested than when exposed by physical contact (Suchail *et al.*, 2000).

Contaminated water

Honeybees may be exposed to agro chemicals when they gather water to cool their hives on warm days or to dilute their honey to feed to offspring's (Jennifer *et al*., 2012). The unknown respective amounts of water consumed by foragers (whether coming from puddles, surface, leaves and/or axils) and the unknown amount and fate of water inhaled by in-hive bees did not allow to characterise the risks (EFSA, 2012).

Guttation fluid

Guttation fluid is the water given off by the plant in the morning, as droplets at the tip of the plant or around the edges. Honeybees and other pollinators may collect these droplets from plants treated with systematic insecticides (Jennifer *et al.*, 2012).Girolami *et al*. (2009) determined that guttations of seed-treated corn plants can contain high concentrations of imidacloprid, clothianidin and thiamethoxam, and these droplets are highly toxic to honeybees. There are a number of factors affecting the residues in guttation fluid, e.g. formulation, metabolism within the plant, application methods, adjuvant, and solubility of the active ingredient and plant species. Metabolism within the plant also affects residues levels; the

systemic herbicides dichlobenil and vernolate are extensively metabolised in plants, and dichlobenil has a high affinity for plant tissue (Bickers *et al*., 1996). A problem with this type of water collection occurs in agricultural areas where plants are treated with systemic insecticides. Bees collecting guttation drops can be poisoned by systemic pesticides flowing through the xylem. Worse, sub lethal, but potentially harmful, doses of pesticide can be carried back to the hive and fed to the developing larvae by way of the nurses. Researchers are currently trying to determine the type and frequency of damage this may cause to honeybee colonies (Dorothy, 2010).

2.5. Risk of poisoning from poisonous plants to local honeybees of Ethiopia

A number of plant species are poisonous to honeybees. A recently emerged red color flower locally called *Ababbo Diima* (Abiyu Zewdie, 2011) was reported to kill worker bees during their flowering stages. Plant species belongs to families of Ranuculaceae, Solanaceae, Acanthacae, Euphorbiaceae and Phytolacaceae were reported to poison honeybees (Nuru Adgeba, 2002). Similarly the nectar or pollen of poisonous plants such as *Cassia siamea, Croton macrostachyus, Aloe brahana, Zizyphus mucronata, Phytolacca dodecandra* and *Susbania* species were reported to be toxic to the bees themselves and those in which the honey produced from their nectar are toxic to humans (Kerealem Ejigu *et al*., 2009). Similarly honey from *Datura arborea* is reported irritates human beings when eating and *Euphorbia cottinifolia* is known to kill honeybees (Awraris Getachew *et al.,* 2012). These studies indicated the emergence of new enemies of honeybee pest plants; this needs a scientific study on the real impact of these pest plants on health of honeybees their influence on the productivity to mobilize community in identification and eradication of the pest plants form the environment. Therefore poison plants which have an economic important on honeybees in our country should be investigated and studied to take the right cautions.

CHAPTER 3: MATERIALS AND METHODS

3.1. Description of the Study Area

3.1.1. Description of Amhara Region

The Amhara National Regional State is one of the regional states in the federal democratic republic of Ethiopia which extends from 9° to 13° 45'N and 36° to 40° 30'E. It covers approximately 161,828.4 Sq.km in area and is moderately compact in shape. Its coverage is 11% of the country's total area. This land consists of three major geographical zones. These are the highlands (above 2,300 masl), the mid-lands (1,500 to 2,300 masl) and lowlands (below 1,500 masl) accounting 20%, 44%, and 28% of the region's total area coverage respectively (ANRS BOFED, 2011).

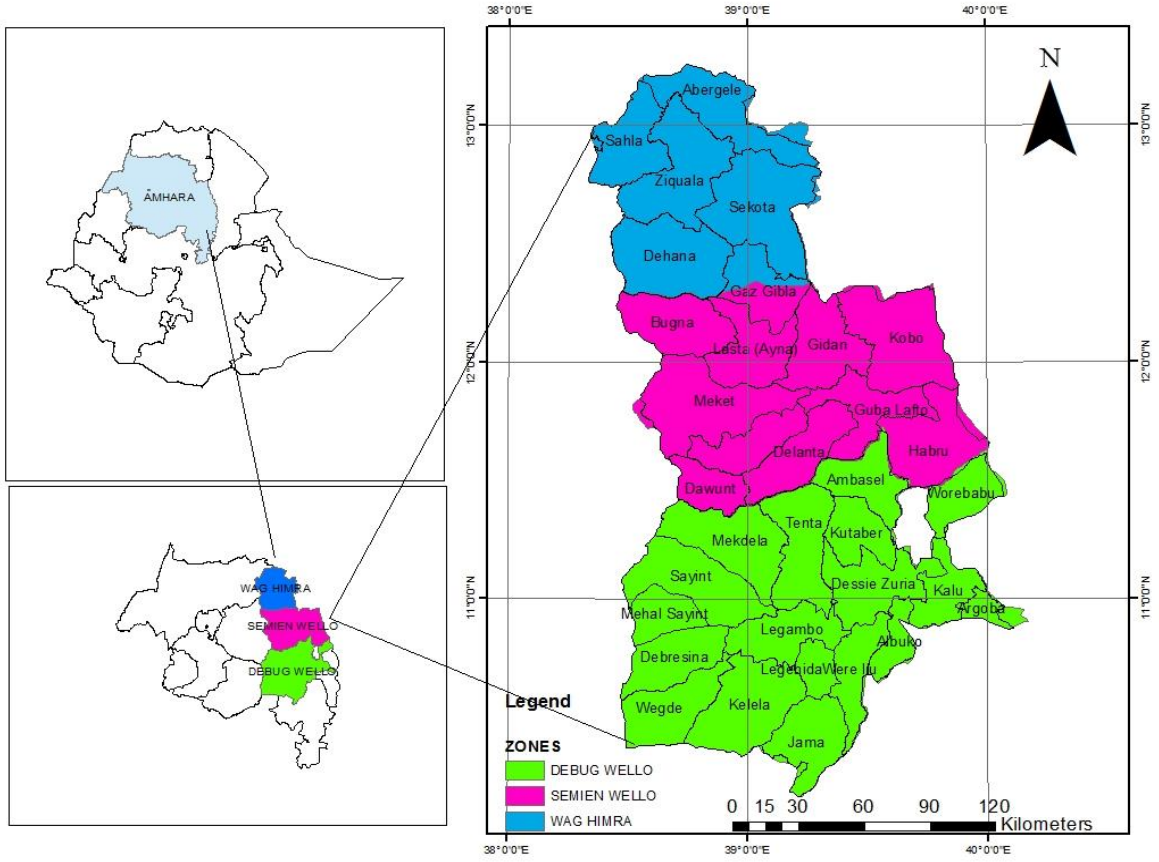


Figure 5. Map of the study area

The regional state is made up of 11 administrative zones categorized into Western Amhara (East Gojjam, West Gojjam, Awi, Bahir-Dar, South Gondar and North Gondar administrative zones) and Eastern Amhara (Waghimra, North Wollo, South Wollo, Oromiya and North Shewa administrative zones). These zones are divided into a total of 113 woredas and 3,216 kebeles. The region's topography embraces plains, gorges, plateaus, hills and mountains. The altitude ranges from as low as 500 metres to 4,620 meters at the peak of Ras Dashen.

3.1.2. Description of South Wollo Zone

South Wollo is also one of the 11 Zones of the Amhara National Regional State (Figure 2). The administrative capital of south Wollo, Dessie, is located at about 400 kms North of Addis Ababa. The zone is bordered in the South by North Shewa and Oromia Region, in the West by East Gojjam, in the North-west by South Gondar, in the North by North Wollo, in the North-east by Afar Region, and in the East by the Oromia Zone and Argobba special districts. The zone covers three main agro-ecological zones, namely: '*Dega*' (above 2,500m), '*Weyna dega*' (between 1,600m and 2,500 m a.s.l) and '*kolla'* (from 1,300-1,600m) within 17 rural and two urban districts. Most parts of the zone are characterized as mountainous and the rest as hilly. The land is highly degraded and the area is deforested in terms of indigenous trees but does have substantial eucalyptus plantations. Alpine species unique to extreme highland areas are also found in some parts. The zone is densely populated with 150-250 persons per km^2.

In most of the areas of South wollo, rainfall is bimodal, with short *'belg'* rains from March to April and longer '*keremt'* rains from July to September. The area has a long-term mean (1162mm) rainfall per annum. The monthly average maximum temperature is 26.4^0C whereas the minimum is 12.6^0C.

Like Waghimra zone, livestock in general and small ruminants and bee colonies in particular are playing a very important role for the farming community in the study area. Regarding the number of livestock, South wollo is estimated to possess 1,564,091 cattle, 1,948,943 sheep, 720,700 goats, 1,662,389 poultry, 112, 656 bee colonies, 86,221 horses, 380,608 donkeys, 13,101 camels, and 29,229 mules (CSA, 2013; Table 2).

Table 2: Amhara Region bee colony zonal distribution and regional share of each zone

Zone	Area coverage		Bee Resource		Bee colony per km^2
	Km^2	Regional share	Bee colony	Regional share	
North Gonder	45,561	28.26	227,463	22.02	4.99
South Gonder	20,061	12.44	153,746	14.88	7.66
North Wolo	10,177	6.31	58,352	5.65	5.73
South Wolo	17,462	10.83	112,656	10.91	6.45
North Shewa	17,698	10.98	54,314	5.26	3.07
East Gojam	14,705	9.12	125,354	12.14	8.52
West Gojam	13,910	8.63	165,390	16.01	11.89
Waghimra	8,421	5.22	53,193	5.15	6.32
Awi	8,579	5.32	75,084	7.27	8.75
Oromia	4,665	2.89	6,416	0.62	1.38
Total/Regional	161,239		1,032,927		6.41

Source: CSA, 2013

3.1.3. Description of Waghimra Zone

Waghimra Zone is one of the 11 administrative zones in Amhara National Regional State (ANRS) that is inhabited by the Agew ethnic group comprising six woredas (Figure 2). The woredas are: Sekota, Dehana, Ziquala, Abergelle, Sehala and Gazgibla. Sekota town is the capital of the zone. Sekota is located between 12^0 23' and 13^0 16' N latitudes and 38^0 44' and 39^0 21' E longitudes (Adefress *et al*., 2000). With an area of 9,039.04 square kilometers, Wag Hemra has a population density of 47.15 persons per km^2.

Livestock in general and small ruminants and bee colonies in particular are the ones who are playing a very important role for the mainstay of the farming community in the study area. Regarding the number of livestock, Waghimra is estimated to possess 333,225 cattle, 162,068 sheep, 467,816 goats, 420,267 poultry, 53,193 bee colonies, 98 horses, 69,575 donkeys, and 2,942 mules (CSA, 2013; Table 2).

In Waghimra, the annual rainfall, which is erratic in distribution and amount, varies between 350 and 650 mm (AMAREW, 2006). The numerous hills and rugged topography found are the ones seriously limiting access to the various parts of the zone. The forest and bush cover of the area is concentrated in specific areas most of which are communally owned or possessed by churches. The topography is dominated by a number of deep gorges, ups and downs and series of rugged massif (uneven mountains).

However, beekeeping is a common culture and farming practice in the study area. The deep gorges and series of rugged massif topography resulted in the presence of different ecologies adjacent to each other promoting diverse vegetation types to grow at closest distance giving wider choices for bees to forage on. Cultivated plants, herbs (weeds) and bushes/shrubs are the main bee forages in wet seasons.

3.2. Methods of Data Collection

3.2.1. Scope, coverage and sample size

This honeybee diseases, pests and poisoning effect research project was comprising two main components: component one was a Cross sectional Study and component two was a Seasonal Monitoring of varroa mite.

Component One: The Cross sectional study

The survey covers two zones of the Eastern Amhara Region (Waghimra and South Wollo). From each zone, three districts and from each district three rural kebeles were purposively selected based on their altitude differences, beekeeping potentials and accessibilities. The sample size (number of respondent beekeepers) required for the survey was determined based on sample size determinations for populations that are large, developed by Cochran (1963) to yield a representative sample for proportions using the following equation.

$$n_0 = \frac{Z^2 pq}{e^2}$$

Where n_0 is the sample size, Z^2 is the abscissa of the normal curve that cuts off an area α at the tails ($1 - \alpha$ equals the desired confidence level, e.g., 95%), e is the desired level of

precision, p is the estimated proportion of an attribute that is present in the population, and q is 1-p. Accordingly a total of 384 farmer beekeepers were selected for a questionnaire survey and it was distributed to the selected districts and rural kebeles proportionally. The respondent farmers were then selected randomly.

The sample size required to select each of the colonies from sampled apiaries for the diagnosis purpose were determined based on sample size determination in random sampling methods for infinite population, using expected prevalence of disease and pests and 5% desired absolute precision, according to Thrusfield (2005) using the following formula.

$$n = \frac{1.96^2 P_{exp}\left(1 - P_{exp}\right)}{d^2}$$

Where: n= required sample size

P_{exp}= Expected prevalence

d = Desired absolute precision

To estimate prevalence of bee diseases and pests using 50% expected prevalence with 95% confidence interval at 5% absolute precision, the number of hives required and calculated were 384. Accordingly a total of 384 honeybee colonies were selected for a diagnostic survey using random sampling methods and it was distributed proportionally to each of the selected apiaries. In general, a total of 2 zones, 6 districts, 18 rural *kebeles*, and 384 beekeeping farmers were selected for the diagnostic survey. A total of 384 bee colonies were also inspected during this diagnostic survey.

In order to compare the different colony management systems (backyard and established beekeeping) during the diagnostic survey, colonies were selected randomly from each of the sampled and categorized apiaries in each of the rural kebeles addressed. Honeybee colonies were inspected internally and externally and data on the health status of the colonies were collected. During this survey, both adult honeybees and sealed brood samples or empty old brood combs were also collected from each of the inspected colonies for further laboratory analysis.

Table 2. Proportional multi stage sampling for selecting the sampling sites

			Colony			Beekeepers		
Zone	Districts	Rural '*Kebeles'*	No	Proportion	sample	No	Proportion	Sample
Wag Himra	Dehana	Chilla	632	0.36	24	373	0.33	24
		kewzba	491	0.28	19	267	0.23	18
		Amdework	609	0.35	23	504	0.44	33
	Sekota	wolleh	523	0.24	19	236	0.25	19
		Fikre selam	937	0.43	34	477	0.51	38
		Tsemera	733	0.33	27	226	0.24	18
	Ziquala	Tsitsika	1640	0.46	21	192	0.36	16
		Netsanet melkam	1133	0.32	15	197	0.37	16
		Addisu firie	811	0.23	10	140	0.26	12
South Wollo	Dessie Zuria	abaso kotu	277	0.35	28	85	0.35	30
		Gelsha	157	0.2	16	69	0.28	24
		Asgedo	248	0.45	35	92	0.37	32
	kalu	Addis mender	118	0.31	17	46	0.24	14
		Tekaki	144	0.37	21	73	0.39	23
		Woraba	124	0.32	18	69	0.37	21
	Tehuled erie	Jari	128	0.41	24	71	0.4	20
		015	105	0.34	20	59	0.33	15
		Gobeya	80	0.26	15	48	0.27	13

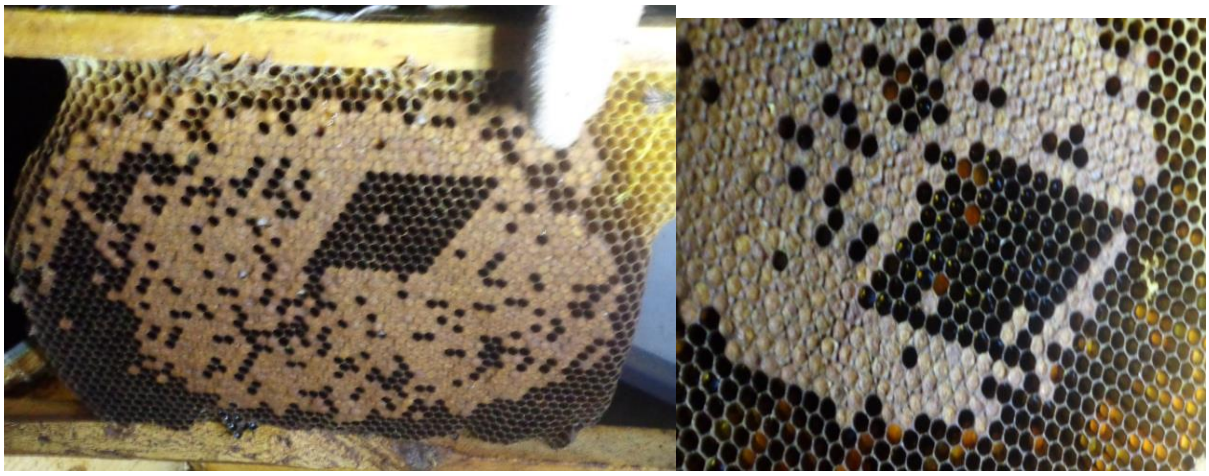
A digital GPS instrument was used to record the geographical positions of each of the localities where colonies were sampled. Moreover, to identify and characterize the more suitable ecologies for the occurrence of the economically important diseases and pests, samples were taken from different agro-ecologies (lowland, midland and highlands). Number of inspected colonies, absence, and presence and infection/infestation rate of diseases and pests were recorded. To determine favorable factors for perpetuation of the agent, metrlological data like rainfall and temperature were taken from metrological stations during the study period. Finally, distribution rates, favorable ago-ecologies and weather conditions were determined using chi square and logistic regression models.

Component two; <u>Monitoring of Infested colonies</u>

In this component, the correlations between the degree of infestation and colony performance and also seasonal dynamicity of varroa mite were determined. From each district, one apiary (i.e a total number of 6 apiaries from all the districts addressed) with a total number of 60 colonies were assigned for monitoring.

