

Studying causes of honey bee mass death in western Amhara Region, Ethiopia

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ABSTRACT

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The study was conducted with the objectives of identifying the causes of honey bee mass death (many individual bees dying at once) and indicating the amount of money losses due to honey bee diseases and pests. Two types of data collection techniques were used. The first was a survey with a semi-structured questionnaire and the second was a case study through monitoring. Five zones; South Gondar, North Gondar, West Gojjam, East Gojjam, and Awi zones were covered by the survey study. On top of this, case reports were present from west Gojjam in three districts (locally called *Woreda*) containing seven *kebeles* (administrative units under district). Then, laboratory investigations were used for confirmation of the causes. From data collection until data analysis SPSS and excel software was applied. According to the survey, the major causes of honey bee mass death were agrochemicals, agrochemical & wax- moth, and agrochemical & *Mich* (local weather difference) with the respective percentage of 21.5%, 24.05%, and 20.25%. But the laboratory result confirmed that the causes were Varroa mite and Wax-moth on brood and Nosema on adult bees, also poisoning from agrochemicals was also suspected. These combination stressors lead to mass death in the honey bee. Therefore, we recommend that adopting continuous management practice should better to minimize the identified honey bee stressors including harmonization of agrochemical application with beekeeping activities. Besides, it would be preferable to develop proactive prevention and control strategies for the identified diseases and parasites. Furthermore, even if *Mich* is the result of the weather change, further investigation should be done to determine how it can kill honey bees.

1. INTRODUCTION

Honey bees are susceptible to a wide variety of parasites and pathogens including mites, protozoa, fungi, viruses, and bacteria. They can also be infested with a wide range of pests and other phenomena endangering their health and life. A growing body of research shows that the principal factors involved are parasites, pathogens (Christian et al 2015; Dolezal et al 2019; Berenbaum and Liao 2019), reduced diet diversity (Dolezal et al 2019), environmental stressors (Bianco et al 2014; Klein et al 2017), mono-crop farming (Bianco et al 2014; Branchiccela et al 2019), widespread use of pesticides and phytochemicals (Bianco et al 2014; Berenbaum and Liao 2019). Clinical symptoms are not enough to determine which disease threatens the bee colonies (Erban et al 2017). This is because many bee diseases show similar clinical symptoms although they have different causative organisms.

In Ethiopia, the presence and distribution of more than 16 honey bee pests and three microbial diseases have been studied (Begna 2015). According to Kebede et al (2008), some diseases reported to exist in Ethiopia are Nosema, Amoeba, and Chalkbrood diseases. Amoeba is not directly killing honey bees, but it is serious and impairs the functioning of the Malpighian tubules, which act as a kidney of bees (Rossi et al 2020). Nosema causes bee decline by changing honey bee behavior and

2. MATERIAL AND METHODS

2.1. Description of the Study Area

The study was conducted in the western Amhara Region. The western Amhara Region includes 6 administrative zones: North Gondar, South Gondar, East Gojjam, West Gojjam, Awi, and Bahir Dar special zone. The annual total rainfall of the western Amhara Region varies from 878 mm to 2,100 mm (Taye et al 2013). The annual mean minimum and maximum temperatures are between 9.2°C and 32.7°C (Taye et al 2013). The total estimated population size was 12,299,763 people and it covers an approximate area of 98335.07 km² (BoFED 2014). It is bounded by Tigray Region in the north, Oromia Region in the south, Benishangul-Gumuz Region and Sudan in the west, and eastern Amhara in the east direction.

physiology (Goblirsch 2018; Duquesne et al 2021). The chalkbrood disease causes death and mummification of honey bee brood (Dessalegn 2006; Aronstein and Murray 2010; Castagnino et al 2020). Also, beekeepers in the Amhara Region of Ethiopia recognized that their colonies could suffer from a disease locally known as '*mich*' or '*mushen*' which results in mass death of bees in the hive, although the causes were not known (Ejigu 2005). A nationally coordinated honey bee diseases and pests diagnostic survey was conducted from March 27 to April 18, 2009, attempting to identify the types, distribution, and prevalence of honey bee diseases and pests in the region. The result confirmed that Nosema, Amoeba, American, and European foulbrood diseases

were not found in any of the sampled colonies (Democratic Republic of Ethiopia 2010).

The findings of this survey conflict with previous positive reports of these diseases in the region, so that the picture of honey bee diseases of the region remains unclear. Thus, to refine this result additional study was needed. This study was developed to identify the causes of honey bee colony death and to indicate the amount of money losses due to honey bee diseases and pests in the Amhara Region of Ethiopia.

