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Major Honey Bee Health Problem with Particular Emphasis to Anti-Varroa Investigation of Propolis in Toke-Kutaye District, Ethiopia

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Abstract: A cross sectional study was carried out from November 2014 to March 2015 for problems associated with Major Honey Bee Health problem in Toke-kutaye, District, West showa Oromia Regional State, Ethiopia with Particular Emphasis to Anti-varroa Investigation of Propolis. Purposive sampling was used in each kebele and a total of 40 beekeepers out off 5 kebeles were selected and Sampling was split proportionally and varies with the number of hives available between each beekeeper apiary site. Direct observation, inspection and laboratory examination was the main data collection techniques. Executing petr-dish bio assay set up were the main data collection techniques used to gather the information of various concentrations of propolis extracted in 55% ethanol. Laboratory examination results revealed that all diagnosed kebeles had varroa mite with infestation rate ranging from 80% to 92.3% and the presence of Nosema 39% and amoeba 62.3% in honey bees in 82 colonies tested out of 5 studied kebeles. All inspected hive had pests like wax moth, 82(61%) and spider (*Lactrodectus mactan*), 82 (59%). Antivarroa investigation of propolis as through bio assay revealed that the length of narcosis and rate of mortality of varroa dependence on the concentration of propolis used, the duration of contact time and the origins of propolis. Thus, treatment with a 20%, propolis solution in 55%ethanol resulted in 100% mortality rate at a contact time of 5s regardless of the origins of the propolis. However, treatment with 5% of propolis (Eastern hararge) narcosis lasted from 37.8 ± 4.41% to 30 ± 5% at 5s and 10s contact times respectively and 100% mortal at 20s contact time. Further research in this field needs to be encouraged, it is therefore very important that the existing problems are well managed to maintain bee health and that the risks and consequences of pests and diseases are well understood and appropriate plans in place to deal with any such honey bee health problem. This will help to sustain the health of honey bee already established in the bee keeper's apiary in the district.

Key words: Antivarroa · Honey Bee · Narcosis · Propolis · Varroa mite

INTRODUCTION

Beekeeping is an important component of agriculture and rural development program in many countries and useful small-scale efforts have been made to encourage beekeeping interventions throughout the world [1]. It plays a role in providing nutritional, economic and ecological security. The business almost requires no land, capital and does not take much part of the farmers' time and does not compete with other components of farming systems for resource. Directly, it contributes in the values of the outputs produced, including honey, bee wax, queen and bee colonies and other products such as pollen, royal jelly, bee venom and propolis in cosmetics and medicine [2,3].

Large and diverse botanical resources combined with suitable climatic conditions make Ethiopia conducive for beekeeping business [4,5] and the country is the leading honey producer in Africa and is one of the ten largest honey producing countries in the world [6, 7]. The most important species for bee keeping in East Africa and the wider sub Saharan Africa is the *Apis mellifera scutellata* (the African Honey Bee) [8].

Able to survive in a wide range of climates and environments, honey bees are among the most successful organisms on the planet [9]. And now inhabit most areas of the world occupied by humans. Accompanying the spread of bees globally has been a host of pathogens, parasites, pests and viruses. Of these organisms, such as

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Nosema apis only just beginning to be understood, while *Varroa destructor* despite being well known, remain at forefront of bee research [10].

The varroa mite is a parasite with the most pronounced economic impact on beekeeping industry [11]. It is a major pest of the honeybee, spreads very quickly and causes serious damage to its host colonies where it reproduces inside the capped brood cells [11,12].

Two microsporidian species infect honey bees worldwide: *Nosema apis* and *Nosema. ceranae*. The term nosemosis is considered to be the infection of ventricular cells of adult honey bees by *Nosema. apis* and reduces worker longevity by 22-44% [13]. *Nosema apis* are the causative agent of nosemosis in honeybees (*Apis mellifera*). This disease is widespread and found in every beekeeping country [14,15]. The world trade in honey-bee products and beekeeping materials may play an important role in the dispersal of infective spores of *N. ceranae* from apiary to apiary over different geographical areas [16].