The degree of infestation was determined using sugar shake methods. To determine the variation in infestation levels, samples of 200-250 live adult bees were collected from each colony and diagnosed in the laboratory for six months (September, October, November, August, May and June). Seasonal dynamicity of the pests was also assessed through determination of the pests' population. Moreover, the health status, flight condition, wing deformity and foraging activity of the colonies were assessed during arranged honeybee's early and late foraging time observations.

Hygienic behaviour of honeybees was also determined through the the pin test method (counting of uncapped and removed pin killed broods) (Figure 6). Besides, data on bee colony strength indicator parameters like number of frames covered by bees, brood area, and pollen and nectar storage areas were estimated every month using liebfelder method to evaluate the performance of each experimental colony in relation to seasonal dynamicity of the environment. Both adult bees and brood samples collected from sampled colonies were examined and confirmed at the laboratory according to the OIE procedures (2008).



Figurce 6. Pin killed dead brood removal (hygienic behavior) of local honeybees

3.2. 2. Study design

A cross-sectional study was designed to determine prevalence of varroa mite and other honeybee diseases and pests, and to find out associated risk factors. During sampling, one bee hive was considered as one colony. Types of hives, metrological variables, agro-ecologies, and types of management were considered as explanatory variables (risk factors), and tested whether they have an impact on occurrence of varroa mite or not. Based on altitude, they were categorized as lowland (less than1500), midland (1500 to 2500) and highland (2500-3000) meters above sea level. Based on the management systems observed, hives were categorized as backyard and established apiary colonies. Bee colonies were randomly selected and examined for the presence of varroa mite and other pests and diseases.

3.2.3. Diagnostic test for varroa mites

Adult honeybees

Collected samples were examined for the presence of varroa mite and its level of infestation using the standard methods for varroa mite research described in Dietemann *et al* (2013). During the cross sectional study, 200-300 adult honeybees were shaken off or brushed off from their brood combs in a colony directly into a sample container and 75% ethanol solution was added immediately. The collected bees in a 75% ethanol solution were then vigorously shaked for one minute to dislodge the mites (Figure 7). The mites were then collected by pouring the bees in a 75% ethanol solution into a double sieve and the mites were transferred to an absorbent paper immediately after washing them off to help them dry up. A magnifying hand lens was used to examine the presence of mites on the absorbant whitish paper (Dietemann *et al* 2013).

Finally, the numbers of collected mites per sample and total number of sampled honeybees were counted. In order to determine the proportion of infested individuals, the total counted number of mites was divided by the number of bees in the sample and then was multiplied by 100 to obtain number of mites per 100 bees (Dietemann *et al* 2013).

Figure 7.Alcohol wash method for varroa mite examination

For the component of monitoring of this study, to dislodge the mite from the bees for analyzing varroa population dynamics throughout the whole season, lower numbers of individuals (100 bees per sampling date) and sugar powder method were used.

Brood for varroa mite

From a brood containing frame, 200 randomly selected capped cells (i.e a pupae comb which is 5cm x 5cm) were cut, rubbed with a plastic bag and transported to the laboratory for further anlysis. Each of the cells in a 5 cm by 5cm brood comb were then opened, larvae or pupa were pulled out using soft forceps and examined for the presence of mites and its infestation level (Figure 8). Mite infestation was diagnosed by observing the mites themselves or from their dejection (white rubbery material, most of the time located on the two upper walls, towards the bottom of the cell). Examinations for varroa mite on the surfaces of the pupae was aided by a magnifying hand lens and number of varroa per diagnosed sample was then recorded.

In order to determine the proportion of mite infested cells, the total number of cells opened and infested cells were counted. And then the numbers of infested cells were divided by the

total number of opened cells and multiply by 100 to obtain the proportion of mite infested cells.

Figure 8. Varroa mite examination in the brood cell

During the monitoring period, colony strength were measured using a method described by Delaplane *et al.* (2013) on the same dates as sampling for determining mite infestation rates (Fries et al., 1991, Dietemann *et al 2013*). With this information, the mite distribution between the phoretic and reproductive phases were determined as the proportion of either mites on adults, or mites in brood.

3.2.4. Diagnostic test for Acarine mite

After diagnosis of the adult bee samples for varroa mite, a sample of 30-50 bees were taken randomly from the whole sampled bees in a container and their abdomen were removed. Furthermore, by securing bees on their back or holding with a thumb and first finger, the heads and forelegs and the collar surrounding the neck opening were removed using soft forceps to expose the tracheae *(*Sammataro *et al 2013)*. Then a thin transverse section was cut from the anterior face of the thorax. The tracheae nearest to the spiracle (as mites enter

through the spiracle) were checked for the presence of the mites. These thin disks were further treated to clear muscle tissues with gentle heating in a 5-10% potassium hydroxide solution for approximately 20 minutes to dissolve the surrounding tissues. The contents then were passed through a fine strainer over a sink and rinsed with cold water to remove dissolved matter. The disk-trachea suspensions were examined for *Acrapis woodi* under a dissecting microscope at 10 magnification power (Sammataro *et al*, 2013).

3.2.5. Diagnostic test for (Nosema and Amoeba)

The examination of samples for the presence and infection levels of Nosema spp. was performed according to OIE guidelines (OIE, 2008; Fries *et al*., 2013). Accordingly, samples of 60 older adult worker bees were collected from the under sides of the top lid, from the cluster formed outside or from the hive entrance just before or after flight. The sampled bees were killed using 70% alcohol. The abdomens of the bees were then removed and grounded with a pestle and mortal until an even suspension is formed. A drop of suspension was added onto a microscope slide and covered with slide cover and was examined under high dry objective (X400) in a compound microscope to check for the presence of Nosema and Amoeba spores (OIE, 2008; Fries *et al*., 2013).

3.2.6. Diagnostic test for bacterial diseases (AFB and EFB)

Clinical symptoms

During colony inspection the combs were being carefully watched for the presences of crossword puzzle appearance, discolored, darker, sunken and punctured capping of sealed brood cells and decayed or dried scale of dead brood.

3.2.7. Diagnostic test for chalk brood diseases

Chalk brood disease can be easily identified by its gross symptoms. It is characterized by infected brood; called "mummies," which, when removed from the comb, appear to be solid clumps, reminiscent of chalk pieces. The mummies can vary in color from white to dark gray or black. Broods attacked by this disease first swell then shrink and dry. Examination of samples for the presence of chalk brood disease was done according to the standard methods for fungal brood disease research protocol described in Jensen *et al* (2013). Worker bees take

out these broods from the combs and discard them, which in most cases can be found under the hive entrances of the chalk brood affected colonies. Thus, chalk brood mummies were checked at the bottom board of the hive entrance, in the comb cells and on the ground beneath the hive entrance. Collected mummies were then moistened with distilled water and a drop of the mix was placed on a microscope slide, covered with cover slide and examined under light microscope for mycelia, spore ball cysts and spores of *Ascosphera apis* (Jensen *et al* 2013; OIE, 2008).

3.2.8. Diagnosis of pests

The occurrence of honeybee pests in the study areas were determined through hive inspection and beekeepers interview using a semi-structured questionnaire. For instance, the presence of bee lice was checked using the same procedures used to dislodge varroa mite from adult honeybees mentioned above (Dietemann *et al* 2013). The occurrence of the small hive beetles and wax moths in the hive were also checked through thorough bee hive inspection. The presence of mass of webs and debris, larvae tunneling through, comb cells covered with webs signifies the presence of wax moths (Ellis *et al.,* 2013). Whereas, the presence of dark brown to black adult beetles and their creamy white larvae in the hive and honey combs are used as indicators for the presence of small hive beetle (SHB) infestation (Neumann *et al.,* 2013).

3.2.9. Poisoning of honeybees

A survey questionnaire was prepared comprising mainly of the types of agrochemicals in use, their spray doses, stage of application, etc. Likewise, structured questionnaire was prepared in such a way to assist the collection of a comprehensive information on suspected local and/or exotic poisonous honey plants in the area including flowering season and duration of identified plants, plant habits, and their effects.

3.3. Data management and statistical analysis

The collected data in this study was organized, coded and tabulated using the statistical package for social science (SPSS) version 20 software and Microsoft excel version 2007. Thus, the statistical analyses were employed by using SPSS version 20 and MS excel depending on the type of variables and information available. SPSS version 20 software was

used to analyze the survey and laboratory data collected. A *p*-value <0.05 was considered as a significant difference among variables. Presence of association between risk factors and varroa mite was tested by using chi-square and logistic regression model using the following regression formula.

$$L = \ln(0) = \ln\left(\frac{p}{1-p}\right) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \cdots + \beta_p X_p + \epsilon$$

Where,

P = is the proportion of successes,
O = is the odds of the event
L = is the ln (odds of event),
x_1, x_2,…..x_p are the independent variables,
β_0 = is the intercept and
β_1, β_2… β_p are the slope coefficients (i.e., the expected change in Y relative to one unit change in xi), and
ἐ = is the random error

Prevalence and infection/infestation rates of identified honeybee diseases and pests were analyzed according to the following formulas.

A) Distribution rate/prevalence in colonies (%) = $\frac{\text{Number of positive colonies}}{\text{Total number of colonies surveyed}}$ X 100

B) Prevalence of Varroa mite in inspected apiaries (%) = $\frac{\text{Number of positive sites}}{\text{Total number of sites surveyed}}$ **X 100**

C) Infestation rate (number of mites per 100 bees) = $\frac{\text{Number of mites counted}}{\text{Number of bees in the sample}}$ **x 100**

CHAPTER 4: RESULTS AND DISCUSSION

4.1. Socio- Economic Characteristics of Households

4.1.1. Household characteristics

According to the results of the study, the majority (91.1%), of sampled respondents interviewed to generate qualitative and quantitative data in beekeeping were males and the rest 8.9% were females (Table 3). This indicated that majority of the beekeepers in the study area were males, although beekeeping is an activity which can be done regardless of sex differences. The participation of very limited number of females in beekeeping we found in the study area was in agreement with Abebe Jenberie (2008), Adebabay kebede *et al.*, (2008) and Kerealem Ejigu et al. (2007). Our finding in this regard is also in line with reports of Hartmann (2004) who noted that beekeeping is men's job in Ethiopia. This might be due to the fact that although females have significant involvement in all or parts of beekeeping, it has been reported that beekeeping is duties and responsibilities of men which underscores beekeeping to be men's job due to physical reasons it requires.

The age group between 15 and 60 years are generally considered to be economically active age group in many findings (Melaku Minale, 2005). From the result of this study, we have confirmed that the majority (89.6%) of the households interviewed were categorized in this age group (Table 3). The study revealed that the average age of the beekeepers was 45.02± 13.3 years (ranging from 20 to 80). Our finding in this regard is in line with Adebabay kebede et al (2008) who reported the mean age of 44.61years and 84.08% of the beekeepers were found between the active age group in Amhara region. Keralem Ejigu (2005) and Tessega Bellie (2009) also reported the mean age of beekeeprs was 47.2 and 41.46 years at at Enebse and Burie districts of Amhara region respectively.The younger age group (less than 30 years old) in the study comprised, only 17.4% of interviewed beekeepers. Furthermore, 27.4% of the respondents were found in between 31 and 40 years of age, while the remaining 55.2% were over 40 years old (Table 3). Most of the beekeepers were found in 41 - 60 years age category represented about 44.8% of the respondents (Table 3). This result showed that people at younger and old age are actively engaged in beekeeping activities (Gichora, 2003), but still it seems the practice is run more by the oldies.

Of the total households interviewed, 95.8% of the respondents were married while 2.9%, 0.3% and 1% were single, divorced and their husband or wife has died respectively (Table 3). Based on the results of this study, people regardless of their marital status, they have been observed to undertake beekeeping activities in the study area.This result is in line with Adebabay Kebede et al, 2008 and Tessega Bellie (2009) who reported majority of the beekeepers (97.4 % and 97.5%) were married in Amhara Region and Burie districts respectively.

Table 3. Household characteristics of the respondents (n=384)

Socio economic indicators (parameters)	Variable	Frequency	Response (%)
Sex of household head	Male	350	91.1
	Female	34	8.9
Age of household head (years)	20-30	67	17.4
	31-40	105	27.4
	41-60	172	44.8
	>60	40	10.4
Marital status	Single	11	2.7
	Married	368	95.8
	Divorced	1	0.3
	Widow	4	1.0
Familly size	1-5	148	38.5
	6-10	225	58.6
	>10	11	2.9
Level of education	Illeterate	141	36.7
	Basic education	62	16.1
	Grade 1-4	78	20.3
	Grade 5-8	83	21.6
	Grade 9-12	20	5.2
Land holding (ha)	<0.5	42	11.5
	0.5-1	212	58.3
	1.1-2	100	27.5
	>2	10	2.7

Regarding family size, respondents had an average of 6.18±2.00 persons per beekeeper (which is slightly higher than the national average of six persons per household) ranging from 1 to 12 people per family. Furthermore, the majority of the respondents (61.5%) had a family size of greater than five (Table 3). This, in turn, has revealed that households with large family size (both female and male) were most benefited to perform different agricultural activities including most common beekeeping activities such as hive inspection, settling swarms, water and feed provisions, assisting the household during honey harvesting and so forth (Adebabay Kebede et al.,2008).

4.1.2 Educational status of respondents

Education is believed to be an important and one entry point for faster transfer of knowledge on improved beekeeping technologies (Adebabay kebede *et al*, 2007). Moreover, the role of education is obvious in affecting household income, adopting technologies, demography, health, and as a whole the socio-economic status of the family as well (Kerealem Ejigu, 2005). Gichora (2003) has also noted that more advanced beekeeping is dependent on a good grasp of bee biology, behavior, and exercising a better way of colony management. Regarding educational status of the sampled respondents, about 36.7% of them didn't receive education while 63.3% of them were literate (starting from read and write to high school level) (Table 3). This result has been found to be slightly higher than results noted by Adebabay Kebede *et al*., (2008) for the regional survey which have found that more than 60% of the sampled respondents were literate. From the study, we could understand that very few (5.2%) of respondent beekeepers had got a chance to receive a high school education and the rest (41.9%) had got an opportunity to attend a formal education at elementary and Junior levels (Table 3).

The illiterate level observed in this study (36.7%) was lower than Enebse dstrrict (55%) and higher than Amaro district (22.2%) and Bure district (15.1%) reported by keralem Ejigu (2005) and Tessega Bellie (2009) respectively. It also differs from the report of Abebe Jenberie (2008) who has found that 76.7% of the respondents of sekota district were illiterate. The high level of illiteracy (36.7%) limits the effectiveness of formal training programmes and requires more practice to be placed on potential demonstration of essential concepts especially in improved beekeeping (Adebabay Kebede et al.,2008; Keralem Ejigu, 2005).

4.1.3. Land holding of respondents

The average land holding of the sample respondents during the study period was 0.99 hectares which is slightly lower than the National average which is 1.0 - 1.5 hectares of land. It was also less than the regional average (1.45 hectars), 1.25 hectar of Enebse and 1.77 hectar of Bure districts reported by Adebabay Kebede (2008), Keralem Ejigu (2005) and Tessega Bellie (2009). About 5.2% of the sample respondents have no private land holdings. This result was in line with the general fact that beekeeping can be exercised by landless people and where land is a very limiting factor. Majority (69.8%) of the beekeepers had less than one hectare of land and very few (2.4%) of them had owned more than 2 hectares of land (Table 3). This indicated that households with limited plot of land may invest more on beekeeping activity since the sector is relatively less land-resource demanding.

4.2. Beekeeping Practice of the respondents

4.2.1. Reason for involvement in beekeeping

Beekeeping is an important agricultural activity and a major component of livelihood in the study area. As far as the driving forces to engage in beekeeping business is concerned majority of the respondents (94.8%) have noted that they assume the beekeeping agribusiness had a useful role both as a source of income for the household immediate expenses and for home consumption. A very small number of respondents 2.9%, 1.8% and 0.5% were noted that they have started beekeeping since it have contributed as an income source, home consumption and other unknown functions it has respectively (Table 4). All the sampled respondents have agreed on the point that use of honey at home served as a source of food while 97.1% and 94.1% of the respondents have noted that the honey they produce has contributed as a medicine against various ailments and for local beverage makings for household members respectively. Very small number of respondents (14.1%) have clarified that honey is used at home for some cultural and ritual ceremonies in their localities (Table 4).

Table 4. Beekeepers Experience, source of starter colonies, driving force to start beekeeping

Parameters	Variables	Frequency	Response (%)
Experience of beekeeping (years)	>15	158	41.1
	10-15	51	13.3
	5-9	83	21.6
	1-4	92	24.0
Source of colonies	Gift from parents	63	16.4
	Catching swarm bees	193	50.3
	Buying	125	32.6
	1+2*	3	0.8
Driving force to engage in beekeeping	Income	11	2.9
	Home consumption	7	1.8
	Both	364	94.8
	others	2	0.5
Home use of honey	As food	384	100
	As medicine	373	97.1
	As a beverage	347	90.4
	For cultural and ritual ceremony	54	14.1

* Both from gift from parents and catching swarm bees

4.2.2. Beekeeping Experience of respondents

The level of beekeepers' experience is the number of years that an individual was continuously engaged in beekeeping. Accordingly, the majority of the beekeepers (41.1%) had exercised beekeeping for more than 15 years.This resut was in line with the findings of Abebe Jenberie (2008) who reported that the average experience of the beekeepers in sekota ditrict was 16.5 years. The recently engaged beekeepers that have less than 5 years of experience were only 24% of the total respondents (Table 4). Furthermore, the observations that we had during the study period to those beekeepers who are much more experienced in providing shelter, protect colonies from pest attack, supplementary feeding during dearth period, swarm management, colony multiplication etc, has confirmed that as experience increases, the quality of colony management also increases. Adebabay kebede et al. (2008) reported that young beekeepers were actively engaged from an early age in helping older beekeepers to undertake basic tasks and based on this exposure young people gradually move onto become independent beekeepers as soon as they can obtain their own hives. They

continue accumulating experience by seeking technical advice from fellow beekeepers whenever necessary (Gichora, 2003).