2.2. Methods of Data Collection

Survey:

Two techniques were used to collect data: a survey with semi-structured questionnaires and case-study through monitoring. The first data collection technique, a survey, was conducted in 2012 and covered all agro-ecological zones (high, mid, and lowlands) of the study area. Study areas were selected based on their beekeeping production potential (honey and wax), road accessibility, and presence of honey bee mass death report. After selecting the study area, interviewees were selected based on relative beekeeping experience among beekeepers either in traditional (made from mud, clay soil, or bamboo), top-bar (made from bamboo and timber), or frame hives (made from timber). Honey bee colony strength data was collected depends on beekeepers' observation, but we guided them to estimate the number of frames

covered by bees, number of top-bars covered by bees, and size of traditional hive occupied by bees. A total of 104 respondent beekeepers from nine districts (administrative unit under zone) were used for the interview. Because of finite population, the sample was determined using Yamane statistical formula (yamane 1967). The formula is;

$$n = N / [1 + N (e)^2]$$

Where; n = the sample size

N = the finite population

e = the level of significance at 0.05

1 = unit or a constant

Case-study:

The second type of data collection was a case study through monitoring conducted from 2012 to 2016 for five consecutive years. Occurrence of cases of mass honey bee death is provided by administrative units of experts in each district, locally called *woreda* and *kebeles*. To collect the data on case reports, semi-structured questionnaires and contact addresses were distributed to each district expert. The semi-structured questionnaire was distributed after discussing the severity and sign of the disease. Through the monitoring, three major cases of mass honey bee death were captured and characterized below.

The first case was reported from the Bure district in the West Gojjam zone. Samples collected from the suspected hive were then sent to Bahir Dar Animal Health Diagnostic and Investigation Center (BDAHDIC) located in Bahir Dar, Ethiopia for laboratory examination. The second case was also reported from Bure and Jabi Tehnan districts. Samples collected from suspected hives were sent to Bahir Dar Animal Health Diagnostic and Investigation Center (BDAHDIC) for laboratory examination. In the third case, honey bee mass death was reported in the Degadamot District. Samples collected from suspected hives for laboratory examination were diagnosed in Sekota Dryland Agricultural Research Center (SDARC), located in Sekota, Ethiopia. In general case reports were presented in seven *kebeles* via; four *kebeles* from Bure, two *kebeles* from Jabi Tehnan, and one *kebele* from Degadamot districts. In Jabi Tehnan district both brood and adult bees from seven hives, in Bure district, both brood and adult bees from three hives, and in Degadamot district three hives for adult bees and two hives for brood samples were collected (Table 1).

Table 1: Number of hives and samples collected for case studies

District	Kebele	Number of beekeepers interviewed	Number of colonies inspected	Samples collected	
				Adult	Brood
Jabi Tehnan	Wegan-Gesa	6	10	5	5
	Agomamite	5	2	2	2
Bure	Denbun	4	-	-	-
	Tiatia	3	4	1	1
	Wan-Gedam	3	1	1	1
	Teniga	2	1	1	1
Degadamot	Agaminiger	3	6	3	2
Total		26	24	13	12

Note: – = data not available

Samples in each case were taken from abnormal bees, immediate dead bees, and unusual broods. A total of 100-150 immediate dead honey bees for tracheal mite examination, 200-300 live honey bees for varroa mite, *Nosema*, and *Amoeba* (because a minimum of 10 adult bees is sufficient for each protozoal disease), and 5×5 cm area of brood for varroa mite per hive were collected as a sample. For an accurate diagnosis, all adult bees were preserved in alcohols. Hives reported by the development agent for the occurrence of the case were carefully inspected and diagnosed for clinical symptoms of honey bee disease. For bacterial, viral, and fungal disease identification, only clinical symptoms were used for diagnosis (laboratory tests were not performed for confirmation). For protozoa (*Nosema apis* and *Malpighamoeba mellificae*) and parasitic tracheal mites (*Acarapis woodi*), microscopic examinations were made through the cutting of worker honey bee abdomen and tracheas respectively. Additionally, honey bee pests were identified through eye aid, but, a magnifying glass was used for differentiating varroa mite from bee-louse (they resemble each other when seen without a magnifying

glass). All case studies were done using appropriate diagnostic procedures developed by Shimanuki and Knox (2000).

Statistical Analyses

With the use of the SPSS (version 20) program, the collected data were entered, organized, and then analyzed using descriptive statistics. Additionally, the graphics were presented and disease prevalence was calculated using excel software.

3. RESULTS AND DISCUSSION

3.1. Demographic Characteristics

Most of the sampled households (85.6%) were male-headed while female-headed households constituted about 14.4% (their husbands had either died or left). The age of respondents ranged from 22 to 80 years old, with the mean age being 45.29 years old. From a total 104 respondents 23.1%, 33.7%, 11.5%, and 31.7% of the respondents kept honey bees for <5, 6-12, 13-18 and >19 years respectively. As a result, 33.7% of all respondents had beekeeping experience ranging from six to twelve years (Figure 1). This indicated that many new inexperienced beekeepers are getting into beekeeping activities.