Despite the diversity of infectious diseases and their agents that can cause bee mortality, surveillance has been fragmented and thus it has been difficult to gain a clear historical view of bee health [17]. Symptoms seen in a colony vary according to the type of organism causing the disease. Many of these symptoms are none specific and so the only way of confirming pathogen is through appropriate laboratory diagnostics. Their severity largely depends upon the vigor of the affected colony [12]. Even though varroosis once established in the apiary it destroyes colonies within 2 to 5 unless treated [18]. Since forces beekeepers around the world to treat their colonies with different types of acaricides which applied to minimize mite infestation rate and subsequently reduce or prevent colony death. However, these acaricides applications led to emergency of resistant mites [19] [20] and undesirable residue problems [21,22].

Therefore diagnostic surveys are conducted in different parts of the country at different times by various investigators on honey bee health problem. Similarly, the works of laboratory analysis reported two adult honeybee diseases nosema and amoeba and determined their distribution and the work of investigation of propolis on varroa mite. According to the damage caused by Varroa mite, Nosema and amoeba and pests on honeybee colonies, the recent statistics about varroa infestation, epidemiological studies in variant geographical regions of beekeeping provides set information on major findings in relation to the works of diagnostic survey and characterizations of major honeybee health problems in Tokke Kutaye Districts and investigation of anti-varroa action of propolis.

Therefore the main objective of the study was to verify major honeybee health problems of beekeeping in Toke-kutaye woreda with major emphasis to determine antivarroa actions propolis collected from different geographic origins of the country.

Objectives

Main Objectives:

- To identify the major honeybee health problems in tokke kutaye districts.
- To determine antivarroa actions propolis collected from different geographic origins of the country.

Specific Objectives:

- To identify and verify the infestation rate of varroa mite, Nosema and amoeba
- To verify the infestation rate of pests through hive inspection in Tokke Kutaye District.
- To assess type of propolis caused narcotizing and effect of different concentration (%w/v) of propolis on varroa mite.

MATERIALS AND METHODES

Study Area Description: The study was conducted in Toke Kutaye districts, West Shewa Zone of the Oromia Regional State and Investigation of antivarroa actions of propolis was conducted in Holeta bee research center located in Holota, Oromia Special Zone around Finfine, Ethiopia. The Toke Kutaye woreda has 32 rural kebeles and 4 administrative towns. This woreda located at 126 km to the west from the capital city Addis- Ababa and geographically located at 8° 58'N, 37° 46'E and altitude of ranging from 1380m to 3194m above sea level. The area receives a mean annual rainfall of 900 mm (800 to 1000 mm) and annual temperature ranging from 15 to 29°C with average temperature of 22°C. West Shewa zone is generally a highland whose topography gave the area a characteristic climate that is conducive for the apiculture. Toke Kutaye woreda has number of livestock on the bases of species as 13 7180 cattle; 46,556 sheep; 22,611 goats; 79,504 poultry; 26792 equines and 17,269 bee hives (Toke kutaye woreda livestock and agricultural offices, 2013).

The Holota town is located 25 km west of Addis Ababa situated at latitude of 9°3’ N and longitude of 38°30’ E. The area has annual rain fall and daily temperature, which ranges from 834 mm to 1300 mm and 5°C to 28°C, respectively.

Study Population: The study populations were honey bee colonies from purposely selected 5 kebeles of the district and propolis from diverse geographical origins.

Sample Size Determination: The five districts were selected for the study following purposive sampling approach considering potential in honey bee production. A multi stage sampling technique was used in this study. In the first and second stage, honey bee potential kebeles and 8 beekeepers from each kebeles and total of 40 registered beekeepers from livestock and agricultural office of woreda have been identified purposively and in the third stage, sample size of 82 honeybee colonies was set for this study using quota method.

Study Design: Cross sectional study (diagnostic survey) and anti-varroa investigation of propolis was carried out from November 2014- March 2015. The honey bee colonies of study area were sampled for laboratory investigation for their potential health problems and the colonies were inspected in connection with factors that potentially affect bee health and the anti-varroa actions of propolis with different levels of ethanol extracted, anti-varroal property of propolis from different agro ecological zones and type, level of extracts and time of efficacy identified.