4.2.3. Source of starter colony and placement of the hive

In order to be engaged in beekeeping business, majority of the respondents (50.3%) have revealed that they have obtained their starting colonies from a swarm catch while 32.6% and 16.4% of the respondents have got their starter colonies from buying and as a gift from parents respectively (Table 4). However, very few numbers of respondents (0.8%) have explained that their source colonies to start beekeeping were both from swarm catches and as a gift from parents. From this result, it can be concluded that catching swarm is the main sources of honeybee colonies in the study area.The present result is inline with the findings of Adebabay kebede et al (2008) who reported that 53.1% of the beekeepers in Amhara Region have got their establishment colonies by hanging bait hives on trees and 73.6% of them had experience of catching incidental swarms to be transferred to other hives for increasing own bee colony stock. Keralem Ejigu (2005) also reported 90% of the respondent beekeepers from Amaro and 62% from Enebse started beekeeping by catching swarms. Marta Zelalem (2013) also reported that 55% of the respondents at mecha district have got their establishing colonies by catching swarms.

Based on this survey result, the majority of sampled respondents (94.7%, 100% and 95.5%) were placing their hives (traditional, transitional and movable frame hives respectively) at the backyard and under the eaves of the house in the vicinity of the homestead under traditional management system (Table 5). In areas where the climatic condition is cold, only 0.9% of the respondents noted that they have placed traditional hives inside the house with a small opening on the wall as a hive entrance. Relatively very small numbers of respondents (1.5% and 0.5%) were placing their traditional and movable frame hives at the edge of irrigation sites (Table 5). The resut of the study agree with the findings of Adebabay Kebede et al (2008) who reported that 94.7% of the respondent beekeepers in Amhara region keep their colonies around the homestead (back yard). Keralem Ejigu (2005) and Tessga Bellie (2009) also reported that majority of the beekeepers at Enebsie and Burie districts kept their colonies under the eaves of the house around the homestead. Tewodros Alemu (2010) reported that hives were exclusivelyplaced at the back yard at Sekota district. Keeping honeybee colonies

under the roof of the house and at the backyard make inspection of colonies and other hive management easier compared with free apiaries (Keralem Ejigu, 2009). However, anti malaria (DDT) is applied around the house including their backyard which will poison honey bees and the most serious problems to honey bee colonies occur when hives are directly sprayed with chemicals.

Table 5. Placement of different hive types of the respondents

Site or placement of the hive	Traditional hive (%)	Intermediate hive (%)	Movable frame hive (%)
Back yard	61% (208)	82.9% (34)	72.1% (142)
Under the eaves of the house	33.7% (115)	17.1% (7)	23.4% (46)
Inside the house	0.9% (3)	-	-
edge of irrigation site	2.9% (10)	-	4.1% (8)
established closure area	1.5% (5)	-	0.5% (1)

4.2.4. Honeybee colony holdings

Based on the levels of technology and management practices used by the beekeepers, three beekeeping production methods were identified in the study area: traditional, transitional and modern honeybee production systems.

Acordingly, majority of the honeybee colonies of the area (74.02%) were kept in traditional hives (Table 6) which is lower than the findings of Adebabay Kebede (2008) who reported 99.7% of the respondent beekeepers in Amhara region were kept in traditional hives. This in turn approved that the number of honeybee colonies in traditional hives is still higher compared to the modern and transitional hives in the study area. Of course the number of traditional hives is decreasing from year to year as the beekeepers are transferring their colonies to improved hives. About 3.08% of the honeybee colonies in the study area were kept in transitional hives (Table 6). Moreover, the distribution and ownership of transitional hives by the interviewed respondents have been observed to be lower than the modern hives. More specifically, about 22.9% of the honeybee colonies were hived in modern hives (Zander

hives) (Table 6). Low adoption and dessimination of movable frame hive attributed to many factors like weak extension, initial high costs, for demanding its own seasonal management techniques and other accessary equipments, poor economic back ground of the beekeepers, lack of Know how and the likes(Adebabay Kebede *et al.*,2008).

Table 6.The number of honeybee colonies of the respondents (n=384)

Hive type	Number of colonies				
	2004	2005	2006	Total	Percent (%)
Traditional	2201	2024	1836	2020	74.02
Transitional	55	42	156	84	3.08
Modern	559	670	645	625	22.9
Total	4580	4741	4643	2729	100

Based on this study, 84.9% of the respondents have agreed on a point that there is a decreasing trend in the number of honeybee colonies and their products from time to time due to the availability and occurrence of various threatening factors which had an adverse effect on honeybee health and their production potentials. More specifically, 96%, 69.2% and 64% of the sampled respondents have identified that presence of pests and predators, agrochemicals application on field crops and lack of bee forage as a result of deforestation were the main reasons (threatening factors) for the colonys' decreasing trends observed respectively (Table 7). However, some respondents (5.8%, 4.5%, 3.9% and 2.9%) have indicated that scarcity of water especially during dearth periods, colony absconding due to various reasons and the occurrence of unidentified brood diseases (locally named as "*Mich*" and death of adult honeybees) were the the associated factors for colony decline respectively (Table 7). These agree with the rsults of Adebabay Kebede et al (2008), Keralem Ejigu (2005), Tessega Bellie (2009) and Tewodros Alemu (2010) who reported the decreasing trend of honeybee populations and their products in Amhara Region, Enebse, Bure and Sekota districts respectively due to multitude reasons like shortage of bee forage, draught, pesticide and herbicide application, lack of water, poor management and diseases.

Table 7. Trends in the number of colonies and possible reasons for a decreasing trend

Parameters	Variable	Frequency	Response (%)
Trends in the number of colonies you owned	Decreasing	326	84.9
	Increasing	58	15.1
Reason for decreasing trend	Lack of bee forage	212	64(3)
	Scarcity of water	19	5.8(4)
	Pests and predators	320	96.7(1)
	Diseases	13	3.9(6)
	Pesticides and herbicides	229	69.2(2)
	Absconding	15	4.5(5)
	Death	11	2.9(7)

NB: ( ) Order of importance

Table 8. Number of colonies kept with different hive types by respondents (from 2004-2006)

parameters	Variable	Mean	SE	Minimum	Maximum
No of colonies kept with traditional hive	2004	7.39	0.545	1	50
	2005	6.51	0.464	1	45
	2006	5.74	0.419	1	40
No of colonies kept with intermediate hive	2004	2.62	0.405	1	6
	2005	2.13	0.531	1	6
	2006	4.11	0.855	1	20
No of colonies kept with movable frame hive	2004	3.14	0.272	1	19
	2005	3.53	0.3	1	30
	2006	3.31	0.279	1	28

4.3. Survey results of major honeybee pests and diseases

4.3.1. Ants (Chuchan /Gundan)

According to the results of this study, almost in all sampled localities (99.7%), ants have been reported to be a serious beekeeping problem responsible for colony number and productivity decreasing trends explained which has ranked first in the study areas (Table 9). As indicated in Table 10, ants have ranked 1st in the region too. This agrees with the results of Adebabay et al (2008) and Tessega Bellie (2009) who reported ants as the most harmful pest in Amhara

Region and Burie districts respectively. In Atsbi-Womberta, Ahferom and Wukro districts of Tigray regional state, there was reported on the occurrence of ants as a serious problem in beekeeping (Etsay Kebede and Ayalew Kassaye, 2001) and the problem was also considered to cause a major problem in the adoption of improved beekeeping technologies (Workneh Abebe, 2007). In Tigray, Amhara and SNNP regional states and Gomma district Jimma zone beekeepers ranked ants as the first problematic pest in honeybees (Amsalu Bezabih *et al.*, 2010). In contrary, Tewodros Alemu (2010) has reported waxmoth as the most harmful pest in Sekota district. Ants have been observed nesting under the ground around the apiaries, continuously getting into the hive, disturbing the colony and causing absconding. However, no significant difference in the prevalence of ants among the agroecologies was observed (χ^2 =2.379; p=0.304).

Table 9: Honeybee pests responsible for a decreasing trend of honeybee colonies

Parameters	Variables	Lowland	Midland	Highland	Total	χ^2	sig
Ants	Yes	100	99.1	100	99.7	2.379	0.304
	No	0	0.9	0	0.3		
Wax moth	Yes	100	90.9	50.9	76.1	100.99	0.000***
	No	0	9.1	49.1	23.9		
Hamagot	Yes	100	39.1	91.3	78.2	143.19	0.000***
	No	0	60.9	8.7	21.8		
Spider	Yes	100	91.8	100	97.6	21.968	0.000***
	No	0	8.2	0	2.4		
Wasps	Yes	37.6	26.4	0	12.1	87.45	0.000***
	No	63.4	93.6	100	87.9		
Birds	Yes	100	93.6	100	98.1	16.99	0.000***
	No	0	6.4	0	1.9		
Beetles	Yes	0	2.9	2.5	1.9	2.77	0.25
	No	100	97.1	97.5	98.1		
Death Head Hawks moth	Yes	97	48.5	46	60.8	30.64	0.000***
	No	3	51.5	54	39.2		
Lizards	Yes	99	86.4	97.5	94.8	20.74	0.000***
	No	1	13.6	2.5	5.2		
Toads	Yes	0	0	2.5	1.1	5.025	0.081
	No	100	100	97.5	98.9		
snakes	Yes	33.7	0	10.6	14.1	49.751	0.000***
	No	66.3	100	89.4	85.9		
Mice	Yes	51.5	0	51.6	37.4	81.483	0.000***
	No	48.5	100	48.4	62.6		
Bee lice	Yes	10.9	31.3	6.2	14.4	32.734	0.000***
	No	89.1	68.7	93.8	85.6		
Varroa mite	Yes	46.5	69.7	45.3	52.4	16.482	0.000***
	No	53.5	30.3	54.7	47.6		

In order to control ants, beekeepers in the study area have explained that they have an experience in using fresh ash, cleaning of hive areas and use of fire to destroy ant nests. It has been also understood that respondents are using a combination of two or more of above controlling mechanisms. Adebabay Kebede et al (2008) has reported different experience of beekeepers in the Amhara region like Placing fresh ashes around the base of a hive stand, plastering hives stands with mud, putting dead snake on the nest of ants, spraying garlic juice, burning the ants with fire, destroying ants nests, use of white eucalyptus leaves as repellant, plastering of thin rubber sheets and metals between the hive and hive stands, pour used engine oil around the hive stand and keeping weeds well away from the base of the hive stand.

Table 10: Priority ranking of pests and predators perceived as economically important based on their level of damaging effect

Pests and Predators	**Index Score**	**%**	**Rank**
Ants	8.45	10.08	1
Waxmoth	8.26	9.86	2
Birds	8.21	9.79	3
Varroa mite	6.98	8.33	4
Wasps	6.86	8.18	5
Lizards	6.83	8.14	6
Spider	5.79	6.9	7
Bee lice	5.78	6.9	8
Death Hawks moth	5.64	6.73	9
Hamagot	5.43	6.48	10
Bettles	4.91	5.86	11
Mice	4.06	4.84	12
Toads	3.8	4.53	13
Snakes	2.83	3.37	14
Total		**100**	

4.3.2. Wax Moth (‘*Sembel Til’*)

In this survey work, it has been observed by 76.1% of sampled beekeepers interviewed that wax moth was one of the major problems in beekeeping (Table 9), which ranked 2nd in its priority in causing colony weakening and absconding (Table 10). This agree with the findings of Adebabay Kebede et al (2008) andTessega Bellie (2009) who reported wax moth as the second most important problematic pest next to ants in Amhara Region and Burie district

respectively. Similarly wax moths (*Gallera mellonela*) and the night flying sort of wax moth (*Aphomia sociella*) were reported in honeybees in the study carried in Tigray regional state in three district of Atsewonberta, Aheferom and Wukro (Etsay Kebede and Ayalew Kassaye, 2001). In the five regional states of Ethiopia wax moths ranked among the disastrous pests of honeybees by bee keepers (Amsalu Bezabih et al, 2010).

The result also has indicated that mid altitude sample areas were more significantly affected by this pest than lowland and highland areas (χ^2 =100.99; p<0.01). This pest has been considered as a sign of poor colony management as observed from its damage caused on honey combs during its larval growth stage and even to the hive body during its pupae stage. This phenomenon could explain that awareness creation and provision of adequate trainings focusing on seasonal colony management for the control of wax moth is a mandatory.

4.3.3. Honeybee Eater Birds (*'woff')*

Our survey result has confirmed that honeybee eater birds are the 3rd most economically important predator of the honeybees in the study area (Table 10) supported by 98.1% of the respondents for the decreasing trend in honeybee colony numbers (Table 9). Their effects have been noticed to be more prominent when birds came in large numbers (supported by their seasonal movements) and weaker colonies are the ones more prone to the effect of honeybee eater birds. In most cases, the birds are using the nearby trees or branches of fences to land prey the forager bees. This agree with the findings of Adebabay Kebede et al (2008) andTessega Bellie (2009) who reported honeybee eater birds as the third most important problematic pest next to ants and waxmoth in Amhara Region and Burie district respectively.

Figure 9. Honeybee eater bird

The seasonal and migratory movements of these birds have been observed to cause a significant effect in lowland and highland areas than midlands (χ^2 =16.99; p<0.01).Tewdros Alemu (2010) reported bee eater birds were very common in the highland in sekota districts. Regarding the control of these birds, beekeepers have explained that they have experienced some techniques like chasing of the birds using '*wonchif*', killing the birds using local traps extracted from sticky gums and destroying the bird's nest in the ground. This agrees with the results of Adebabay Kebede et al (2008) who reported that Killing using 'wonchif and whipping are the protection techniques against bee eater birds experienced by respondents in the Amhara region.Similarly, Tewodros Alemu (2010) has also reported the experience of sekota beekeepers to protect bee eater birds by placing gums of plants where the birds rest and near the apiary, killing the bird using smoke at their nest and by chasing away the birds. Tessega Belie (2009) also reported putting something (cloth, festal etc) and spin around the hive and killing using wochif as experience of beekeepers at Burie district.

4.3.4. Varroa mite (locally called "Yenib *mezger"*)

Varroa mite, as one of the major honeybee parasite in the study area, 52.4% of the respondents have claimed as one of the factors responsible for colony number decreasing trend (Table 9). In this case, the pest has ranked fourth among the group in its importance (Table 10). More specifically, these parasites are causing a considerable amount of damage through colony weakening. However about 47.6% of beekeeping respondents claimed that they didn't know the impact of the pest on their honeybee colonies. Furthermore, higher numbers of respondents (69.7%) from midland representation areas had better knowhow on the effect of varroa mite on beekeeping business than lowland and highland beekeepers. This may be due to the fact that negative impacts of the pest on honeybee colonies was more visible in the midland than the other agro-ecologies which may be favourable for the negative actions of the pest in the midland.

4.3.5. Wasps

Respondent Beekeepers especially from lowland and midland confirmed that Wasps are among the factors responsible for a decline in colony population supported by 12.1% of the interviewed beekeepers as a prey to foragers (Table 9). According to the survey result, this

pest has been ranked fifth in its economic importance to beekeeping (Table 10). However, it was not reported from highland represention of the study area. Furthermore, most beekeeping respondents have explained that they didn't have a controlling mechanism to this pest.

4.3.6. Lizards (*'Enshashlit'*)

It has been revealed that lizards were widely distributed and observed to prey on honeybees at the hive enterance by 94.8% of the respondents (Table 9). Lizards were also considered as one of the factors contributing to colony population decreasing trend. Moreover, lizards have ranked 6th in their economic importance to beekeeping (Table 10). Lizards were found to be more common in lowland representing areas than midland and highland ones and showed significant difference among agro-ecologies (χ^2=20.74; p<0.01). Killing of lizards using sticks, use of traps and good apiary management (including cleaning) have been proposed by the respondent beekeepers as a possible control mechanism against the pest.

4.3.7. Spider (*'shererit'*)

Spiders, as one of the major honeybee predators in the study area ranked 7th (Table 10) in its economic importance to beekeeping and its wide distribution was supported by 97.6% of the interviewed respondents (Table 9). The occurrence of spiders in the colonies and/or apiaries has been also considered as a sign of poor apiary management. In apiaries which were not clean, spider webs and traped honeybees have been observed during colony inspections. By doing so, the pest had a considerable effect on decreasing colony population and honey yield.

4.3.8. Bee Lice (Yenib *kimal*)

Laboratory diagnostic result of bee lice prevalence and distribution has been presented in the next sections. About 14.1% of beekeeping respondents have claimed bee lice as one of the factors contributing to colony population decline in the study area (Table 9) and has ranked 8th among the other pests and predators identified (Table 10). However their degree of damage was not considered as serious as that of ants, wax moths, honeybee eater birds, and spiders identified in the study area. The pest is known to attach to the body of the adult bees including the queen, more conspicuously during the dearth periods of the year. As a controlling mechanism to this pest, some beekeepers have suggested that use of cow dung, tobacco and

straw as a smoking material while others didn't know an option as a controlling measure. The result also has indicated this pest is more common at the mid altitude than lowland and highland areas with the significant difference among the three agroecologies (χ^2 =32.73; $p<0.01$). This disagrees with the finding of Tewodros Alemu (2010) who reported bee lice were very common in lowlands of Sekota district.

4.3.9. Death head Hawks Moth *('Embabra')*

Death head Hawks moth was one of the pests responded by 60.8% of the interviewed beekeepers and ranked 9th for its effect on honeybee health (Tables 9 and 10) among the list of pests identified in the study area. The pest gets entered into the hive through wider hive entrances and different openings and could cause colony absconding due to its nuisance from a continuous faster wing vibration. Regarding the control measures to this pest, some respondents have explained that they are trying to kill the pest at night using a hand tourch or light sources and decrease the size of the hive enterance.

4.3.10. Hamagot *('Megoza')*

Even though hamagot has a wide distribution supported by 78.2% of the respondents and ranked 10th (Tables 9 and 10), the degree of damage is not as serious as the previous pests and predators. Most of the time the predator is known to destroy a colony in traditional hives than hived in other hive types. The majority of the beekeepers have also explained that their colonies could be prevented from the damage by this predator using dogs and fencing of apiaries as a safeguard. This agree with the findings of Adebabay *et al* (2008) who reported beekeepers at Amhara region has an experience of protecting honey badger by killing,fencing and chasing with dogs.