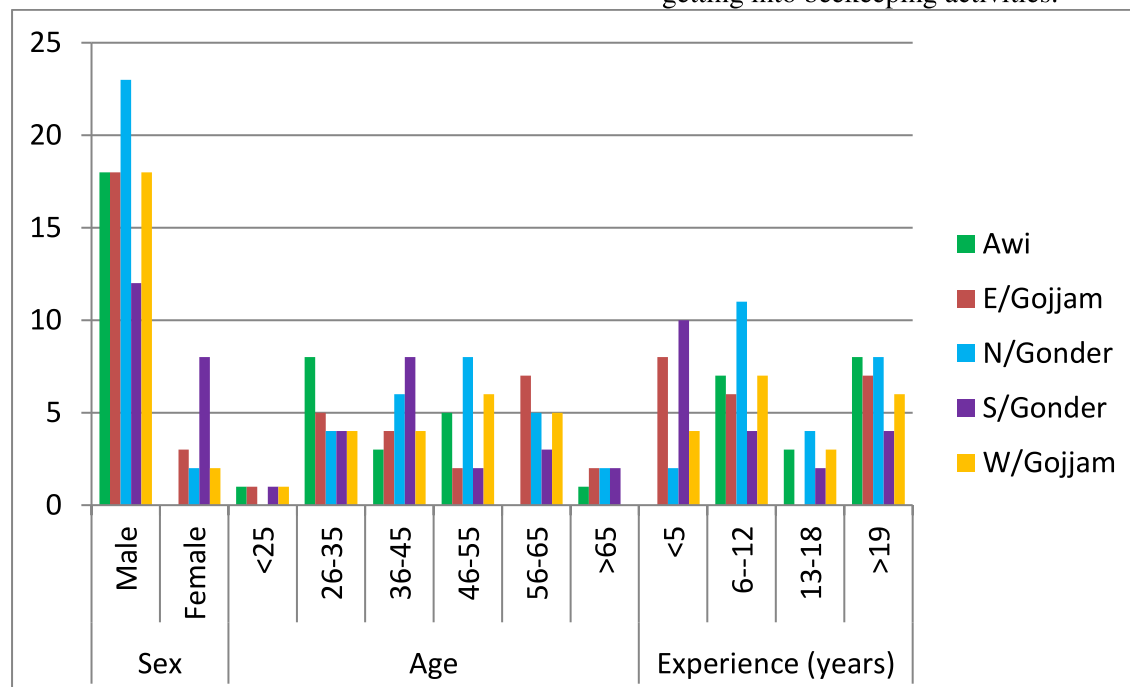


Figure 1. Household information of the study area

3.2. Beekeepers' Perception on the Cause of Honey bee Mass Death and its Mitigation Techniques

A total of 79.8% of the respondents reported the mass death of honey bees. The other 20.2% responded that they never observe the mass death of honey bees in their apiary sites. However, among respondents that reported mass honey bee death, 95.2% knew and could explain the cause. The remaining respondents (4.8%) simply observed the honey bee's mass death but could not explain the causes.

According to the respondents, wax-moth, *Mich*, and agrochemicals were to blame for the mass death of honey bees (Figure 2). A similar result was reported previously where the number of bee colonies either died or absconded from their hive due to extensive use of agrochemical (Beyene and Verschuur 2014). It was also confirmed that mass honey bee death has been linked to Colony Collapse Disorder (CCD),

which was brought on by a number of pesticides, parasites, and diseases (Fleck 2013). Even on field experiment in Japan insecticide was reported as a causes of mass honey bee death (Kimura et al 2014). The cause of honey bee mass death due to *Mich* was supported by the finding of Ejigu (2005) that bees could suffer from a disease locally known as '*Mich*' or '*Mushen*' which results in honey bee mass death in the hive. Additionally, the respondents were reported that wax-moths had to blame for honey bee mass death on honey bee brood. The presence of wax moths might be caused by improper frame hive management, notably by failing to replace old combs with new combs. These result was in line with other studies that the dark old comb containing broods are at risk (Charrière and Imdorf 1999; Vijayakumar et al 2019). However, it goes against the other finding that the wax moth only uses abandoned combs for its own purposes (lays large numbers of eggs, from which larvae emerge and eat the comb) (Somerville 2007).