Sampling Methods: Livestock Development and Health District agencies of Toke-Kataye Woreda were consulted to selected sampling site. Accordingly 5 sampling sites and 40 beekeepers having bee colonies were selected purposely and honey bee colonies randomly sampled and examined from selected kebele. Proportional sampling method was used to fix sample size for each beekeeper and the diagnosis and examination was conducted on 82 randomly drown brood and adult honey bee represented honey bee colonies of selected apiary site. Propolis samples used in experiments were obtained from the Holota Bee Research Center which was collected from beehives of different areas of the country.

Study Methodology: The colonies were examined for pests according to OIE [23] methods and disease diagnosis was carried out according to Shimanuki and Knox [24] on the colonies and broods to verify the levels of infestation rate with varroa mite, presence of bee lice, Amoeba and Nosema.

About 200-300 bees and brood cape cells were collected from each selected hive in a jar, using bee brush from hive entrance (externally), killed and preserved in 70% ethanol and from brood comb capped brood cells (internally) labeled accordingly. Number and Type of hive, type of sampled hive, presence of wax moth and spider were recorded corresponding to the label and then transported to Holeta bee research center laboratory for infestation rate of varroa mite and about 30 adult honey bee from collected adult bee were diagnosed for the detection of amoeba and nosema spore which represents sampled colonies.

Examination Procedure: In the laboratory, preserved worker and brood were examined.

Adult Examination: In the laboratory, preserved bee samples were taken and a detergent solution was poured into each of the jar containing bees up to half of the jar. Then the jar was shaken for 1 minute, until the mite separated from honey bees. The solution strained through a ladle (8-12 mesh) to remove the bees and then sieving the solution through tea strainer. Finally, the tea strainer was examined and counted for varroa mite.

Brood Examination: The mites themselves should be sought for confirmation, by examining the bottom of the cell and the brood for attached mites. Brood examinations were done by random opening of 200 to 300 brood cells. The brood was removed from the cell with a fine forceps and the cell was inspected for the presence of mite using by naked eye.

Microscopy: A laboratory method consists of the individual examination of the colonies for the simultaneous detection of Nosema spores and Amoeba cysts using 30 bees per colony was employed

The abdomens of the bees to be examined were separated and ground/grind up in 2 ml of water. Three drops of the suspension were placed on a slide under a cover-slip and examined microscopically at ×400 magnification, under bright-field or phase-contrast optics.

Propolis Extraction and Preparation: Propolis samples used in experiments were obtained from beehives in the colonies in the apiary site of Bako, Eastern harage and Toke kutaye woreda and extracted and investigated in Holeta Bee research Center. In preparation for extraction, weighed and frozen propolis samples were homogenized using a coffee mill. Samples were frozen before homogenization since unfrozen propolis, due to its sticky nature, does not lend itself to easy homogenization. The homogenate powder was then suspended in 70 %

ethanol, in a ratio of 1:9 (w/v) for effective extraction [25] and extracted in a rotary evaporator at 60 degrees Celsius for 2 h. After the allocated extraction time, the suspension was cooled at room temperature for about 1 h and suction filtered.

The filtrate was dried in an incubator at 40°C to weight constancy, which was achieved in 2 weeks time. The dried 70% ethanol extract was dissolved in 55% ethanol to make a 10% (w/v) stock solution of propolis. Even though 70% ethanol was used for extraction purpose, 55% ethanol was employed as a solvent in the bioassay in order to reduce the effect of strong ethanol solution on the experimental organisms. The little amount of precipitation observed while suspending the 70% extract in 55% ethanol was brought into solution by agitation. The stock solutions were stored in a refrigerator for later use.

Different concentrations of propolis were prepared for the bioassay by diluting the stock solutions with ethanol of the concentration used to prepare the corresponding stock solution.