4.4. Survey Result of Varroa mite

4.4.1. Varroa presence and infestation

As it has been indicated in table 11, the majority of respondents (54.5%) have never observe/notice Varroa mite in their colonies while 45.5% of the total respondents observed Varroa mite in their colonies and have noticed its infestation (Table 11). In this case, a

significant difference (χ^2 =1.316; p=0.518) was not observed in detecting the Varooa and its infestation among the agro ecologies addressed. Moreover, our survey data has explained that infestation rate of the colonies with this pest has considerably increased in the last three years (2012 – 2014) (Fig. 7).

Table 11. Varroa mite observation in colonies at different agro ecologies

Parameters	Variables	Lowland	Midland	Highland	Total	χ^2	Sig
Have you ever Observed varroa mite in your colonies?	yes	45.4	49.6	42.6	45.5	1.316	0.518
	No	54.6	50.4	57.4	54.5		

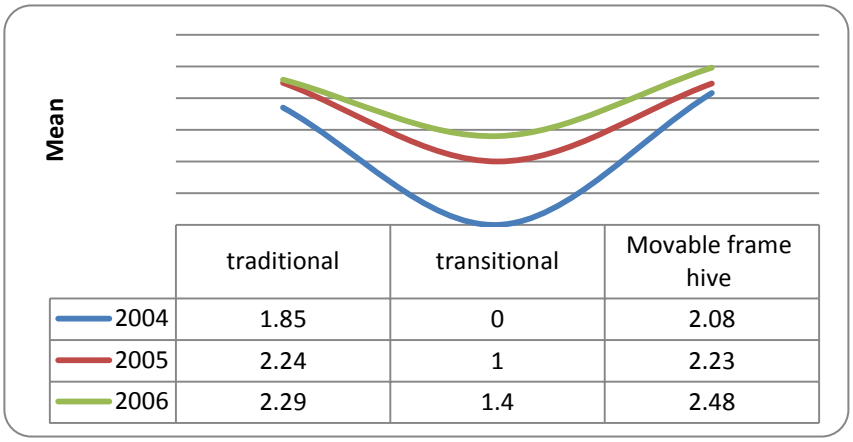


Figure 10. Mean number of colonies infested by varroa mites

As indicated in table 12, majority of the respondents (78.9%) had explained that their colonies had started to suffer from varroa mite infestation within these recent years from 2009-2014. This might be related to high mobility and marketing of honeybees for the transfer of colonies from traditional to the modern hive promoted by the extension program. However, for some respodents, the time when the respondent beekeepers started to notice mites on the bees were traced back during 2004-2008 and 1998-2003, supported by 12.1% and 9% of the respondents respectively (Table 12). This shows varroa mite might were introduced in to the study area some years back without being noticed by the majority of the beekeepers in the representations.The presence of Varroa mite was first detected in 2008 when conducting

nation wide diagnostic survey in Ethiopia (Abebe Jenberi et al., 2010). Likewise the presence of Varroa mite in Kenya was first detected in 2009 (Muli *et al.*, 2014).These newly introduced pests to Africa might have long term implications for the honeybees populations. As these new parasites become more widespread, as pesticide use increases and as land scape degradation increases due to increased urbanization and climate change we expect to see the combination of all these factors negatively impact the bees in the future.

Table 12. Year when respondent's colonies start to suffer from varroa mite infestation

Parameters	Variable	Frequency	Response (%)
Since when did your colonies start to suffer from varroa mite infestation?	1998-2003	15	9
	2004-2008	20	12.1
	2009-2014	131	78.9

4.4.2. Behavioral change of colonies due to varroa mite

The most common behavioral changes as a result of varroa mite infestation reported by interviewed beekeepers were colony dwindling, reduced honey yield, reduced foraging activity supported by 92.6%, 52.8% and 35.21% of the respondents respectively and showed a significant difference among agro ecologies (χ^2=12.003; p=0.017; Table 14). Furthermore, increased absconding tendency, presence of irregular brood pattern, deformed wing, frequent colony disturbances, and in severe cases loss of the entire colony were also observed behavioral changes among varroa infested colonies supported by 16.5%, 11.7%, 10.1%, 1.2%, and 2.5% of interviewed respondents respectively (Table 14). Symptoms of varroa mite infestation in a colony may include restless behavior, spotty brood patterns, discarded pupae at the hive entrance, malformed and discolored and drones (MAREEC, 2004). Detection of dead pupae with discolored, shrunken and decreased body size was reported by Desalegn Begna, 2015. However in this particular study, it was not confirmed in the monitoring apiaries for any signs of this syndrome within the six month period. Therefore the presence of parasitic mite syndrome has to be confirmed by expernmental evidence through a prolonged monitoring period.

Table 13. Behavioral changes in varroa infested colonies

Behavioral changes	Variables	Lowland	Midland	Highland	Total	χ^2	sig
Irregular brood pattern	yes	2	37.2	1.7	11.7	32.5	0.000***
	No	98	67.3	98.3	88.3		
Disturbance of colonies	yes	4	0	0	1.2	4.536	0.104
	No	96	100	100	98.8		
Dead bees and brood on entrance	yes	0	21.2	1.6	7.3	24.708	0.000***
	No	100	78.8	98.4	92.7		
Dwindled colonies	yes	94	88.5	95.2	92.6	12.003	0.017*
	No	6	11.5	1.8	7.4		
Absconding	yes	18	23.1	9.7	16.5	6.834	0.145
	No	82	76.9	90.3	85.5		
Infested not yielded	yes	48	53.8	55	52.8	0.594	0.743
	No	52	46.2	45	47.5		
Reduced foraging activity	yes	46	50	13.3	35.21	20.132	0.000***
	No	54	50	86.7	64.8		
Deformed wing	yes	4.5	25.9	0.00	10.1	24.738	0.000***
	No	95.5	74.1	100	89.9		
Loss of the entire colony	yes	0	7.7	0	2.5	8.676	0.013*
	No	100	92.3	100	97.5		

4.4. 3. Time of varroa mite occurrence

The beekeepers involved in this study reported that they (71.8%) have observed varroa mite in their colonies during the time from March to May in most cases when colonies were starved and weakened due to a prolonged dearth period. The respondents varroa mite observation time in their bee colonies showed a significant difference (χ^2=61.545; p=0.000) among the respondents at different agroecologies. In relation to the presence of an extended dearth period at the lowlands than mid and high lands, the mite was also detected from June to August (34%) (Table 15). The observation time reported by the beekeepers (March to May) was found to be different from the seasonal monitoring of this result which was higher at active season with a peak at November. The difference might be due to the beekeepers frequently observing their colonies when they are less defensive at dearth than their defensing time (active season).

Table 14. Time when varroa mite was observed in the respondent colonies in the different agro ecologies

Parameters	Variables	Lowland	Midland	Highland	Total	χ^2	sig
When do you most likely observed varroa mite in your colony?	Sep-Nov	8..5	0	16.7	8.5		
	Dec-Feb	0	9.4	6.1	5.7		
	Mar-May	55.4	81.3	74.2	71.8	61.545	0.000*
	June-Aug	34	9.4	3	13.6		

4.5 Laboratory test result of Varroa Mite

4.5.1 Prevalence of varroa mite

In this survey, the occurrence of Varroa mite has been observed in the largest areas of the two sampling zones of the eastern parts of the Amhara National Regional State. Out of the 6 sampled districs in the eastern Amhara, 100% of them were found to be Varroa positive indicating a wide spread in many areas of the region where beekeeping is in-practice. From the total colonies examined (384) for the presence of varroa mite, the laboratory diagnosis has confirmed that 85.9% (330) were found to be varroa mite infested. The present result was found to be comparable with the findings of Muli *et al.*(2014), who reported 83% varroa mite prevalence in Kenya and Desalegn Begna, (2014) who has also reported 82% varroa prevalence in Tigray region, Ethiopia. This result is also higher than 56% prevalence at colony level reported by Allisop (2006) in South Africa and 48% prevalence reported by Zee *et al* (2015) in Tanzania.

In our study, the highest varroa prevalence (94.2%) was observed in movable frame hives. This might be associated with higher number of brood combs constructed by the colonies at the brood chamber which might favor mite reproduction enormously and the possible exposure of frame hives to drifting and robbing when kept close with minimal spacing between hives. Prevalence of varroa mites in honeybee colonies showed a significant difference ($p<0.01$) among the colonies hived in different hive types (Table 16).

Furthermore, higher varroa mite prevalence was observed from honeybee colonies in the midland altitude representations than colonies in the low and highland areas (Table 16). The higher varroa mite prevalence in the midlands than in lowland and highland representations might be associated with differences in colony mobility and marketing experiences of the three agroecologies adressesed in this study. However, there was no a statistically significant difference (χ^2=1.348; p>0.05) among the agro ecologies addressed.

Table 15. Prevalence of varroa mite and the different risk factors

Risk factors	samples					χ^2	Sig.
	Total (n)	positive (n)	positive (%)	negative (n)	negative (%)		
Type of management							
Backyard	241	206	85.5	35	14.7	0.113	0.736
Apiary	143	124	86.3	19	12.7		
Altitude							
Low land	102	88	86.3	14	13.7	1.634	0.442
Mid land	139	123	88.5	16	11.5		
High land	143	119	83.2	24	16.8		
Hive type							
Traditional	182	145	79.6	37	20.4	14.998	0.001**
Intermediate	47	39	84.8	8	15.2		
Movable frame	155	146	94.2	9	5.8		
District							
Ziquala	46	38	82.6	8	17.4	22.217	0.000*
Sekota	80	66	82.5	14	17.5		
Dehana	66	63	95.5	3	4.5		
Dessie zuria	77	56	72.7	21	27.3		
Tehulederie	59	57	96.6	2	3.4		
Kalu	56	50	89.3	6	10.7		
Total	384	330	85.9	54	14.1		

The prevalence of varroa mite was also found to be higher in bees kept in the apiary management system (86.3%) than bees kept in the backyard (Table 16). However, statistically significant differences (χ^2=0.305; p>0.05) were not observed among varroa mite prevalence in the different colony management systems (apiary and backyard). Furthermore, the higher varroa mite prevalence observed in apiary management system might be associated with the different contacts among colonies and the introduction of unkown sources of colonies for transfeing to the modern hives. In most cases beekeepers sell colonies with inferior in their performance and/or weakened by parasites infestation. As a result of the introduction of such types of honeybee colonies in the apiaries, the distribution within the apiaries increased. Colonies who were arranged very close to each other in the apiaries have been believed to facilitate transmission of varroa mite among the colonies through swarms, drifting and robbing activities. Beekeepers probably spread an infestation from one colony to another through frequent apiary manipulations. Infestations also are spread as a result of drifting (especially drifting drones) from one apiary to another and swarming bees (MAREEC, 2004). In regions with a high density of honey bee colonies the population dynamics are influenced by a permanent exchange of mites when foragers or drones enter foreign colonies or by robbing (Goodwin et al., 2006). It is interesting to note that the robbing bees will ‘‘receive” the mites from the victim colonies, which often are already weakened through a high Varroa infestation, and that the effective ‘‘robbing distance” is more than 1 km (Renz and Rosenkranz, 2001).

Tehulederie district has the highest prevalence of 96.6% (57/59) of varroa mite in the investigation area followed by Dehana district of 95.5% (63/66) among the sampling districts while Dessie Zuria experienced comparatively with low prevalence of 72.7% (56/77) with significant difference (χ^2=22.217; p<0.01) among the sampling districts (Table 16).

Moreover, the major risk factors for the prevalence of varroa mite were also assessed using the logistic model, and the analysis indicated that 89% of the total variation for varroa prevalence was explained by logistic model. Chi-square also showed that the parameters were significantly different from zero at p<0.01. The explanatory variables that fit the model: type of management, hive type, agro- ecological zone, altitude and rainfall were found to be significant as hypothesized (Table 17).

Type of management was found to be an important variable in the prevalence of varroa mite in the study area. Colonies kept under the apiary management system were 2.755 times more likely to be infested by varroa mite than those colonies kept under the backyard management system (Table 17). Hive type was also another factor, which was significantly associated with dependent variables and was significant at 1% significance level.

Colonies kept in traditional hives were 0.222 times less likely to be infested by varroa mite than those kept in movable frame hives. On the other hand, logistic model results showed that agro- ecological zones where colonies were located affected the presence and/or absence of varroa mite significantly at 5% significance level (Table 17).

Table 16. Logistic regression for factors influencing prevalence of varroa mite

Variable	B	SE	Wald	DF	Sig.	Exp(B)
Type of management						
Apiary *vs.* Back yard	1.014	0.435	5.421	1	0.020**	2.755
Geographic zone						
Waghimra *vs.* South wollo	1.761	1.856	0.900	1	0.343	5.816
Hive type						
Traditional *vs.* Frame	-1.507	0.477	9.973	1	0.002**	0.222
Transitional *vs.* Frame	-0.705	0.755	0.873	1	0.350	0.494
Agro - ecological zone						
Lowland *vs.* midland	-6.273	2.286	7.530	1	0.006**	0.002
highland *vs.* Midland	-4.228	1.178	12.891	1	0.000***	0.015
Altitude(m.a.s.l)	-0.007	0.002	21.153	1	0.000***	0.996
Mean temperature(^{o}c)	0.310	0.414	0.561	1	0.454	1.363
Rainfall (mm)	0.010	0.012	0.648	1	0.006**	1.010
Constant	3.492	22.15	0.025	1	0.875	

Nagelkerke R square = 0.337, $\chi 2$= 67.552***; **, *** significant at p<0.05, and p<0.01, (n=384)

The odds of varroa prevalence decreased by a factor of 0.002 and 0.015 for bee colonies located at lowland and highland respectively than the midland. It was also apparent from the result that altitude significantly and negatively related to the dependent variable at 1% significant level. A one unit increase in altitude decreased the odds of varroa prevalence by a factor of 0.996 (Table 17). Among the climatic variables also mean temperature and rainfall associated with varroa prevalence positively at 5% and 1% respectively. A one unit increase

in the amount of rainfall increased the odds of varroa prevalence by a factor of 1.01 (Table 17).

4.5.2 Infestation rate of varroa

Concerning to the infestation rate of varroa mite on adult bees per hundred bees calculated in colonies located in the high, medium and low altitude areas, higher infestation rate was observed from colonies located in the highland areas both in phoretic (6.26±0.51) and reproductive (9.73±0.97) phases (Table 18 & 19).There was a statistically significant difference among the three agro-ecoogies in the infestation rate of varroa mite in the phoretic ($F=13.86$; $p<0.001$) and reproductive (brood cells) phase ($F= 3.592$; $P<0.05$). The higher varroa mite infestation rate per hundred bees observed in higher altitude areas than the other altitudes might be associated with the abundance of pollen source plants and tendency of bees to rear brood for a relatively extended period of time in the highland areas which might also favor higher mite reproduction in this altitude than colonies in mid and lowland representations.

The laboratory diagnosis confirmed that higher varroa infestation rate per hundred brood cells (9.23±1.5) was observed in apiary management systems than the backyard beekeeping systems (6.88±0.57). However, varroa mite infestation rate showed no statistical significant difference between bees kept in apiaries and backyard management system in the phoretic ($F=1.88$; $p>0.05$) and reproductive (brood cell) ($F=3.002$; $p>0.05$) phases (Table 18 & 19). This in turn indicated that colonies kept at the backyard have been observed to be better adapted to the prevailing environment and disease and parasite resistance than the newly established apiaries. Moreover, the honeybee colonies brought to new apiaries have been observed to be more prone to varroa mite infestation.

Similar higher varroa infestation rates were observed from intermediate (5.12±0.55) and movable frame (4.98±0.45) hives than traditional hives (4.02±0.34) in a phoretic phase. A mean infestation rate of 9.46±1.85 and 7.64±0.79 was recorded from brood cells in intermediate and movable frame hives respectively, while it was 7.28±1.17 for the traditional hives. Though, our data didn't show a significant difference in varroa mite infestation rate among the honeybee colonies hived in different hive types both during phoretic ($F=1.88$;

p>0.05) and the reproductive (brood cells) (F=0.578; p>0.05) phases (Table 18&19). Lower varroa mite infestation rate of traditional hive than the other two hive types might be associated with the frequent removal or cutting of the combs might reduce the residues of the pathogen or break the life cycle of the agent. behavioural adaptation of bees for frequent swarming due to overcrowding of its small volume of traditional hive which favours reduction of its varroa load along with the departed daughter colonies.

When we calculate the number of varroa mites per hundred bees in honeybee colonies from the six districts (ziquala, Sekota, Dehana, Dessie zuria, tehulederie and kalu), higher infestation rate was observed in honeybee colonies from Dessie zuria during the phoretic (8.1±0.92) and reproductive (10.1±1.24) phases; while the least infestation rates were observed in colonies from Ziquala during the phoretic (2.74±0.46) and reproductive (2.55±0.37) phases (Tables 18 and 19). A statistically significant difference was observed in varroa infestation rates among the six sampled districts both during the phoretic (F=10.94; p<0.001) and reproductive (F= 3.28; P<0.05) phases (Table 18 &19).