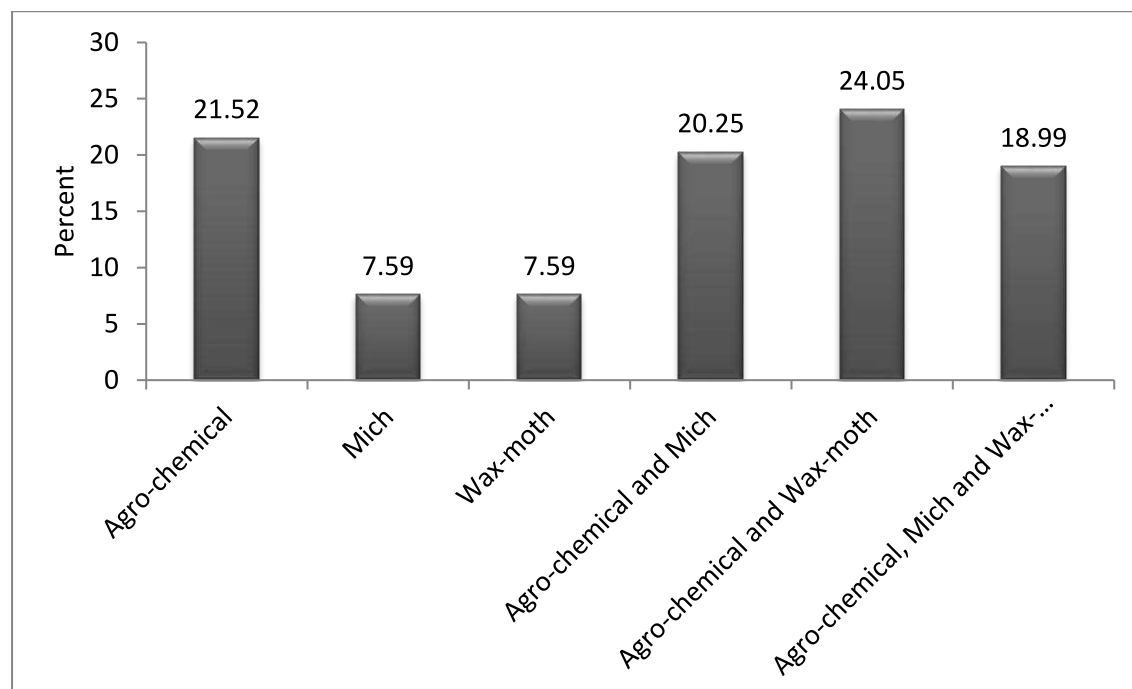


Figure 2. Cause of honey bee mass death reported by beekeepers

From the interviewed beekeepers, 71.08% of them took one or more measures when they observed honey bee mass death in their apiary. Some of the measures undertaken by the sampled respondents were listed in Table 2.

Table 2: Measure taken by the beekeeper to overcome honey bee mass death

Cause of honey bee mass death	Measures taken
Agrochemicals	Relocating and closing of bee entrance
<i>Mich</i>	Not opening the hive during May and not cleaning of hive underground during the rainy season
Wax moth	Removing old combs, Fumigating with barley straw (<i>Hordeum vulgare</i>), teji sar (masarot), woyra (<i>Olea Africana</i>), area cleaning, and breaking Wax-moth webs

Mich is the result of local weather variations that occurred towards the end of the dry season and the start of the wet season. Therefore, beekeepers felt that opening hives during that season and cleaning up any debris that has fallen around the apiary site would hasten the mortality of honey bee colonies. In the case of wax-moth, beekeepers also believe that fumigation of the beehive with *Hordeum vulgare*, masarot, and *Olea Africana* repels wax moth.

In general practice, 87.5% of the respondents use a management practice to maintain the health of honey bees. Those management practices were dearth period supplementary feeding, watering, external inspection (flight status of bee, activity of worker bee bringing pollen into the hive and examination of died bees at hive entrance), and internal inspection (presence of rodents, availability of bee reward (pollen and nectar), presence of bee and brood mortality). The remaining 12.5% of the beekeepers did not perform any management practices (Figure 3).

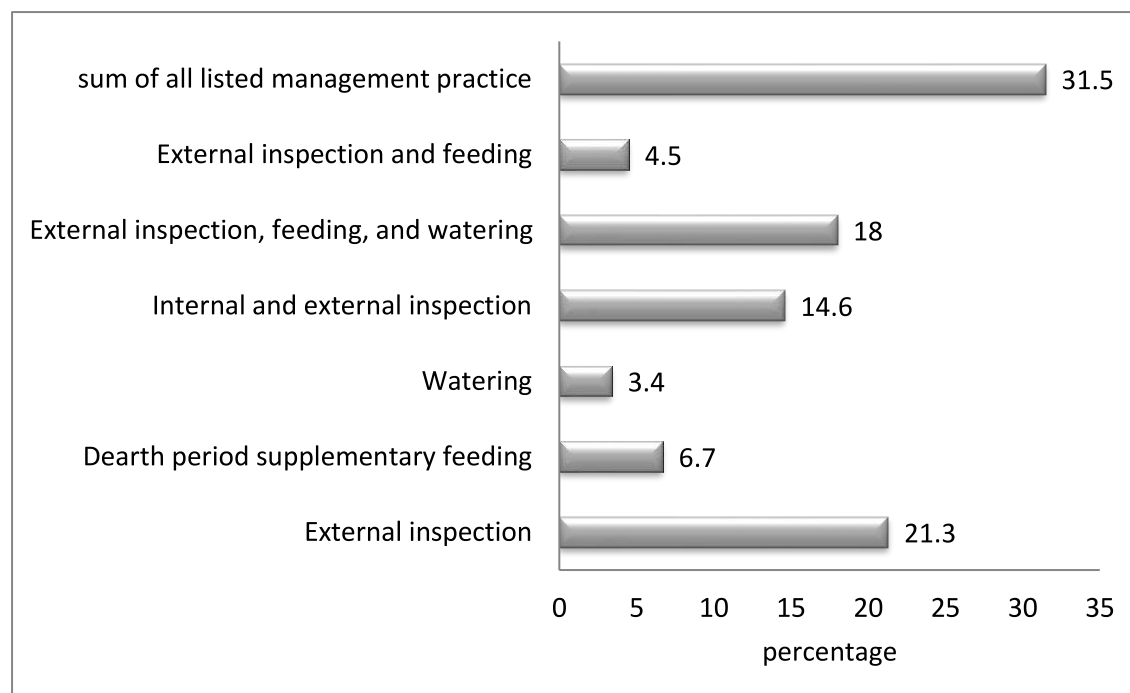


Figure 3. Management practices followed by beekeepers to keep the health of honey bees