Mite Collection: Mites were collected from infested colonies in the apiary site of Holeta Bee research Center. Infested combs or pieces of combs containing drone broods were brought to the laboratory and Varroa mite were collected from capped cells by opening and inspecting individual cells. In order to avoid starvation of the mites during the collection process collected mites were kept in a Petri dish on bee larvae or pupae. Collection of mites was done from both the larval and pupal stage of healthy brood, since mites at the different developmental stages of the brood did not show any significant difference in their sensitivity for pyrethroids in a laboratory bioassay [20]. Newly moulted adult mites, identified by their pale colour, relatively smaller size and weak locomotion activities were excluded from the experiment since they may have a different response as hardening of the cuticle is still in progress. Mites, which seemed physically weak and abnormal and also those obtained from diseased brood were discarded.

Petri Dish Bioassay: In order to observe the effect of contact time of different propolis concentrations on the mortality rate [26] of V. destructor mites, six mites per experiment were placed on a 3 cm x 3 cm single layer tissue paper in a Petri dish. The mites were treated by applying 250 micro liters of the concentrations to be used in the bioassay: 5%, 7.5%, 10%, 15%, 20% (w/v) of propolis on the tissue paper using micropipette. The treatment was stopped after the allocated time by removing the mites from the Petri dish and Control experiments for each experimental group were run by treating the mites, for the corresponding treatment times, with 55% ethanol solution and also with distilled water.

An individual was considered dead if it showed no leg movement and/or movement of any other body part when gently prodded with a probe. If it showed movement, whether it was partially paralyzed or normal, was counted as alive. Each treatment was repeated three times and the mean ± S.D values were used in the presentation of results.

Data to Be Collected: Proportion of narcotized mites just after treatment, Time at which the treated mites narcotized, Effect of different concentrations (%w/v) of propolis, Type of propolis caused effective narcotizing

Data Management and Analysis: Data analysis was conducted using SPSS version 20. All collected data was entered into Microsoft Excel 2007 and descriptive statistics such as mean, standard error; frequency, percentages were used to analyses the data using SPSS (version 20). Chi-square (÷2) was used to determine the statistical association between factors. The statistically significant associations between variables were considered if the calculated p-value was less than 0.05 with 95% confidence level.

RESULTS

Adult Bee Diagnosis: This study showed that varroa mite infestations were found in 70 (85%) out of 82 sampled colony of studied area. From the total 82 bee colonies diagnosed, *varroa* mites were detected in 70 with overall infestation rate of 85%. An average of 262 ± 22 bees per colony was examined through adult bee colonies and an average of 7 ± 6.7 (ranging from 0-14) *varroa* mites were recovered. Although there is no apparent difference, infestation rates were high for Melka-nega-denbi 92.3%, Himela-dawe-ajo 86.9%, Toke-meti 84.2%, Lenca 81.82% and Negafile 80% with decreasing infestation rate. However, per colony recovered the average number of *varroa* mites was high for Melka-nega-denbi and Negafile and low for Lenca (Table 1). The mite infestation cannot be established whether due to poor hive management or climatic factors or related to the geographical location of the apiaries.

Table 1: Adult bees sampling kebele with percentage bee colonies tested positive for varroa mites.

Sampled area	Total number of bee colonies sampled	Number found positive to varroa mite	% infestation	Average number of bees sampled per colony	Average number of varroa recovered per colony
Negafile	15	12	80	255	11
Melka-nega-denbi	14	13	92.3	265	11
Himela-dawe-ajo	23	20	86.9	261	4
Toke-meti	19	16	84.2	264	6
Lenca	11	9	81.82	266	3
Total	82	70	425.22	1311	35
Average		14	85.044	262.00	7
STDEV		.356	4.421	22.831	6.740

Table 2: Total number of sampled bee colonies per kebele and obtained results through

Sampled area	Total number of bee colonies sampled	Number found positive to varroa mite	% infestation	Average number of bees sampled per colony	Average number of varroa recovered per colony
Negafile	15	12	80	255	11
Melka-nega-denbi	14	13	92.3	265	11
Himela-dawe-ajo	23	20	86.9	261	4
Toke-meti	19	16	84.2	264	6
Lenca	11	9	81.82	266	3
Total	82	70	425.22	1311	35
Average		14	85.044	262.00	7
STDEV		.356	4.421	22.831	6.740

Table 3: Total number of sampled bee colonies per kebele and obtained results through microscopy diagnosis