Table 17. Mite infestation rates per hundred adult bees of the different variables during the cross sectional study

Variables	Adult bees sampled				Infestation rate/ sample of bees			Infestation rate /100 bees		
	N	Mean±SE	Min	Max	Mean±SE	Min	Max	Mean±SE	Min	Max
Type of management										
Backyard	241	190.07±5.13	100	457	8.14±0.6	1	34	4.57±0.34	0.31	32.14
Apiary	143	225.69±6.85	102	426	9.31±0.73	1	41	4.57±0.25	0.25	20
F stat		1.5			17.78				1.88	
P value		0.22			0.000***				0.154	
Altitude										
Low land	88	165.07^{b}±5.99	100	340	6.31^{b}±0.76	1	30	4.02^{b}±0.45	0.45	19.8
Mid land	124	186.62^{b}±7.33	112	426	7.67^{b}±0.67	1	41	3.33^{b}±0.3	0.25	18.7
High land	120	248.62^{a}±6.77	108	457	11.18^{a}±0.85	1	54	6.26^{a}±0.51	0.37	32.14
F stat		42.724			10.21				13.86	
P value		0.000***			0.000***				0.000***	
Hive type										
Traditional	146	179.45^{b}±5.48	100	426	6.36^{b}±0.53	1	30	4.02^{a}±0.34	0.75	98.53
Intermediate	43	244.5^{a}±10.9	112	365	11.91^{a}±1.29	1	31	5.12^{a}±0.55	1.56	63.00
Movable frame	135	218.9^{a}±6.86	100	457	9.85^{a}±0.81	1	54	4.98^{a}±0.45	0.65	32.00
F		17.8			10.61				1.88	
P value		0.000***			0.001**				0.154	
District										
Ziquala	39	205.96^{b}±9.66	104	340	6.23^{b}±1.33	1	30	2.74^{b}±0.46	0.45	10.18
Sekota	66	246.96^{a}±8.49	116	426	7.39^{abc}±1.03	1	41	3.14^{b}±0.47	0.35	18.1
Dehana	63	236.47^{ab}±9.13	115	457	10.87^{ab}±1.07	1	40	4.64^{b}±0.43	0.37	12.73
Dessie zuria	56	143.9^{c}±4.64	103	266	11.55^{a}±1.44	1	54	8.1^{a}±0.92	0.8	32.14
Tehulederie	57	250.86^{a}±12.1	112	398	7.96^{abc}±0.82	1	31	3.55^{b}±0.36	0.25	11.21
Kalu	49	131.48^{c}±3.46	100	203	6.37^{bc}±0.87	1	27	5.04^{b}±0.69	0.49	19.81
Total	330	203.34±4.2	100	457	8.57±0.46	1	54	4.57±0.26	0.25	32.14
F		4.12			41.63				10.94	
P value		0.001**			0.000***				0.000***	

Table 18. Mite infestation rate per hundred brood cells of the different variables

variables	Number of cells opened				Number of infested cells			Brood infestation rate / 100 cells		
	N	Mean±SE	Min	Max	Mean±SE	Min	Max	Mean±SE	Min	Max
Type of management										
Backyard	209	130.24±1.51	95	194	9.07±0.76	1	72	6.88±0.57	0.67	48.65
Apiary	115	129.66±2.82	92	246	11.82±1.93	1	134	9.23±1.5	0.65	98.53
F stat		0.46			2.4				3.002	
P value		0.83			0.122				0.084	
Altitude										
Low land	89	130.25^{a}±2.98	100	246	7.82^{a}±1.07	1	42	5.56^{b}±0.7	0.75	29.09
Mid land	111	127.39^{a}±2.43	95	206	8.89^{a}±1.04	1	134	7.32^{ab}±1.57	0.67	98.53
High land	124	132.33^{a}±2.0	92	188	12.74^{a}±1.21	1	72	9.73^{a}±0.97	0.65	63.0
F		1.14			3.279				3.592	
P value		0.32			0.039^{*}				0.029^{*}	
Hive type										
Traditional	146	128.91^{a}±1.89	92	206	9.6^{a}±1.58	1	30	7.28^{a}±1.17	0.75	98.53
Intermediate	43	126.0^{a}±3.3	100	174	11.28^{a}±2.0	1	31	9.46^{a}±1.85	1.56	63.00
Movable frame	135	132.61^{a}±2.4	95	246	10.17^{a}±1.04	1	54	7.64^{a}±0.79	0.65	32.00
F		1.42			0.202				0.578	
P value		0.24			0.818				0.562	
District										
Ziquala	46	120.26^{c}±2.29	100	154	3.2^{b}±0.5	1	12	2.55^{b}±0.37	0.75	9.38
Sekota	69	126.1^{bc}±2.87	100	206	10.6^{ab}±3.05	1	134	8.54^{ab}±2.32	0.89	98.53
Dehana	86	129.1^{abc}±3.11	92	184	11.29^{ab}±1.6	1	63	9.3^{a}±1.5	0.65	63.0
Dessie zuria	68	134.97^{ab}±2.5	100	188	14.5^{a}±1.8	1	72	10.1a±1.24	0.93	48.65
Tehulederie	42	131.14^{abc}±5.2	95	190	5.75^{ab}±1.11	1	19	5.0^{ab}±1.15	0.67	19.0
Kalu	43	140.93^{a}±4.39	100	246	12.56^{a}±1.81	1	42	8.65^{ab}±1.18	0.81	29.09
Total	324	130.06±1.39	92	246	10.08±0.86	1	134	7.74±0.66	0.68	98.53
F		4.32			3.95				3.28	
P value		0.001^{**}			0.002^{**}				0.007^{**}	

When the infestation level of varroa infestation level was calculated based on the two management system, out of the 122 and 206 colonies positive for varroa mite infestation from the apiaries and backyard type of management, 41(33.6%) and 57(27.7%) of the colonies were infested with > 5% infestation level respectively, with no significant difference between the two management system in the infestation level (χ^2=2.117; p= 0.548).

Table 19. Varroa mite infestation level of sampled colonies and the association with different risk factors

Risk factors	Varroa mite infestation level										χ^2	Sig
	<1%		1-3%		3.1 -5%		>5%		Total			
	N	%	N	%	N	%	N	%	N	%		
Type of management												
Backyard	44	21.4	57	27.7	48	23.3	57	27.7	206	100	2.117	0.548
Apiary	22	18	36	25.4	23	18.9	41	33.6	122	100		
Altitude												
Low land	23	26.1	26	29.5	16	18.2	23	26.1	88	100		
Mid land	33	27	39	32	29	23.8	21	17.2	122	100	31.621	0.000***
High land	10	8.5	28	23.8	26	22	54	45.8	118	100		
Hive type												
Traditional	38	26.2	43	29.7	29	20	35	24.1	145	100		
Intermediate	6	14.6	7	17.1	10	24.4	18	43.9	41	100	11.737	0.068
Movable frame	22	17.1	43	30.3	32	22.5	45	31.7	142	100		
District												
Ziquala	16	41	12	30.8	3	7.7	8	20.5	39	100		
Sekota	21	31.8	21	31.8	15	22.7	9	13.6	66	100		
Dehana	9	14.5	18	29	11	17.7	24	38.7	62	100		
Dessie zuria	1	1.8	10	17.9	15	26.8	30	53.6	56	100	52.943	0.000***
Tehulederie	12	21.4	18	32.1	14	25	12	21.4	56	100		
Kalu	7	14.3	14	28.6	13	26.5	15	30.6	49	100		
Total	66	20.1	93	28.4	71	18.6	98	29.9	328	100		

Of the total 118 colonies positive for varroa mite in the high altitude areas, majority of colonies 54(29.9%) were infested with > 5% infestation level of varroa mite and there was a significant difference between the three agro-ecologies (χ^2=31.621; p<0.001). As it is shown in the table 20, out of the 41 and 142 colonies positive for varroa mite from colonies kept in the intermediate and movable frame hives 18(43.9%) and 45(31.7%) of the colonies respectively were infested with > 5% infestation level of varroa mite with no significance difference in infestation level among the three hive types (χ2=11.737; p=0.068).

Based on the calculated infestation level in different districts, 30 (53.6%) and 24 (38.7%) of the total positive colonies from Dessie zuria and Dehana districts respectively were found to be infested with >5% infestation level. With this, there was a significant infestation level differences between the six districts (χ^2=52.943; p<0.01). Furthermore, in the United States (US), it has been recommended that honeybee colonies need to be treated against Varroa mite when 5–20 mites have been detected in 300 bee samples during the fall (Ellis and Macedo, 2001). However the survival of our local honeybee colonies with out any medication for long might evidenced the tolerance of the local honeybee races for higher varroa loads.

4.6. Relationship between Tested Parameters during seasonal Monitoring of varroa Mite

4.6.1 Colony Strength and Varroa

The results in the relationship between the major parameters for the honeybee colony strength and varroa mite infestation levels on colonies assigned for monitoring at six different apiaries (Jari, Gerado, Harbu, Jinkaba, Tsitsika and Kewzba) has been presented in table 22. Number of frames covered by bees was higher in Gerado (9.83±0.17) and Jari (8.67±0.16) locations and was significantly (p<0.01) lower in three locations; Jinkaba (6.78±0.22), Tsitsika (6.20±0.21) and kewzba (6.97±0.32). Besides, brood area was higher in Jari (2339.58 ±80.76cm^2) and Gerado (2283.33±154.1cm^2) apiaries and lower in the other four apiaries (Table 22). The amount of pollen grains stored as a protein source for brood rearing was higher for Jari (871.88±18.83cm^2 and Gerado (763.33±58.23cm^2) sites; and was significantly lower in Tsitsika (386.67±39.75cm^2), Jinkaba (386.67±39.75cm^2) and Harbu (483.33±37.69 cm^2) apiaries. However, nectar storage didn't show a significant difference among the representative apiaries (F=36.96, p<0.09). The lowest level of varroa mite infestation was

reported from Tsitsika (3.90±0.38 mites per 100 bees) while the highest infestation level was from kewzba (10.31±0.28 mites per 100 bees) locations and showed a significant difference among representative apiaries (F=17.72, p<0.001). Furthermore, dead brood removal (%) in 24 hours has been observed to be higher in Tsitsika (98.39±0.58%) and significantly (F=10.191, p<0.001) lower in kewzba (87.90%±1.42). The overall mean dead brood removal percentage was 90.83±0.7% (Table 22).

Table 20. Mean frames covered by adult bees (AB), brood area (BA), pollen area (PA), nectar area (NA), dead brood removal percentage (DBRP) and number of mites per hundred bees (NMPHB)

Monitoring sites/apiaries	NFCB (Mean± SE)	BA (Mean± SE)	PA (Mean± SE)	NA (Mean± SE)	DBRP (Mean± SE)	NMPHB (Mean± SE)
Jinkaba	6.78±0.22^{d}	1676.39±61.87^{b}	394.2±35.93^{b}	1262.32±91.68^{a}	95.95±0.67^{ab}	7.73±0.51^{b}
Tsitsika	6.20±0.21^{cd}	1640.00±59.03^{b}	386.67±39.75^{b}	1256.67±170.71^{a}	98.39±0.58^{a}	3.90±0.38^{d}
Kewzba	6.97±0.32^{cd}	1781.00±66.57^{b}	728.59±83.26^{a}	1573.91±83.26^{a}	87.90±1.42^{c}	10.31±0.28^{a}
Jari	8.67±0.16^{ab}	2339.58±80.76^{a}	871..88±18.83^{a}	1239.13±10.81^{a}	86.54±1.6^{c}	5.53±0.31^{cd}
Gerado	9.17±0.14^{a}	2283.33±154.1^{a}	763.33±58.23^{a}	1386.67±29.45^{a}	89.32±1.28^{bc}	8.51±0.7^{ab}
Harbu	7.81±0.38^{bc}	1791.67±100.82^{b}	483.33±37.69^{b}	1362.86±40.39^{a}	89.93±1.61^{bc}	6.4±0.58^{bc}
Total	7.71±0.11	1979.32±41.84	634.34±20.1	1348.74±38.92	90.83±0.70	6.88±3.81
F stat	20.84	13.19	34.6	7.84	10.191	17.72
P value	0.000	0.000	0.000	0.000	0.000	0.000

NB:

- Frames covered by adult bees was calculated from number of adult bees in a 5x5cm^2 unit area
- Values followed by different letters with in a column are significantly different at =0.05 using Tukey test

The analysis of data recorded in different months between September 2014 and June 2015 is depicted in (Figure 8). Varroa infestation level on worker bees was lower in September (5.88 mites per 100 bees). This may be due to the availability of high brood area in colonies when the mite entered to the brood cell for reproduction and hence mites at the phoretic phase reduced. Mite infestation was observed to be relatively higher starting from November (7.63 mites per 100 bees) in which brood area was dramatically reduced and the mite gets attached to the bees and stayed for long in the phoretic stage. Following the short rain starting from April, brood rearing commences due to the flowering of some pollen source plants thus

worker bees infestation level has been observed to reduce (6.16 mites per 100 bees). Further, mite infestation has been observed to slightly increase after the fall in brood rearing activities from May (6.61mites per 100 bees) to June (7.01mites per 100 bees) (Figure 8).

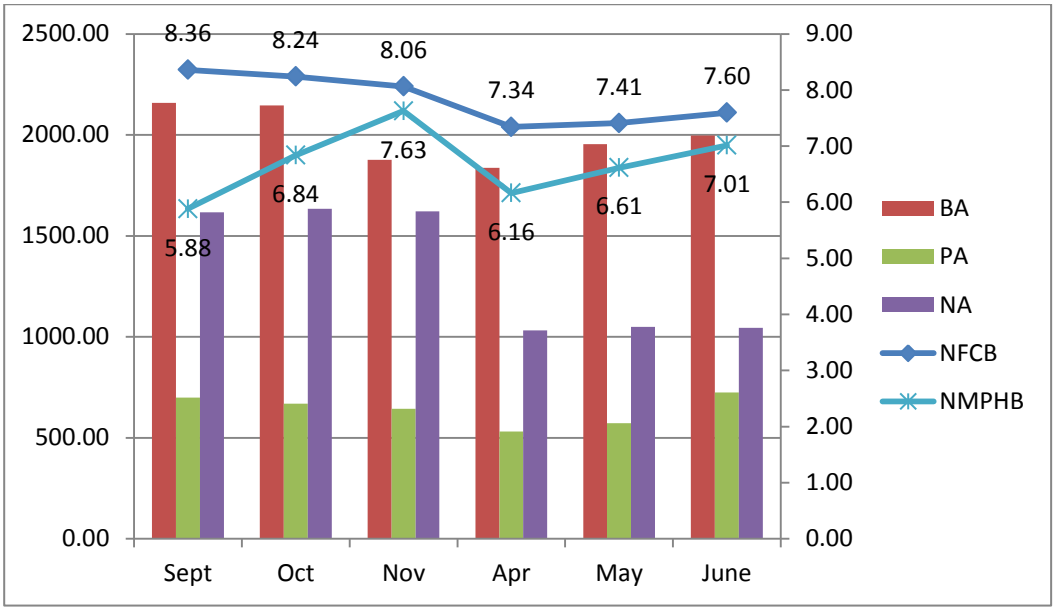


Figure 11. Seasonal dynamicity of varroa mites in different seasons in relation to colony population growth pattern

The present findings have been, partially, found to be in agreement with Allisop(2006) who reported the peak infestation level at November(8.7 mites per hundred bees) in south Africa and those of Ghoniemy, *et al*, (1991) and the percent infestation levels on worker bees were 2.9%, 10.2%, 13.2% & 5.1% for summer, autumn, winter and spring respectively with significant difference among all values. Allam (1994) reported that infestations reached their highest levels during autumn and spring, followed by winter and recorded their lowest numbers during summer. Harbo and Zuhlke (1988), in the USA, found in early February, that the number of mites per 100 adult bees ranged from 7 to 136 (average 19). Matthes *et al*. (1991), in their 2-year study, also found that maximum Varroa infestation level in worker brood was in March (101.7 female mites/100 cells) and January (67.5 mites/100 bees).

The result indicated that the highest brood rearing activity was observed in September while the least was observed during November to April. The smallest brood area coverage has been observed to coincide with seasons of rainfall. This result indicated that at the beginning of

September and during the active season when flowering plants are at bloom, colonies tend to build up their populations and as a result, the numbers of frames covered by bees were increased from September to November (figure 8).

Table 23 below shows the correlation matrix between the tested colony strength parameters and infestation rate of varroa mite. Accordingly, Varroa infestation levels have a significant weak negative correlation with bee colony strength parameters such as number of frames covered by bees (r= - 0.133, p= 0.021) and brood rearing area (r= - 0.156, p= 0.008) but no significant negative correlation was observed between pollen grain (r= - 0.045, p= 0.453) and nectar (r= - 0.007, p= 0.913) storages of the colonies. The weak relationship observed might suggest that the effect of varroa mite on colony strength was at its early stage and/or Varroa alone does not appear to strongly impact honeybee colonies in the Eastern Amhara. Besides, the effect of varroa mite was more prominent on weaker colonies than stronger ones.The present result agree with Allisop(2006) who reported varroa level were negatively correlated with number of frames covered by bees,amount of drone brood and stored pollen in south Africa.

Table 21. Correlation between parameters: number of frames covered by bees (NFCB), Brood area (BA), Pollen area (PA), nectar area (NA), and number of mite per 100 bees (NMPHB)

	NFCB	BA	PA	NA	NMPHB
NFCB	***	0.532**	0.551**	0.319**	-0.133*
		0.000	0.000	0.000	0.021
BA		***	0.569**	0.114	-0.156*
			0.000	0.060	0.008
PA			***	0.164**	-0.045
				0.006	0.453
NA				***.	0.024
					0.693
NMPHB					***

4.6.2 Climatic Variables and Varroa mite

There was a significant positive correlation between *Varroa* levels with elevation (r= 0.42, p< 0.001) and rainfall (r=0.17, p< 0.003), suggesting that environmental factors (climate, landscape ecology) may play a key role in mediating this host-parasite interaction, and perhaps honeybee health in general. Though, the effect of these environmental factors needs to be explored in much more detailed and larger scaled studies. This result is in agreement with the findings of Muli et al., (2014) who reported that Varroa levels were positively correlated with elevation (r(53)=0.44, p=0.001 in Kenya.