3.3. Application of Agrochemicals

Beekeepers reported that honey bee poisoning occurs in their locality. They observed honey bee mortality in fields treated with agrochemicals, as well as increased honey bee mortality at the hive entrance during foraging at the time of agrochemical application. About 86.6% of them responded poisoning of honey bees occurs due to agrochemical mainly by insecticides and herbicides. However, around 13.4 % of them stated that honey bee poisoning happens as a result of the combination of pesticides, herbicides, and poisoning plants. The result was in line with previous studies in Ethiopia, agrochemicals cause mass death of honey bees (Bekele et al 2021) and the poisonous plant also causes honey bee mortality (Gifford 2011; Tesfa et al 2013). Other challenges encountered in survey

districts were the participation of beekeepers themselves in the application of agrochemicals. Because beekeeping in Ethiopia is a sideline activity with other farming activity, beekeeper themselves are crop producers (Enbiyale 2018).

As indicated in Figure 4, the type of chemicals used by beekeeper were Actellic, malathion, 2, 4-D, 2, 4-D+roundup, and DDT (Dichlorodiphenyltrichloroethane). But, the severity of honey bee mass mortality due to such chemicals varied depending on time of the day and season of the year (Figure 5 and 6). The odd thing is that DDT is still employed in our nation as an anti-malarial agent despite being prohibited everywhere else (Biscoe et al 2004; Van Den Berg 2009).