Sampled area	Number of samples colonies	Number positive to Nosema spore	%of Nosemosis within site	Number positive to Amoeba cyst	%of Amoeba within site
Negafile	15	6	40.0%	9	60.0%
Melka-nega-denbi	14	5	35.7%	11	78.6%
Himela-dawe-ajo	23	9	39.1%	14	60.9%
Toke-meti	19	8	42.1%	10	52.6%
Lenca	11	4	36.4%	7	63.6%
Total	82	32		51	317
Average			39.0%		62.2%
df			4		4
P			.996		.676

Brood Bee Diagnosis: From the total of 82 bee colonies diagnosed for brood, 70 bee colonies accounting 85% of the diagnosed brood were tested positive for *varroa* mites. The result from the diagnosed brood showed that the sampling localities were infested with *varroa* mites with the infestation rate ranging from 80% to 92.3%. On the average 16 ± 8.320 bee colonies were sampled for brood diagnosis from each kebele and an average of 14 bee colonies were found positive to *varroa* mite. Likewise, on the average 262 ± 22.154 brood cells were opened from each bee colony with an average of 16.15 ± 19.946 *varroa* mite detection. Through the brood analysis, Melka-nega-denbi and Negafile kebele were found with high and low *varroa* mite infestation rates respectively (Table 2).

The average mite load per brood recovered range from 9 to 31 which was highest in and low in Lenca respectively (Table 2).

Microscopy Examination and Inspection: *Nosema* spores were found in five sampled sites with a total of 82 (39%) colonies tested and *Amoeba* cyst were found with total of 82(62.2%) colonies tested (Table 3). The %of *Nosema* spores within sites in the honeybee colonies range from 36% to 42% and Amoeba range from 53% to 79% (Table 3).

Based on colony status of diagnosed honey bee colonies within kebele, the highest nosemosis 42.1% observed in Himela-dawe-ajo while highest amoeba infection 78.6% was observed in Melka-nega-denbi.

Table 4: Total number of sampled bee colonies per kebele and obtained results through colony inspections

Sampled area	Number of sampled colonies	Number wax moth present	% of wax moth	Number spider present	% of spider
Negafile	15	10	66.7%	10	66.7%
Melka-nega-denbi	14	10	71.4%	9	64.3%
Himela-dawe-ajo	23	13	56.5%	14	60.9%
Toke-meti	19	12	63.2%	11	57.9%
Lenca	11	5	45.5%	5	45.5%
Total	82	50	303.44	49	295.51
Average			61.0%		59.8%
df			4		4
P			.722		.854

Table 5: Percentage reduction in the mean and mortality rate of V. destructor mites by different propolis concentrations at each contact of treatment time represented by mean ± S: D: values.

Propolis concentration (%, w/v) In 55% ethanol (30s treatment)		Contact time T5s	T10s	T20s	T30s	T40	T60s	T75s	T90s
5%	Mean	3.78	3.00	2.11	1.11	.22	.00	.00	.00
	Std. Deviation	.441	.500	.928	1.054	.667	.000	.000	.000
7.5%	Mean	3.33	3.00	2.00	.67	.00	.00	.00	.00
	Std. Deviation	.577	.000	.000	1.155	.000	.000	.000	.000
10%	Mean	3.00	2.00	1.33	.00	.00	.00	.00	.00
	Std. Deviation	.000	.000	1.155	.000	.000	.000	.000	.000
15%	Mean	2.50	2.00	.00	.00	.00	.00	.00	.00
	Std. Deviation	.548	.000	.000	.000	.000	.000	.000	.000
20%	Mean	2.33	.00	.00	.00	.00	.00	.00	.00
	Std. Deviation	.577	.000	.000	.000	.000	.000	.000	.000
control	Mean	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	Std. Deviation	.000	.000	.000	.000	.000	.000	.000	.000
Total	Mean	2.70	2.00	1.17	.60	.27	.20	.20	.20
	Std. Deviation	1.088	1.050	1.053	.814	.521	.407	.407	.407

There were no significant differences in the infection from colony to colony, site to site in nosema and amoeba infection rate (Pearson Chi-Square =.179, *df* = 4, P =.996 (P > 0.05) and (Pearson Chi-Square = 2.394, *df* = 4, P =.676 (P > 0.05) respectively (Table 3).