Table 22. Correlation between different tasted parameters of metrological variables

	DBRP	EFT	LFT	NMPHB	ALT	MAXT^{o}	MINT^{O}	RF
DBRP	***	-0.185*	-0.339**	-0.327**	0.020	0.262**	0.369**	0.066
		0.023	0.000	0.000	0.811	0.001	0.000	0.425
EFT		***	0.059	0.610**	0.238**	-0.204*	0.081	-0.041
			0.475	0.000	0.003	0.012	0.323	0.620
LFT			***	0.434**	0.008	0.111	0.019	0.140
				0.000	0.925	0.177	0.817	0.088
NMPHB				***	0.421**	0.027	0.007	0.170*
					0.000	0.637	0.909	0.003
ALT					***	-0.115*	-0.012	0.351**
						0.047	0.832	0.000
MAXTO						***	0.510**	-0.207**
							0.830	0.000
MINT^{o}							***	0.170**
								0.003
RF								***

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

DBRP= dead brood removal percentage NMPHB=Number of mites per hundred bees
ALT=altitude MAXT^{O}= maximum temperature MINT^{o}=minimum temperature RF= rainfall
EFT=early foraging time LFT=late foraging time

4.6.3 Hygenic status and Varroa mite

The overall mean percentage of pin killed brood larvae removal within 24 hours was found to be 90.83% being the highest recorded from Tsitsika (98.39%±0.58) and the lowest from Jari (86.54±1.6%) apiary (Table 24). Dead brood removal within 24 hours varied significantly among the sampling sites ($p<0.05$). This result is higher than the low dead brood removal percentage of 75.16 % reported by Gracia *et al*, 2013 for *Apis mellifera scuttelata* races in Brazil. Al Ghamdi (2002) also found that *A.m.jemenetica* removed 100% of the dead brood within 24hrs, while *A.m. carnica* removed only 55% in the same period. According to Spivak and Gilliam, (1998), hygienic behavior is greatly different among and within bee populations and subspecies. Gramicho and Goncalves (2009) also consider honeybees to be hygienic when 80-100% of the grubs killed are removed within 24 hours of the test. Brood removal percentage was positively correlated with temperature ($r= 0.260$, $p= 0.001$) and negatively correlated with varroa mite infestation rate ($r= - 0.277$, $p= 0.001$). No correlation was observed between brood removal percentage and altitude ($r= 0.020$, $p= 0.811$) and rainfall ($r= 0.066$, $p= 0.425$) (Table 24).

4.6.4 Foraging behavior and Varroa mite

As foraging behavior majorly depends on the availability and type of floral resources in the area, the honeybees adjust their foraging activity according to the flowering periods of the available resources in their surroundings. Accordingly, foraging behavior of the *A. m. monticola* honeybee race in Harbu showed a higher competence to the natural resources by exhibiting early foraging as early as 5:41am (Table 25). The colonies were also observed coming back from foraging as late as 6:43pm at Kewzba site. The result showed that there was a significant difference in early foraging time ($F=3.936$; $p<0.001$) among the different monitoring apiaries. However, late foraging time didn't show a statistical difference ($F=0.848$; $p=0.518$) between colonies assigned at different locations.

There was a significant positive correlation between *Varroa* mite load in early ($r= 0.610$; $p<0.001$) and late ($r= 0.434$; $p< 0.001$) foraging times. This result might explain that the influence from the parasite load on the host, which could cause the honeybees to fly longer or may not return back at all, possibly due to impaired orientation, was considered to be one of

the main factors contributing to the forager bees' late returning time. Furthermore, an actual forager bee parasitization may conceivably influence the behavior of the bee outside the colony, causing longer times of absence or even prevent its return (Kralj & Fuchs, 2006). The parasitized foragers dis- play a decreased capability of non-associated learning, prolonged absences from the colony and a lower rate of return to the colony (Kralj and Fuchs, 2006), which may be due to a reduced ability to navigate.

Table 23. Mean and SE of early and late foraging times of bees at sampling districts

Monitoring sites	EFT(am)		LFT(pm)	
	Mean± SE	Earliest	Mean± SE	Latest
Jinkaba	5:50±0:02[ab]	5:44	6:34±0:01[a]	6:37
Tsitsika	5:51±0:04[ab]	5:42	6:37±0:02[a]	6:42
Kewzba	5:59±0:02[a]	5:54	6:38±0:02[a]	6:43
Jari	5:45±0:01[b]	5:43	6:36±0:01[a]	6:38
Gerado	5:48±0:02[b]	5:44	6:36±0:01[a]	6:39
Harbu	5:47±0:02[b]	5:41	6:36±0:01[a]	6:40
Total	5:49±0:01	5:47	6:36±0:00	6:37
F stat	3.936		0.848	
P value	0.000		0.518	

4.7 Prevalence of Bee Lice

From the total colonies examined for the presence of bee lice, the laboratory diagnosis confirmed that 118 (30.7%) were found to be infested with bee lice (Table 26). This result was slightly lower than Gizachew Gemechu et al., (2013) who have reported 42% of colonies infested with bee lice at Holleta and its surrounding area. However, our result was found to be higher than the *Braula coeca* (bee louse) prevalence reported by Adeday Gidey et al., (2012) which was 4% in the brood and 5.5% in the adult honeybees at Wukro District, Tigray region.

When we see the types of hives used and the bee lice prevalence, the highest (46.5%) was observed in movable frame hives (Table 26). This higher bee lice prevalence in modern hives

might be associated with the modern hives', kept close with minimal spacing, exposure to drifting and robbing. Generally, bee lice prevalence in the different hive types used showed a statistically significant difference (χ^2=34.07; p<0.01). Furthermore, our result was found not to be inline with the results of Gizachew Gemechu *et al.*, (2013) who have reported that the highest bee lice prevalence (48.5%) was observed in traditional hives at Holleta.

On the other hand, higher bee lice prevalence was observed in honeybee colonies located in the medium altitude areas (46%; Table 26). In this case, a statistically significant difference was observed in bee lice prevalence among the different altitudes (χ^2 =24.959; p<0.01). This higher bee lice prevalence in the medium altitude areas than to that of the highland and lowland representations might be associated with the difference in environmental factors like temperature which might also affect the multiplication and occurrence of this pest.

Table 24. Prevalence of bee lice and the different risk factors

Risk factors	samples					χ^2	Sig.
	Total (n)	positive (n)	positive (%)	negative (n)	negative (%)		
Type of management							
Backyard	241	53	30.7	188	49.0	23.210	0.000**
Apiary	143	266	69.3	78	20.3		
Altitude							
Low land	102	19	18.6	83	81.4	24.959	0.000**
Mid land	139	64	46	75	54.0		
High land	143	35	24.5	108	75.5		
Hive type							
Traditional	182	31	17	151	83	34.070	0.000**
Intermediate	47	15	31.9	32	68.1		
Movable frame	155	72	46.5	83	53.5		
District							
Ziquala	46	17	37	29	63	48.623	0.000**
Sekota	80	36	45	44	55		
Dehana	66	25	37.9	41	62.1		
Dessie zuria	77	10	13	67	87		
Tehulederie	59	28	47.5	31	52.5		
Kalu	56	2	3.6	54	96.4		
Total	384	118	30.7	266	69.3		

In this study, the honeybee colonies kept in established apiaries showed the higher bee lice prevalence (69.3%) (Table 26). Moreover, there was a significant difference (χ^2 =23.210; $p<0.01$) in bee lice prevalence among the two types of colony management systems (the backyard and established apiary) the higher bee lice prevalence in the established apiary management system, we believe, might be associated with the contact among colonies and the introduction of colonies from unkown sources. The colonies in established apiaries have been found to be close to each other, hence, facilitating the transmission of the pest among the colonies through swarming and drifting.

Tehulederie district has the highest prevalence of 47.5% (28/59) of varroa mite in the investigation area followed bySekota district of 45% (36/80) among the sampling districts while Kalu experienced comparatively with low prevalence of 3.6% (2/56) with significant difference (χ^2=48.623; $p<0.01$) among the sampling districts (Table 16).

4.7. Prevalence of Honeybee Diseases

4.7.1. Nosema and Ameba

Diagnosis made on honeybees in field and laboratory at the study area revealed a very law prevalence of 1.3% (5/384) of nosema infection at Dehana district of Amdework sampling locality with no significant effect on the colony performance which can be explained that there was no nosema incidence in the sampled colonies of the study area.

The diagnostic survey also confirmed that amoeba was detected with an overall prevalence of 8.3% (32/384) of sampled colonies without any significant effect on the colony health at Jinkaba sampling localities of Sekota District, at Chilla sampling site of Dehana district and Gerado sampling localities of Dessie zuria district.

4.7.2. American and European foul brood

In this survey work among all colonies inspected for disease, all inspected colonies in all locations were found to be free of American foul brood, and European foulbrood there was no sign of American and European foul brood disease symptoms both in the brood and in the comb. Even in suspected case of abnormal brood of two colonies from Dessie zuria locations, the negrosine test was negative for both brood diseases.

4.7.3. Chalk brood

In this particular diagnostic survey period, it was able to observe the disease in only 0.78% (3/384) of the total sampled colonies at chila sampling site of Dehana district. From the result, it can be explained that there was no chalk brood incidence in the sampled colonies of the study area, however some beekeepers have reported the occurrence of the disease some three years back.

4.7.4. Acarine Mite

In this survey work, laboratory test was carried out for the presence of Acarine mites that enter and block the respiratory systems of the adult bees. However in all samples tested, there was no positive result indicating for the presence of the Acarine mite.

4.8. Survey on Agrochemicals

4.8.1 Major Crops grown and Attractiveness to bees

According to the results of this survey, the main annual crops grown in the study areas were teff, sorghum, wheat, barley, bean, maize and pea. In addition, Tomato, onion, and potato were also grown in irrigated lands. Among the perennial crops, mango, orange, lemmon, banana, Avcado and papaya were also produced widely in the study area. The area of land covered by different crops has been indicated in table 26. Of the total respondents interviewed, 52.9% of them were using irrigation and 47% of them were producing only rain fed crops. About 42.5%, 51.5% and 6% of the total respondents were producing once, twice and three times a year respectively.

In the current investigation, teff (87.1%), wheat (70.3%), barley (64.8%) and chat (66.7%) have been identified to be non-attractive to honeybees. On the other hand, tomato (86.3%), onion (78%), mung bean (60.7%), potato (51.4%), avocado (69.1%), papaya (51.2%) and apple (60%) were identified to be attractive to honeybees. In addition, sorghum (94.3%), maize (85.5), bean (87.7%), lemmon (98.2%), orange (83.8%), banana (80%) and coffee tree (97.1%) were found to be excellent attractive honey plants to honeybees (table 26). Although sesame, cowpea and Grass pea were not widely grown in the study area, they were found to be excellent attractive crops to honeybees in the lowland areas.

Table 25. Major crops grown, area cultivated and attractiveness to honeybees

Major crops Grown	Response	Respondents		Area cultivated		Attractiveness to bees		
		N	%	Mean (Timad)	SE	Excellent (%)	Attractive (%)	Non attractive (%)
Annual	sorghum	229	59.8	2.66	0.158	94.3	5.7	0
	teff	333	86.9	2.24	0.121	2.1	9.1	87.1
	sesame	58	15.1	3.52	0.569	85.2	14.8	0
	Cow pea	32	8.4			51.3	48.7	0
	Grass pea	40	10.4	0.68	0.139	92.1	7.9	0
	barley	194	50.7	2.11	0.126	20	33.6	64.8
	wheat	208	54.4	1.55	0.088	2.4	27.3	70.3
	bean	168	44	1.38	0.088	87.7	12.3	0
	pea	61	18.9	1.23	0.632	44.6	55.4	0
	Harricot bean	11	2.9	0.77	0.079	54.5	45.5	0
	chick pea	63	13.5	0.97	0.093	54.4	45.6	0
	maize	147	38.4	0.8	0.042	88.5	11.5	0
	Mung bean	28	7.3	0.92	0.101	28.6	60.7	10.7
	tomato	61	15.9	0.64	0.059	13.7	86.3	0
	onion	56	14.7	0.87	0.089	0	78	22
	potato	58	15.1	0.65	0.058	48.6	51.4	0
perennial	mango	113	29.5	15.74	1.994	19.6	78.6	1.8
	avocado	49	12.9	9.07		30.9	69.1	0
	papaya	43	11.2	16.89	3.83	48.8	51.2	0
	orange	69	18	38.61	12.628	83.8	16.2	0
	Lemmon	67	17.5	6.39	1.006	98.2	1.8	0
	zeytun	20	5.5	83.65	42.405	56.5	43.5	0
	banana	63	16.4	121.3	51.925	80	20	0
	coffee tree	37	9.7	39.12	13.073	97.1	2.9	0
	chat	37	9.7	95.81	16.324	0	33.3	66.7
	apple	7	1.8	6.7	1.399	40	60	0

4.8.2 Status of pesticide use in the study areas

In this study, 82.4% of the respondent beekeepers were using agrochemicals in their localities. This result has been found to be higher than results of Desalegn Begna, (2015) who has reported that 54% of his respondents used pesticides and among which about 61% of the

pesticides used by the farmers were identified as herbicides, 21% insecticides and 18% both types at western Amhara. Our study has also verified that 78.9%, 57.6% and 40.4% of the sampled respondents were using pesticides to protect the crops from pests, herbicides to control weeds and chemicals (DDT) as anti malaria respectively (Figure 13). Furthermore, the result has revealed that 91.4%, 5%, 2% and 1.7% of the respondents were applying the chemicals as liquid spray, granules, dust spray and as a wettable powder respectively (Table 27). This agrees with the findings of Desalegn Begna (2015) who reported 85.03% (124/147) farmers apply in liquid (emulsified), 8.84%(13/147) in powder and 4.6% (8/174) both in liquid and powder forms in western Amhara. In general, majority of the respondents (89.6%) were using these agrochemicals for cereals followed by pulses (14.7%) and fruits (14.6%). However, very small numbers of respondents were also found to use these agrochemicals for '*chat*' and leguminous crops (Table 27).

Table 26. Status and reason for agrochemical application by the respondents

Description	Response	Frequency	%
Do you use agrochemical in your locality	Yes	31.3	82.4
	No	6.7	17.6
Why do you apply the chemical	Crop pest control	280	78.9
	Weed control	205	57.6
	Malaria control	144	40.4
Chemical formulations	As liquid spray	275	91.4
	As dust spray	6	2.00
	Granules	15	5.00
	Wettable powder	5	1.7
For what type of crop do you use those agro chemicals?	Fruit	84	14.6
	Leguminous crop	6	1.6
	Cereal	271	89.6
	Pulses	46	14.7
	chat	9	2.9

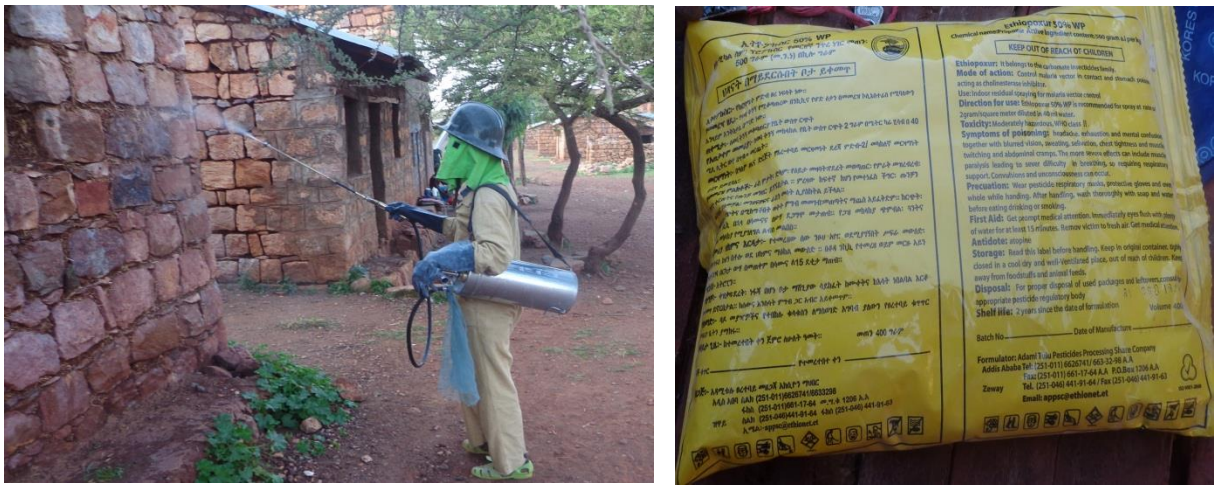


Figure 12. Application of anti-malaria spray in the study area

4.8.3 Type of pesticide Used

Among crops which are more attractive to local honeybees, majority of the beekeeping respondents (60.7%) didn't use chemicals for sorghum crop. But, these respondents have complained that they were facing a threat from wider chemical application by non-beekeepers to this crop (Table 28). Moreover, the rest of the respondents described that they were using karate (22.7%), Malathion (12.7%) and DDT (2.6%) as pesticides. The majority of the respondents were also claiming that they were using Malathion on *Cow pea* and *Grass pea* to control crop diseases and pests. This claim (the application of Malathion on *Cow pea* and *Grass pea*) which caused a devastating effect was higher in the lowland represtations.

The majority of respondents were also using karate (38.2%), DDT (10.9%) and Malathion (1.8%) for the control of pests in maize crop. In the study area, Endosulphan was also widely used on tomato (41.2%) followed by Mung bean (14.5%) crops (Table 28). DDT had been widely used on chat (90%) which is one of the non-attractive plants to honeybees. Moreover, very few respondents were also using DDT on lemmon, orange and maize which are considered to be attractive to honeybees.

Similarly, Desalegn Begna (2015) reported Malathion 50%, phenetratite 50% Ethiothoate 40%, Agrothoate 40%, Diazion 60% EC, Dimethoate 40%EC, Ethiolathion 50% EC or Malathion, Karate 5EC, and herbicides like 2,4-D Amine, Zura, Diazion60% EC, Agro-

Thoate40%, Etho-Thoate40%, Hepta clore, Phenetratite50%, Daconil, Diasnol, Primagram, Roundup, Agroset, Glycell and Terminator were the most used pesticides in the western Amhara. Marta Zelalem (2013) also reported the types of pesticides applied at mecha districts was Malathion (76%) followed by actalic (methyl parathion) (24%).