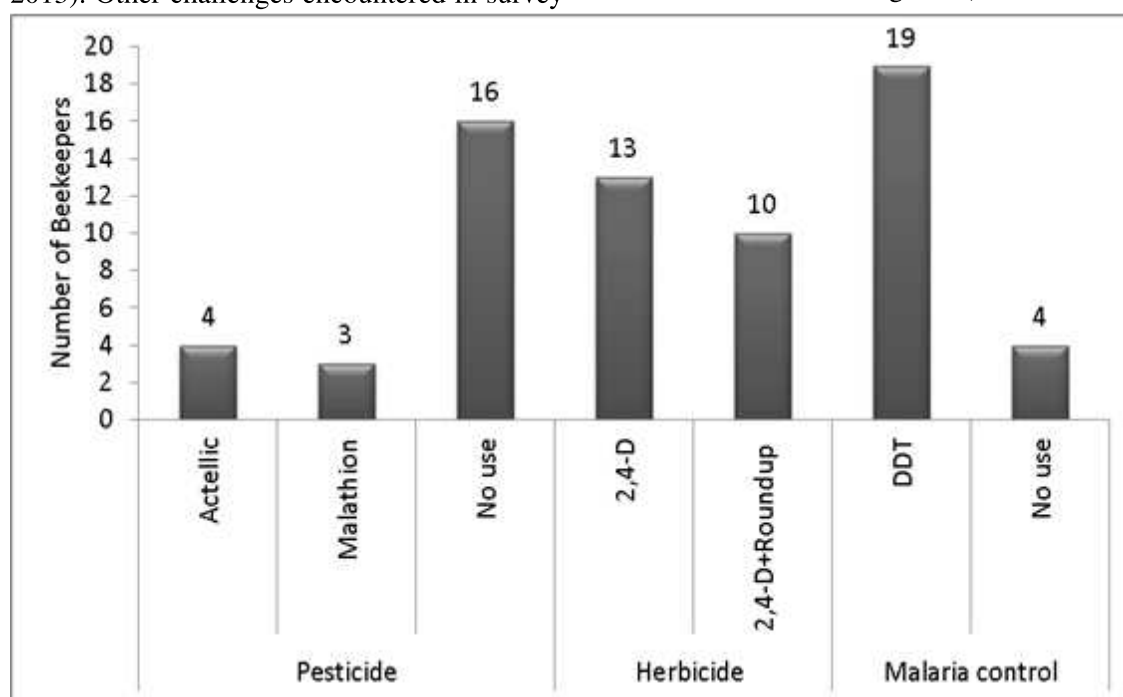


Figure 4. Type of agrochemicals used by respondents

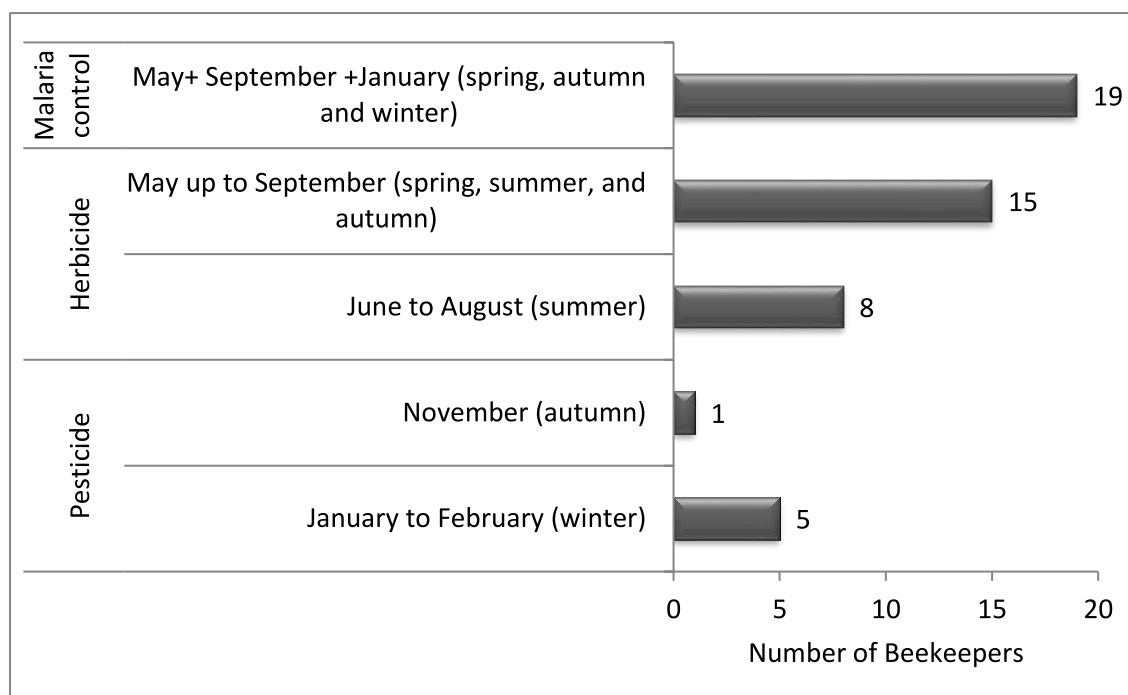


Figure 5. season of agrochemical application

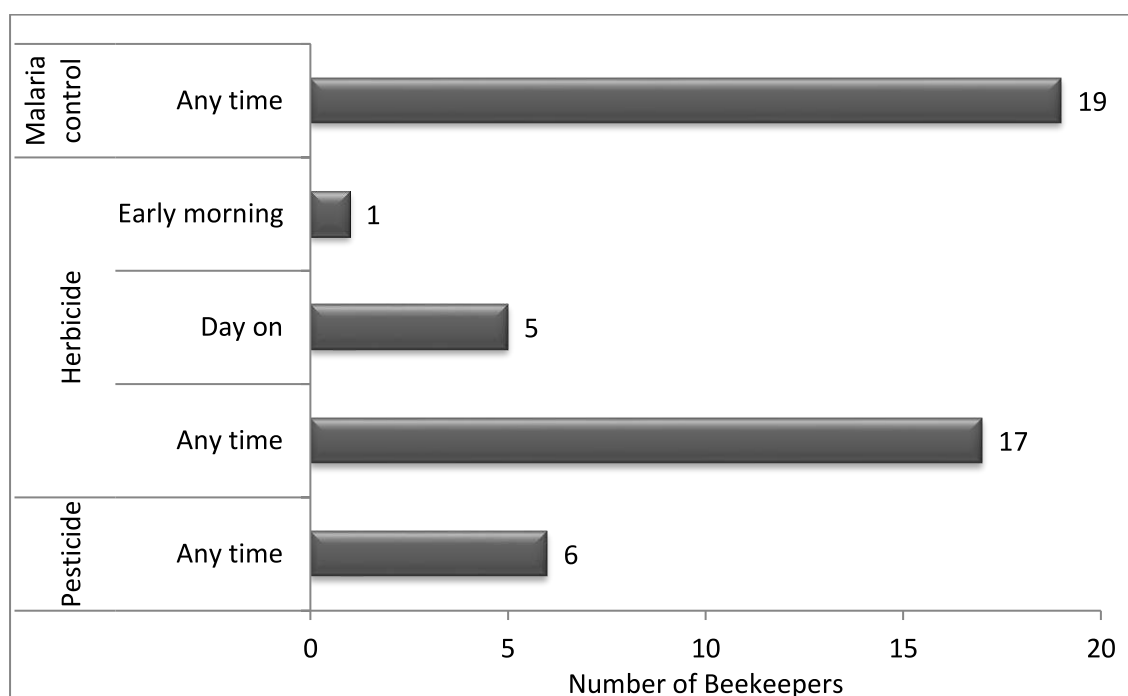


Figure 6. Time of the day agrochemicals applied

3.4. Season of Honey bee Mass Death Occurrences

The highest frequency of honey bee mass death was reported from June to August, followed by September to November. This might be attributed to the use of agrochemicals to manage weeds, insects, and pests in order to

maximize agricultural productivity during rainy and irrigation seasons. Besides, in this time, many crops get bloomed and bees forage on them. While the minimum was happened during December to February (Figure 7).

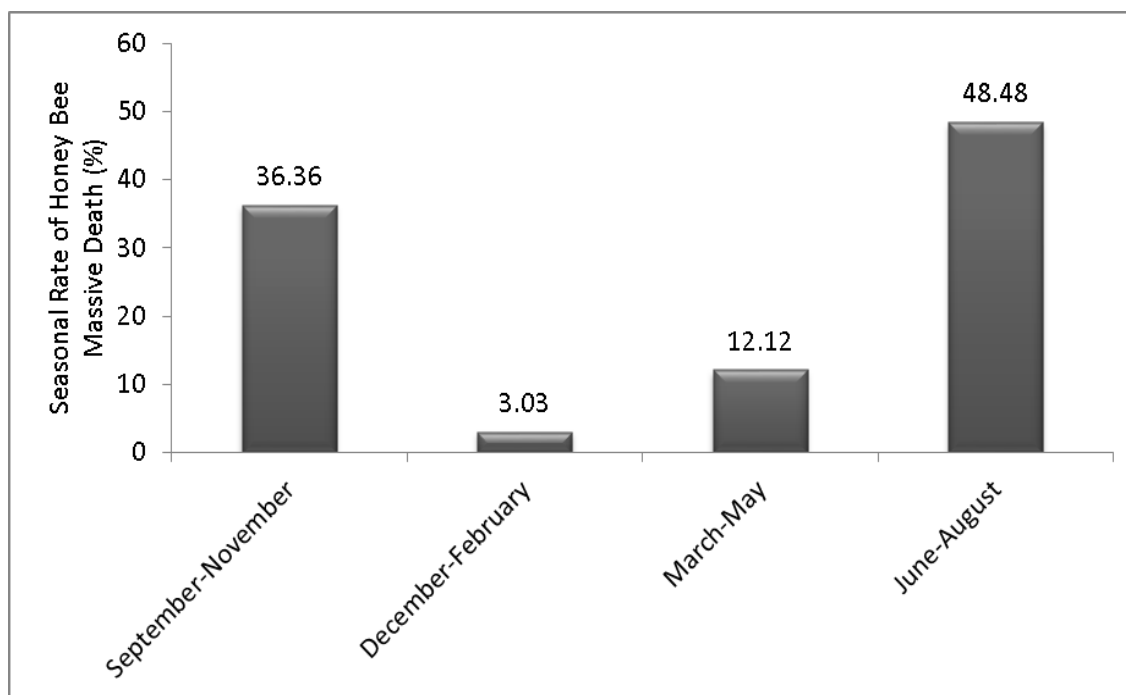


Figure 7. Season of honey bee mass death occurrence

3.5. Status of Honey bee Mass Death in Different Colony Strength

The respondents indicated all types of managed honey bee colonies (weak, medium, and strong) were affected by honey bee mass death, but more pronounced in weak and medium colony (Table 3). The result showed

that strong colonies were less susceptible for mass mortality, which was supported by other studies that weaker colonies have frequently been associated with a higher occurrence of diseases in honey bees (Budge et al 2015)

Table 3: The status of honey bee mass death on colony strength

Parameter	Variables	Frequency	Percentage
Colony strength	Weak	8	27.3
	Medium	1	1.5
	Weak and medium	41	62.1
	Weak, medium, and strong	6	9.1
	Total	66	100

3.6. Trends of Honey bee Colonies Number

A total of 57.7% of the respondents explained that their managed honey bee colonies were decreased alarmingly due to colony mass death. As their explanation, colonies absconded due to mass death (34%), colonies died due to mass death (32.7%) and colonies dwindled due to mass death (33.3%). From 2012 to 2014, the number of managed honey

bee colonies decreased from 1,177 to 747) (Figure 8). But, the result contradicts the findings of CSA data (Central Statistical Authority 2011, 2012) which indicated that the number of managed honey bee colonies in the study areas grew from 911,986 to 965,293 between 2011 and 2012.