Table 27. Types of pesticides on different crop types

Types of crop	Types of pesticides used							
	Malathion (%)	DDT (%)	Karate (%)	Diazinon (%)	Endosulphan (%)	Mancozeb (%)	Rodenticide (%)	Never used any (%)
sorghum	12.7(29)	2.6 (6)	22.7 (52)					60.7 (139)
teff	27(86)	2.2(7)	8.2(26)	2.5(8)				60.2(192)
Cowpea	62.9(22)							31.4(11)
Grass pea	18.9(7)							81.1(30)
barley	15.3(28)						12.6(23)	72.1(132)
wheat	43.2(92)		0.9(2)				1.9(4)	54(115)
bean	1.2(2)							98.8(166)
maize	1.8(1)	10.9(6)	38.2(21)					49.1(27)
Mung bean			50.9(28)		14.5(8)			34.5(19)
tomato			17.6(6)		41.2(14)	41.2(14)		
onion			92.3(24)					
mango		3.3(4)	4.9(6)		3.3(4)	4.9(6)		83.2(103)
orange	13.3(13)	12.2(12)	18.4(18)			6.1(6)		50(49)
Lemmon	2.2(2)	15.2(14)	2.2(2)					100(68)
zeytun		13.6(3)						86.4(19)
chat		90 (36)						10(4)

Malathion is an organophosphorus, synthetic insecticide used widely in agriculture and also against insects to protect public health. This pesticide is categorised as a highly toxic to honeybees (Lowore, n.d.). The Pacific North-west Extension Publication: How to reduce bee poisoning from pesticides has clearly stated that Malathion should never be applied to flowering crops and plants. Furthermore, the chemical has been blamed for its 2-5 day

residual toxic effect on lives. The Apiculture Programme of North Carolina State University stated also that Malathion is a "highly toxic" and a "severe bee losses may be expected" when used in the vicinity of the honeybee colonies. Ri & Bura, (2013) have also explained that Diazinon could have the same effect with its residual toxic effect for about 2 days and should not to be applied on flowering plants.

4.8.4 Stage and Time of application

According to this survey majority of respondents apply agrochemicals on sorghum (52.2%), maize (87%) and chat (100%) before blooming. On the other hand most of the respondents apply the chemical on non-attractive crops to bees like teff (60.7%), barley (58.3%) and wheat (87%), also on attractive crops to bees like *Cow pea* (91.3%), *Grass pea* (100%), *Mung bean* (70.6%), tomato (94.2%), onion (58.3%), mango (60%), orange (100%) and lemmon (100%) at blooming (Table 29). Few respondents also reported their application of pesticides on barley and *Mung bean* after flowering and on maize and sorghum at any stage of the crop growth when disease signs were observed.

Table 28. Stages of application of pesticides

Types of crop	Stages of application			
	Before blooming	At blooming	After flowering	When disease signs are observed
sorghum	52.2%(48)	34.8% (32)		9.8%(9)
teff	33.6% (48)	60.7% (74)		
Cow pea		91.3% (21)	8.7% (2)	
Grass pea		100% (6)		
barley		58.3% (28)	37.5% (18)	4.2% (2)
wheat	5.2% (4)	87% (67)	5.2% (4)	2.6% (2)
bean		100% (3)		
maize	87% (20)			13% (3)
Mung bean	17.7% (3)	70.6% (12)	11.8% (2)	
tomato	5.9% (1)	94.2% (16)		
onion	16.7% (2)	58.3% (7)		25% (3)
mango	40% (4)	60%(6)		
orange		100%(33)		
Lemmon		100%(10)		
chat	100% (35)			

Desalegn Begna, 2015 reported from the total 147 pesticides users, 114(77.55%) apply pesticides before the crops bloom, 25 both before and during the crops in bloom. This indicated that colonies at the eastern Amhara due to the experience of pesticide application during the crops in bloom are more exposed to pesticide risks than the western Amhara honeybees.

According to the result of this survey majority of the respondents apply the chemicals at the early morning (67.9%) of the day and about 40.51% the respondents apply the chemicals during bees' active foraging time including late morning (14.9%), middle of the day (11.3%) and early afternoon (14.31%). Only few respondents (1.6%) were applying the chemicals at the late afternoon (Table 30). According to the results reported by Desalegn Begna (2015) though 64.4% of the users' at wesern Amhara prefer 6:00-9:00am as appropriate spray time, applications times are fixed by Knapsack renters and forced to spray at convenient time of knapsack renters.

Table 29. Time of the day when respondents were applying chemicals on their crops

Time of application	Frequency	%
Early morning	205	67.9
Late morning	45	14.9
Middle of the day	34	11.3
Early afternoon	13	14.31
Late afternoon	5	1.6

4.8.5 Awareness of farmers on the effects of agrochemicals

Most of interviewed beekeepers (56.2%) have found dead bees around the farm after the application of agrochemicals. As it is indicated in the table 31, with regard to awareness of the beekeepers on agrochemicals effect on honeybees, 86.9% of the respondents clarified that they had got this notion from extension agents (63.5%), from their own experience or personal observation (20.8%) and lessons from collogues (9.7%). This result agree with Desalegn Begna, 2015 who reported that 69% of the beekeepers have got an extension services and are already aware of when and how to properly use pesticides without producing effects on the environment and honeybees. Marta Zelalem, 2013 aloso reported 85% of the

total respondents at mecha districts of western Amhara are awared about the effects of agrochemicals.

Table 30. Awareness of farmers and their observation on the effect of agrochemicals on bees

Description	Response	Frequency	%
Did you find dead bees after you apply the chemical?	Yes	157	43.8
	no	214	56.2
Are you aware of agrochemicals effect on honeybees	Yes	271	86.9
	no	41	13.1
Who and how do you get the concept	personal observation	75	20.8
	Awareness from extension	230	63.5
	Lesson from colloquies	35	9.7
	Both (1+2)	21	5.8

4.8.6 Beekeepers and crop/fruit farmers' cooperation

From the total sample respondents about 39.5% of them use anti mala chemical sprays and 42.27 % have observed the effect of anti-malaria after the application which expressed as colony dwindling, loss of honey production and failure to perform a natural reproductive swarming (Table 32). To control poisoning of honeybees by chemicals, beekeepers take different measures like closing the hive entrance during application (29%), covering the honeybee colonies with coarse close (4%). However majority of the beekeepers (93.2%) did not use any control measures againist chemical poisoning. The result is agree with Marta Zelalem, 2013 who reported beekeepers at mecha district have experienced to control poisoning of honey bees by chemicals, nearly of respondents by moving the colonies away from the application area (21.2%), by covering the honey bee colonies with coarse close (9.1%), by closing the hive entrance during application (6%), adjust time of chemical application (3.4%) and do not use any control measures for chemical poisoning (61.1%).

About 93.3% of the beekeepers involved in this survey also described that agrochemical users did not announce the beekeeper before application. Moreover, 94.8% of the agrochemical user farmers especially the non-beekeepers had no willingness to use cultural pest control mechanisms like the application of IPM which were less promoted by the extension service (Table 32). The survey result agreed with the findings of Marta Zelalem (2013) who reported that none of the agrochemical users announce before they apply the chemical in Mecha district. In this regard, Desalegn Begna (2015) pointed out that the effects of pesticides due to none beekeepers indiscriminate uses and the jealously actions are showing absences of governing policy that put in place forcing measures so that the criminals can be penalized.

Table 31. Beekeepers and crop grower's cooperation and measures taken to protect bee colonies

Description	Response	Frequency	%
Do agrochemical users announce the beekeeper before application	Yes	26	6.8
	No	355	93.2
Measures taken to protect bee colonies from agrochemicals	covering with course cloth	15	4
	closing the hive entrance	11	29
	No any option	356	93.2
Willingness of farmers to use cultural pest control mechanisms	Yes	20	5.2
	no	362	94.8

4.8.7 Number of colonies lost due to agrochemicals

According to the result of current study 60.2% of the total respondent lost colonies due to the agrochemicals sprayed on different crops. This is slightly lower than the findings of Marta Zelalem (2013) who reported that 70.8% of the total respondent lost colonies due to the agrochemicals sprayed on different crops at Mecha district of western Amhara Region. The respondents were also pointed out the major signs observed on honeybees due to chemical poisoning like worker bee death at hive entrance (72.8%), massive death (17.7%), dead brood (5.8) and aggressiveness (3.7%). According to the survey result, the mean number of colonies

lost due to agrochemicals was 3.78±0.378, 2.36 ±0.217 and 1.43 for traditional, movable frame and intermediate hives respectively. The estimated amount of honey from lost colonies is shown in Table 33. As a result of this, from the interviewed beekeepers alone a total an estimated price of 834,910 ETB were being lost from unwise use of agrochemicals. Desalegn Begna (2015) reported financial loss incurred due to the dead, absconded and dwindled honeybee colonies in western Amhara was estimated to about 819291.4 USD. Therefore, this increased and substantial loss of local honeybees necessiates the importance of protecting bees from pesticides in the study area (Desalegn Begna, 2015).

Table 32. Number of colonies lost and honey lost with an estimated price due to agrochemical applications

Hive type	N	No of colonies lost			Honey lost in kg			Estimated price		
		mean	SE	sum	mean	SE	sum	mean	SE	sum
Traditional	187	3.78	0.378	707	29.99	3.501	5579	2941.77	349.04	547170
Intermediate	7	1.43		10	15.88	4.27	127	780	195.96	7800
Movable frame	81	2.36	0.217	191	39.22	5.556	3277	3217.7	331.38	279940

4.9. Poisonous plants

About 67.7% of the interviewed beekeepers reported the existence of poisonous plants in the study area (Table 34). Accordingly the major reason for the existence of poisoned honey was resulted from the nectar and or pollen of the source plant (92.1%). Among the respondents 4.1% claimed that they didn't know the reason for honey poisoning. Thirteen plant species: Kulkual (*Euphorobia spps*), kalkalda *(Euphorbia spp.*), kinche (*Parthenium hysterophorus*), Bahirsuf *(Helianthus annuus)*, kinchib *(Euphorbia tirucalli)*, Digita (*unidentified*) and Mech (*Guizotia scarab)* Eret (*Aloea spps*), Chiret (Agave *spps*), Nim *(Azadirachata indica),* ye wof kolo (*Lanthana camara*) and saligna (*Acacia saligna*) belonging to 8 different families (*Asteraceae, Agavaceae, Aloeaceae, Euphorobiaceae, Poaceae, Meliaceae, Fabaceae and Verbanaceae*) were the major poisonous plants reported in the area (Table 34). Nuru (2002) reported some poisonous bee plants from Northern regions of Ethiopia that include the families *Ranuculaceae, Solanaceae, Acanthacae, Euphorbiaceae* and *Phytolacaceae.*

Table 33. Presence of poisoned honey and their possible reasons of its existence

description	response	frequency	%
Have you faced poisoned honey?	Yes	260	67.7
	no	124	32.3
Reasons for poisoned honey	Source plant	245	92.1
	Times of storage	7	2.6
	Container	3	1.1
	I don't Know the reason	11	4.1

The effects of the poisonous plants mentioned below were observed on bees and or humans. Some of plants kill the forager bees by poisoning them and others have the physical body damaging effect. Honeys from some of these plants were also reported to cause an ailment or discomfort to the consumers by causing irritation on consumers' throat, diarrhea. According to the respondents, *Helliantus anus* and *Agave species* have a damaging effect due to their producing a tar which bees get stuck and sometimes expose the foragers to bird attacks. Similarly the effects of *Azadirachata indica* and *Lanthana camara* were reported as repellant and bee killers. On the other hand *Acacia saligna* were responsible for the dwindling of colonies during their flowering period. Honey from *'Digita', Aloea spps, 'kalkalda', Parthenium hysterophorus, kuliza, Euphorobia spps, Guizotia scarab* and *Azadirachta indica* cause irritation on consumers' throat (Table 35).

The result is in agreement with Yetimwork Gebremeskel et al 2015, who reported that plants like *Acacia saligna, Euphorobia spps, Melia azedarach and Azadirachta indica* were identified as poisons in Kilte Awulalo district of eastern Tigray. Keralem Ejigu (2002) reported Gumero, yefrenj Digit (*Cassia slamea*), Bisana (*Croton macrostyches*), Eret (*Aloea brahana*), Foch (*Ziziphus macronata*), Endod (*phytolacica dodecandra*) and *suspania spps* as plants toxic to bees and humans at Amaro and Enebse districts. Tewodros Alemu (2010) also reported the existence of toxic plants like neem tree (*Azardiacha indica*), Bahir Suf (*Helliantus anus*), Tihan tila (*Verrbena officinalis*), kinchib (*Euphorobia tirucalli*), Ater (pisium sativum) and kalkalda (*Euphorobia spps*.) in sekota district. However the kowlegde of beekeepers regarding the damage caused by poisonous plants on honeybees was comparatively very limited. Hence it should be further studied and proved by scientific investigations to verify their poisoning effect and status.

Table 34. Plants known for their poisoning effect, their flowering month, effects and symptoms

Local name	Scientific name	Family name	Flowering month	Plant type	Effects on	Symptoms and cases	Response (%)
Bahir suf	*Helliantus anus*	*Asteraceae*	Oct-Nov	Cultivated crop	Bees	Produce a tar which bees get stuck	12.7
Chiret	*Agave spps*	*Agavaceae*	Dec-Apr	Shrub	Bees	Produce a tar which bees get stuck and expose to bird attack	8
Digita	*Unidentified*	*Unidentified*	Oct-Nov	Herb	Human	Irritating the throat	10.2
Eret	*Aloea spps*	*Aloeaceae*	Sept-Nov	Shrub	Human	Irritating the throat	8.4
Kalkalda	*Unidentified*	*Unidentified*	Sept-Nov	Shrub	Human	Irritating the throat	23.1
Kinche	*Parthenium hysterophorus*	*Asteraceae*	Year round	Herb	Human	Irritating the throat	13.1
Kinchib	*Euphorobia tirucalli*	*Euphorobiaceae*	Nov	Shrub	Human	Irritating the throat	11.6
Kuliza	*Unidentified*	*Unidentified*	Sept-Oct	Shrub	Human	Irritating the throat	21.5
Kulkual	*Euphorobia spps*	*Euphorobiaceae*	May-June	Shrub	Human	Irritating the throat and vomiting	29.8
Mech	*Guizotia scarba*	*Poaceae*	Sept-Nov	Herb	Human	Bitter taste	9.5
Nim	*Azadirachata indica*	*Meliaceae*	March-May	Tree	Bees	Repellent and bee killer	5.8
Saligna	*Acacia saligna*	*Fabaceae*	Oct-Nov	Tree	Bees	Weakened colonies	1.8
Yewof kollo	*Lanthana camara*	*Verbanaceae*	Year round	Shrub	Bees	Repellent	3.6

Figure 13. Some poisonous plants in the study area

4.10. Prevalence of Absconding

Honeybee colonies abandoned their hive at any season of the year for different reasons. According to the respondents, 88.7% were facing a problem of absconding (Table 37) and evidence of relationship with agro ecology was obtained in this study (χ^2=13.388; sig=0.001). More prevalence of absconding was reported at lowland (95%) (Table 36).

From the total colony owned per year by the interviewed beekeepers the number of absconded colonies larger number of absconded colonies (>10) per individual beekeeper were reported from ziquala and sekota districts representing lowland and midland areas of waghimra zone respectively and from Kalu and Tehulederie districts of south wollo zone representing lowland and midland agro ecology. This result shows that more number of honeybee colonies was absconded at the lowland areas and relatively lower at the highland areas.

Table 35. Prevalence of bee colony absonding in different agroecologies

Parameters	Variables	Lowland	Midland	Highland	Total	χ^2	sig
Have you faced a problem of absconding	yes	95	80.3	91	88.7	13.388	0.001
	No	5	19.3	9	11.3		
No of colonies absconded/year	one	100	90.9	50.9	76.1	100.99	0.000***
	two	0	9.1	49.1	23.9		
	three	100	39.1	91.3	78.2	143.19	0.000***
	four	0	60.9	8.7	21.8		
	five	100	91.8	100	97.6		
	Six to ten	0	8.2	0	2.4	21.968	0.000***
	>ten	37.6	26.4	0	12.1		

In this survey, 61.3% of the respondents claimed that the main reason for absconding of honeybee colonies in the study areas was due to poor management followed by the nuisance from pest and predators attack (56 %). Some respondents also reported shortage of food (29.8%) as the other reasons for honeybee absconding (Table 37). The rest 0.9% of absconding was due to lack of shelter. This result is not in line with the result of Adebabay and his groups (2008) and Tessega Bellie (2009) that indicated shortage of bee forage was the main reason for absconding in the Amhara region and Bure districts respectively. Keralem Ejigu (2005) reported invasion of ants (40.5%) and attack by honey badgers (38.7%) as most common causes of absconding at Enebse district.

The interviewed beekeepers involved in the survey indicated that the prevalence of absconding was more prevalent at dearth period especially from March to June with a peak time at June in lowland and midland and at May in the highland. Due to a prolonged dearth at lowland areas, relatively the prevalence of absconding had started earlier starting from January. Besides absconding of bee colonies in the other months of the year starting from July to December was less prevalent. This agrees with the report of Adebabay Kebede et al (2008)

and Tessega Bellie (2009), absconding of colonies in Amhara region and Bure district respectively mostly occur from March to May.

Table 36. Reasons of absconding

Reasons for absconding	Variables	Lowland	Midland	Highland	Total	χ^2	sig
Shortage of food	yes	45.8	21.2	24.8	29.8	16.969	0.000*
	No	54.2	78.8	75.2	70.2		
Bee enemies	yes	59.4	81.8	35.5	56.0	53.334	0.000*
	No	40.6	18.2	62.4	43.2		
Lack of shelter	yes	0.0	0.0	2.2	0.9	4.278	0.118
	No	100	100	97.8	99.1		
Poor bee management	yes	56.2	38.4	81.2	61.3	54.823	0.000*
	No	43.8	61.6	18.8	38.7		

Chapter 5: CONCLUSION AND RECOMMENDATIONS

This research work took place on selected six districts of South Wollo and Wghimra zone of the Eastern Amhara Region with the objectives of examining the effect of major honeybee diseases, enemies and poisoning factors to local honeybees. Despite the potential of the study area for beekeeping business, in recent years, there has been a decreasing trend of colony populations and the local honeybees have been largely threatened by the occurrence of newly introduced pests, unwise use of agro-chemicals and presence of poisonous plants.