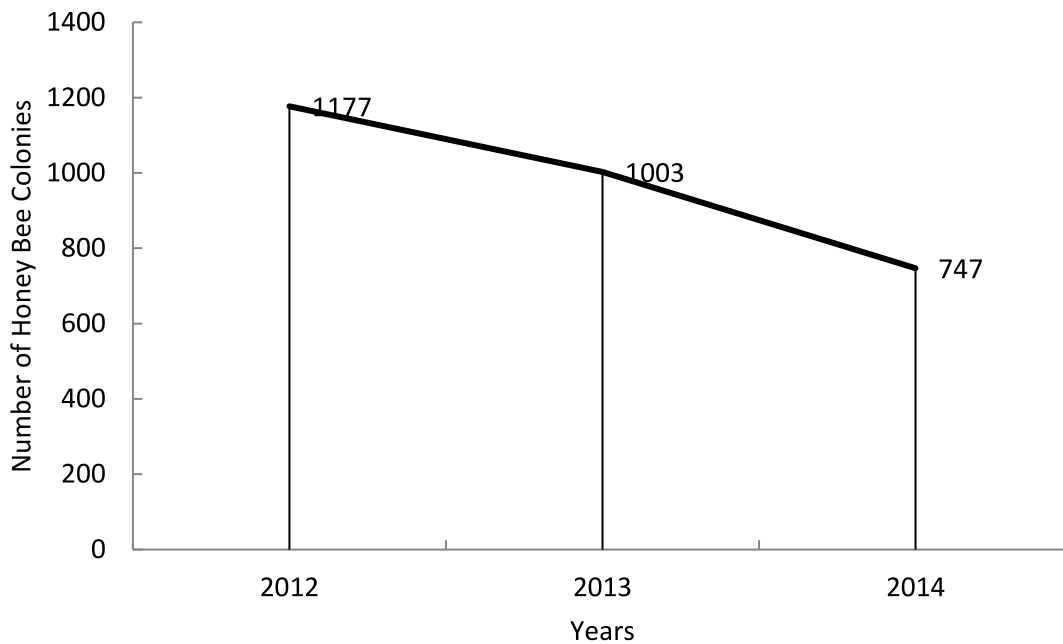


Figure 8. Trends in managed honey bee colonies across years in the survey districts

3.7. Laboratory Result on Causes of Honey bee Mass Death

Upon disease outbreak, types of honey bee disease were investigated and their prevalence was also calculated. The results confirmed that Varroa mite and wax moths on brood and Nosema on adult bees were identified. Moreover, poisoning from agrochemicals was also suspected due to large numbers of honey bees dying at the hive entrance, the occurrence of sudden honey bee loss, and honey bee death mostly happening during the main farming activity season and flowering season of crops.

Such type of pests and diseases were previously reported in the country that the Varroa mites were first reported in Tigray

Prevalence of honey bee disease and pests

Region (Begna 2015) and the Nosema were reported in Amhara Region (Kebede et al 2008). Such results on honey bee mortality were in line with the previous study that Nosema disease results in a large loss of honey yield and increases adult bee mortality (Hong et al 2011; Duquesne et al 2021). Besides varromite causes honey bee mortality by consuming fat body tissue (Ramsey et al 2019). In general both varromite and nosema cause a considerable honey bee mortality globally (Hristov et al 2020). In general, the disease-related hive type is presented in Table

Table 4: Prevalence of Varroa mite, wax moth, and Nosema across Districts in different hive types

Districts	Hive type	Brood prevalence (%)			Adult prevalence (%)	
		Varroa mite	Varroa mite and Wax-moth	Negative (free from disease and pests)	Nosema	Negative (free from disease and pests)
Jabi - Tehnan	Frame	50	25	25	50	50
	Traditional	33.3	-	66.67	66.67	33.33
Burie	Frame	75	25	66.67	33.33	
	Traditional	-	-	-	-	-

Degadamot	Traditional	33.3	-	66.6	33.33	66.67
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Note: ==data not available

3.8. The effect of Honey bee Mass Death

3.9. on Beekeeper Outcome

Within three years of case reported period (2013, 2014, and 2016), the survey results indicate that both honey bee mass death and absconding of honey bee colonies increased from time to time. From the total sampled areas of western Amhara Region, 171

managed honey bee colonies were lost within four years (85 deaths and 86 absconding) (Table 5). The average price of honey bee colony in the study area was 524.73 Ethiopian Birr (ETB) and the amount of money lost becomes 89,728.83 ETB (524.73*171).

Table 5: Number of honey bee died and absconded due to mass mortality by hive type

Year	Honey bees died due to mass death			Honey bees absconded due to mass death			Total
	Frame hive	Top bar hive	Traditional hive	Frame hive	Top bar hive	Traditional hive	
2013	-	-	9	-	-	6	15
2014	4	-	34	-	8	31	77
2016	-	2	36	1	2	38	79
Total	4	2	79	1	10	75	171

Note: ==data not available

4. CONCLUSIONS

Honey bee's mass death had occurred in a few districts of the western Amhara region but it had not a great devastating effect on the colony population in which the occurrence was three case reports per four years within three districts. Agrochemical, *Mich*, Wax-moth and the interaction between them were the main cause reported by participant beekeepers. Therefore, a combination of all factors had a great devastating effect rather than a single cause for the honey bee colony's mass death. On the other hand, the laboratory result showed that the causes of mass honey bee death were Varroa mite and wax moth on broods and Nosema on adult bees, and poisoning from agrochemicals was also suspected from both adult and larvae stages.

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Therefore, a combination of all these stressors might be contributed to honey bee's mass death during the study period; the beekeepers lost about 89,728.83 ETB. With these conclusive remarks, we recommend that adopting continuous management practice should better to minimize the identified honey bee stressors including harmonization of agrochemical application with beekeeping activities. Besides, it would be preferable to develop proactive prevention and control strategies for the identified diseases and parasites. Furthermore, even if *Mich* is the result of the weather change, further investigation should be done to determine how it can kill honey bees.

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