The results of the cross sectional study have identified ants, wax moth, birds, varroa mite, Wasps, Lizards, Spider, Bee Lice, Death head Hawks moth and Hamagot were the major honebee pests and predators in order of their importance.This study also revealed that the presence of real threat to beekeeping from varroa mite infestation. Higher prevalence of the varroa mite in all sample districts showed that there have been negative health consequences as the mite were moving easily through higher mobility or marketing of bee colonies and swarm catching with less or no any cautions. The higher infestation rate of varroa mite especially greater than 5 mites per hundred bees alarmed the local honeybee colonies need close monitoring as infestation levels greater than 10 mites per hundred bees could result a colony collapse in western honeybees. Having a very promising trait in hygienic behavior of the local honeybees in the study area, we could have better opportunity to develop a varroa resistance line. Varroa level reached its peak following the main honey flow season implying that there is a need to close monitoring and take proper control measures.

In the areas, most of the farmers extensively apply different pesticides for different purposes (to control different pests, weeds and mosquitos). As a result, pesticides have caused considerable effects in killing honeybees and their products decline. Those factors which have been identified to cause negative effects on the honeybee production were application of chemicals when bees are actively foraging, spraying on plants that are highly attractive to honeybees at their blooming stage and application of chemicals that are highly toxic to bees with longer residual toxicity. The existence of some poisonous plants grown in the area have poisoning effects on bees while ingesting their nectar or pollen and/or have physical damaging effects while some others had effects on consumers upon consuming honey.

Reommendations:

- general colony seasonal management practices, apiary cleaning and strengthening, regular colony inspection and disease diagnosis shall be considered and advocated as potential possible solutions to minimize honeybee death and colony decline due to honeybee pests and diseases.

- awareness creation using possible methods to advise all actors in the value chain to take important cautions while performing bee colony purchasing, swarm catching, and transporting from doubtful sources to minimize bee pests and disease fast spread

- The presence of Varroa mite in the study area is highly significant and might affect the international market accreditation process of the country.

- Strong national and/or regional enforcements that could regulate the illegal colony movements and marketing should be in placed as soon as possible to hold the pest and minimize the threat posed to the market and growth of the sector.

- Trainings on varroa mite diagnosis and monitoring, its economic importance and means of reducing its transmission and spread, should also be given to different actors.

- Immediate research agendas should be developed and promoted before mite population densities reach the threshold levels in each of the agroecologies.

- The presence of different varroa related diseases (bacterial and viral) in the country in general and the study area in particular should be addressed and studied.

- Non-chemical varroa mite control options like use of a screen bottom board, sanitation (comb culling), drone brood removal, re-queening with resistant stock, use of powdered sugar method, should be tested and verified through research in accordance with the local prevailing conditions.

- There must be due attention in minimizing the effect of agro-chemicals with the involvement of regional government through the development of coppingup strategies and policies to local conditions.

- Advise farmers to avoid the application of bee-toxic agro-chemicals on blooming plants and it is a good idea to check for the presence of other blooming plants which might attract bees.

- We need to be wise and active enough to advise people in selecting and applying less hazardous chemicals to honeybees before blooming and when honeybees are not foraging a food at least to minimize the direct application of the chemicals on honeybees which are working in the field.

- We need to advise farmers also to apply pesticides when honeybees are not flying. Evening application allows time for these chemicals to partially or totally decompose during the night.

- Programmed chemical application could be used as an option according to the prevailing conditions in each of the localities. In this case cooperation from farmers is mandatory to genuinely notify ahead of chemical application in command areas.

- The use of a product with less and short residual toxicity effects to honeybees and of course to human lives shall get due emphasis among users and product providers. More specifically, Malathion and Diazinon, known for longer residual effects shall better be substituted by other products.

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Chapter 7: APPENDIX

7.1. Questionnaire Used in the study

POTENTIAL THREATS TO HONEYBEE HEALTH WITH EMPHASIS ON VARROA MITE IN WAGHIMRA AND SOUTH WOLLO ZONES OF AMHARA REGION, ETHIOPIA, QUESTIONNAIRE

Date _____

Zone _____

District_____

Zonal Description

Altitude _____ fts, Rf. _____ To _____RH_____
_____

Agro ecology:-

Highlands (%) _____ No. Beekeepers _____ No Colonies Trd _____ Trs ____ Mod ____
Midland (%) _____ No. Beekeepers _____ No Colonies Trd _____ Trs ____ Mod ____
Lowland (%) _____ No. Beekeepers _____ No Colonies Trd _____ Trs ____ Mod ____

Woreda description

Altitude _____ fts, Rf. _____ To _____ RH_____
_____

Agro ecology:-

Highlands (%) _____ No. Beekeepers _____ No Colonies Trd _____ Trs ____ Mod ____
Midland (%) _____ No. Beekeepers _____ No Colonies Trd _____ Trs ____ Mod ____
Lowland (%) _____ No. Beekeepers _____ No Colonies Trd _____ Trs ____ Mod ____

Natural Vegetation Category:

- Dominant crops_____
- Dominant fruits_____
- Soil type_____
- Forest/Tree/Bushes/herbs_____

To be completed on the respondents reply

PA_____ Co-ordinate_____

Altitude_____

I. PERSONAL INFORMATION OF HOUSEHOLD

1. Name of respondent _____
2. Sex 1. Male 2. Female
3. Age _____
4. Marital status
   a. Single c. Divorced
   b. Married d. Widow or widower
5. Educational status _____
   a. Illiterate b. Basic education
   c. Grade 1-4 d. Grade 5-8 e. Grade 9-12.

II. FAMILY CHARACTERISTICS

6. Family composition:

Family size: Male ———— Female ————

Age composition	No	
	Male	Female
Number of children below 14 years of age		
Number of youth (15-40 years of age)		
Number of adults (41-60 Years of age)		
Number of old persons (Above 60 years)		

III. CROP PRODUCTION SITUATION

7. Land holding (ha)
   7.1. Total land holding _____
   7.2. Farm land (plantation courage) _____
   7.3. Grazing land _____
8. Do you use irrigation?
   a. Yes b. No
9. If yes how many times of the year do you harvest?
   a. Once b. Twice c. Three times d. More than three times
10. What are the main crops and trees grown in your locality?

Crops grown in rain feed	Crops grown in irrigation	Permanent crop types (Fruits and vegetables)	Forest tree/bushes/herbs

IV. Agrochemical applications

11. Major crops grown and agrochemical applications

No.	Types of crops	Area (ha)	*	Quantities of agrochemicals applied					
				Pesticides		Insecticides		Herbicides	
				Amount	**	Amount	**	Amount	**
1	**Annual**								
1.1									
1.2									
1.3									
1.4									
1.5									
2	**Perennial**								
2.1									
2.3									
2.4									
2.5									

*Attractiveness: - 1-exellent, 2-attractive, 3-non attractive

**Stages of application: a/ before blooming b/ at blooming, c/ at flowering, d/ after flowering

12. What are the major crop production problems you encountered? **(Put in the order of importance)**
    a. Shortage of farmland
    b. Drought (shortage of water)
    c. Soil fertility
    d. Inputs (seed, fertilizer)
    e. Weeds
    f. Insects
    g. Diseases

13. Do you use agrochemicals/chemicals in your locality?
    a. Yes b. No

14. If yes, why do you apply agro chemicals/chemicals?
    14.1. Crop pest control
    a. Yes _____ b. No_____
    14.2. Weeds control
    a. Yes _____ b. No_____
    14.3. Malaria control
    a. Yes _____ b. No_____
    14.4. Tsetse fly control
    a. Yes _____ b. No_____
    14.5. Others (specify): _____

15. Did you find dead bees around the farm after you apply the chemicals?
    a. Yes b.No

16. For what type of crops do you use these chemicals?
    a. Fruits b. leguminous crops c. Cereals d. Pulses e. Other _____

17. In what stage of the crop growth you apply the chemicals?
    a. Early growth stage b. Mid growth stage
    c. at the beginning of flowering d. As disease signs are observed
    e. Other (specify) _____
18. Are you aware of agrochemical effects on honeybees?
    a. Yes b. No
19. Who and How do you get the concept?

_____
_____
_____

20. When do you use agrochemicals/chemicals (months)? _____
    a. Herbicides _____
    b. Insecticides_____
    c. Pesticides_____
21. What type of agrochemicals/chemicals are you using?
    a. Herbicides _____
    b. Insecticides_____
    c. Pesticides_____
22. How do you apply the chemical?
    a. As liquid spray b. As dust spray
    c. As seed treatment d. By other means (specify) _____
23. If you use liquid spray in what proportion did you dilute?
    a. Herbicides _____
    b. Insecticides_____
    c. Pesticides_____
24. At what time of the day do you apply the chemicals?
    a. Morning c. Late evening
    b. Middle of the day d. Night
25. What is your reason to apply at this time? (For the choice of Q no. 24)

_____
_____
_____

26. Have you faced agrochemicals/chemicals effect on honeybees?
    a. Yes b. No
27. What are the major signs observed on honeybees related to these chemicals?
    a. Massive death b. Aggressiveness c. Dead brood
    d. Queen death e.Worker bee's death at hive entrance
28. If your answer for Q.26 is yes how many colonies did you lost due to chemicals? _____ When? (Year and months): _____
29. What is the estimated honey you lose? (Kilograms)

No	Types of Beehive	Effect of Agrochemicals		
		No. of lost colonies by chemicals	Honey lose in Kg	Estimated price/ETB
1	Traditional			

2	Intermediate			
3	Movable-frame			

30. What measures do you take to protect your bee colonies from agrochemicals /chemicals?
    a. By covering the honeybee colonies with coarse cloth
    b. By moving the colonies away from the application area
    c. By applying the chemical at the appropriate time of the day
    d. By closing the hive entrance
    e. By other methods (specify).
    f. No any mechanism
31. Why do you use this method? (Choice for Q. no, 55)

_____
_____

32. Do agrochemical users announce the beekeepers before application?
    a. Yes b. No
33. Are farmers willing to use mechanical and cultural weed and pest control mechanism after awareness?
    a. Yes b. No
34. If no, why? _____
35. How frequent did anti-malaria chemical sprays in your home?

_____
_____

36. Do you observe any effect of this chemical on your honeybee colonies?

_____
_____

37. What are the traditional pest and weed controlling methods?
    a. Herbicides _____
    b. Insecticides_____
    c. Pesticides_____

V. BEEKEEPING SITUATION

38. Do you keep bees a. Yes b. No
39. For how long do you keep bees and practiced beekeeping?
    a. More than 15 years b. 10 – 15 Years c. 5 – 9 years d. 1 – 4 Years
40. How do you get colony to start beekeeping practices? Source of bees
    a. Gift from parents b Catching swarming bee
    c. Buying d. Robbing from caves and forests
    e. Other (specify)
41. What are the driving forces to engage in beekeeping practices?
    a. Income b. Home consumption
    c. Both 1 & 2 d. Others (specify)_____
42. If you use for home consumption, List the home use of honey.
    15.1. As a food a. Yes _____ b. No_____
    15.2. As a medicine a. Yes _____ b. No_____
    15.3. For beverages a. Yes _____ b. No_____
    15.4. For cultural and ritual ceremonies a. Yes _____ b. No_____

15.5. Others (specify):_____

43. No of colonies owned, honey & beeswax yield per year

Hive type	2004			2005			2006		
	Col.	Honey	Wax	Col.	Honey	Wax	Col.	Honey	Wax
Traditional									
Transitional									
Modern									

44. Where did you place your colonies?

No	Site or placement of hive	Traditional	Transitional	Moveable- frame
1	Backyard			
2	Under the eaves of the house			
3	Inside the house			
4	Hanging on trees near homestead			
5	Hanging on trees in forests			
6	Others (specify)			

VI. HONEYBEE DISEASE AND PESTS

45. Is there a decreasing trend in the number of colonies you owned?

46. If there are decreasing trend in no of colonies and hive product over the year, what do you think the case?

Causes	Indicate change you observed for your answer
Lack of bee forage	
Water	
Pest & predators	
Diseases	
Pesticides and herbicides	
Absconding	
Death	
Others, Specify	

47. If your answer above is or includes pests & predators, indicate the major causes and put in order of their economic importance according to your local area.

SN	Common name	Local name	Order of importance	SN	Common name	Local name	Order of importance
1	Ants			8	Death head hawks moth		
2	Wax moth			9	Lizards		
3	Hamagot			10	Toads		
4	Spider			11	Snakes		
5	Wasps			12	Praymanteds		

6	Birds			13	Mice		
7	Beetles			14	Bee lice		
				15	Others		

48. If your answer above is or includes disease, indicate the major causes and put in order of their economic importance according to your local area.

SN	Common name	Local name	Order of importance	SN	Common name	Local name	Order of importance
1	Nosema			6	Chalk brood		
2	Amoeba			7	Sac brood		
3	Paralysis			8	Stone brood		
4	varroatosis			9	AFB		
5	Acarin disease			10	EFB		
				11	others		
				12			
				13			

49. Have you ever observed varroa mites in your colony a. Yes _____ b. No _____

50. If yes indicate number of honeybee colonies infested by varroa mite over the last 3 yrs

Hive Types	No. of colonies infested with varroa mite		
	2004	2005	2006
Traditional			
Transitional			
Modern			

51. since when did your colonies start to suffer from Varroa mite infestation_____

52. Effects of varroa mite

Year	Irregular brood pattern	Absconded	Dwindled	Died death	Infected not yielded	Deformed wing	Disturbance of colonies
2004							
2005							
2006							

53. Average honey yield /yr with regard to varroa mite infestation

1. Infested _____ Trad _____ Trans _____ Mod _____
2. Un infested _____ Trad _____ Trans _____ Mod _____

54. Condition of honeybee colonies before & after infestation;

SN	Condition of Colonies	Before infestation	After infestation
1	Strength	1.Strong ☐	1.Strong ☐
		2.Medium ☐	2.Medium ☐
		3.Weak ☐	3.Weak ☐
2	Cleaning behavior	1.Poor ☐	1.Poor ☐
		2.Good ☐	2.Good ☐

		3.Very good ☐	3.Very good ☐
3	Aggressiveness	1.High ☐	1.High ☐
		2.Medium ☐	2.Medium ☐
		3. Docile ☐	3. Docile ☐
4	Foraging activities	1.Poor ☐	1.Poor ☐
		2.Good ☐	2.Good ☐
		3.Very good ☐	3.Very good ☐

55. Which of your colonies most likely infected by disease and pests?

SN	Condition of Colonies	Diseases	Pests	Varroa mite
1	Strength	1.Strong ☐	1.Strong ☐	1.Strong ☐
		2.Medium ☐	2.Medium ☐	2.Medium ☐
		3.Weak ☐	3.Weak ☐	3.Weak ☐
2	Defensive behavior	1.Docile ☐	1.Docile ☐	1.Docile ☐
		2.Agressive ☐	2.Agressive ☐	2.Agressive ☐
		3.Very Aggressive ☐	3.Very Aggressive ☐	3.Very Aggressive ☐

56. Have you observed any change of behavior on infected colonies? Yes ☐ No ☐

SN	Behavioral changes	Diseases	Pests	Varroa mite
1	Irregular brooding pattern	☐	☐	☐
2	Disturbance of the colonies	☐	☐	☐
3	Dead bees and brood on the entrance	☐	☐	☐
4	Weakened colonies	☐	☐	☐
5	Absconding	☐	☐	☐
6	Infested and not yielded	☐	☐	☐
7	Reduced foraging activity	☐	☐	☐
8	Loss of the entire colony	☐	☐	☐
9	Other specify	☐	☐	☐

57. Which types of bee keeping system is most likely affected by Diseases and pests?

SN	Beekeeping system	Diseases	Pests	Varroa mite
1	Traditional	☐	☐	☐
2	Transitional	☐	☐	☐
3	Modern	☐	☐	☐
4	1+2	☐	☐	☐
5	2+3	☐	☐	☐
6	1+3	☐	☐	☐

58. When do you most likely observe bee disease & enemies in the colony?

SN	Beekeeping system	Diseases		Pests		Varroa mite	
		Observe	No	Observe	No	Observe	No

			infected		infested		infested
1	Sep. - Nov	☐		☐		☐	
2	Dec - Feb	☐		☐		☐	
3	March- May	☐		☐		☐	
4	June- August	☐		☐		☐	
5	Others Specify	☐		☐		☐	

59. What measure have you taken to control varroa mite, disease, pest & predators?

SN	Type of measure	Diseases	Pests	Varroa mite
1	Traditional			
2	Modern			
3	Management			
4	Others specify			

60. Have you faced a problem of absconding?

a. Yes b. No

61. If yes, number of honeybee colonies absconded from the total colony owned/year

1. One 2. Two 3. Three 4. Four 5. Five 6. Six - Ten 7. >10

62. If yes, in which month does absconding occur? (Circle one or More Months)

1. January 2. February 3. March 4. April 5. May 6. June 7. July
8. August 9. September 10. October 11. November 12. December

63.What do you think the reason for absconding?

1. Shortage of food 2. Bee Enemies 3. Lack of shelter 4. Poor bee management

VII. Poisonous plants

64. Have you ever faced poisoned honey?

a. Yes b. No

65.If yes, what do you think the reason? It is due to:

a. The Source Plant b. The Container c. Time of Storage d. The Environment
e. Poisonous agrochemicals f. I don't know the reason g. Others (specify) ----------

66. Plants known for their poisoning effect and symptoms

No.	Plant Name	Flowering Month(s)	Symptoms and Cases
1			
2			
3			
4			