A total of 82 colonies examined were infested with Wax moth and Spider (*Lactrodectus mactan*) (Table 4). Again, 61% colonies were infested with Wax moth and 59% colonies were infested with spider (Table 4). When infestation of wax moth was calculated based on colony status of inspected hive per kebele, the highest wax moth (71.4%) was observed in Melka-nega-denbi, while the spider 66.7(%) was observed in Negafile. In contrary lowest Wax moth 45.5% and spider 45.5% was observed in Lenca (Table 4). However, the difference in infestation of wax moth and spider was not statistically significant among bees of different colony status of sampled site (P>0.05).

Proportion of Narcotized Mites Just after Treatment: Comparison of the mean and mortality rate, from the Petri dish assay experiment displayed propolis concentrations that show considerable effects on the mortality rate (Table 1). The reduction in the mean due to treatment with a certain propolis concentration was with increasing propolis concentrations where the mite was found out to be more sensitive well as the reduction in the movement of mites and mortality achieved except for the experiments, where the mite was found to be recovered (Table 5).

The effect of contact time of different propolis concentrations on the mortality rate of varroa mites as can be seen from Fig. 1, mortality rate increased with increasing contact time for concentrations of 5, 7.5, 10, 15 and 20% in 55% ethanol. In the shortest contact time, for 5 with 20% propolis in 55% ethanol resulted in 100% mortality. In the case of different propolis concentrations in 55% ethanol, mortality rate, even though it was weak seemed to increase with increasing contact time and concentration.

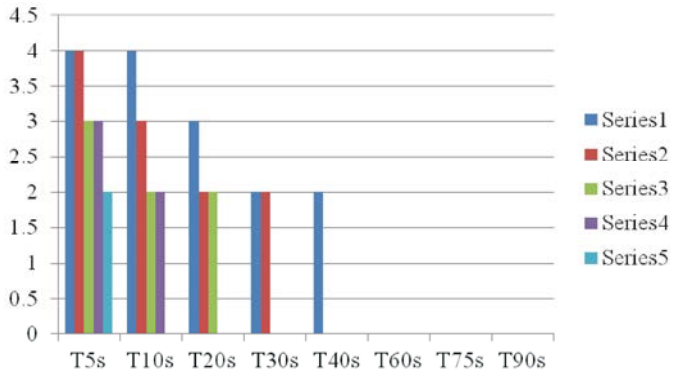

Fig. 1: Effect of contact time of different propolis concentrations (% w/v) in 55% ethanol on the narcotic and lethality of varroa mites: six mites per experiment, n= 3: the bar indicate series 0 = 5%: series 1 = 7.5%: series 2 = 10%: series 3 = 15%: series 4 = 20% concentration of propolis: Y-axis= 0---1 low movement; 2 no movement; or lethal, 3=medium movement and 4= high movement: X-axis= Treatment time

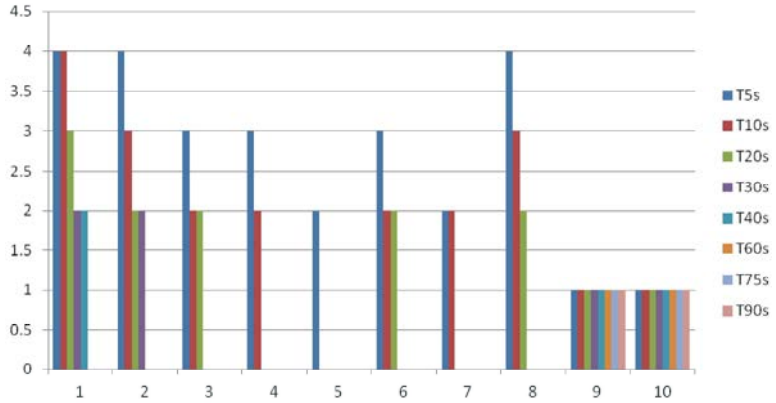

Fig. 2: Antivarroa actions of propolis from different geographic origins and the effect of contact time of different propolis concentrations on the mortality rate of Varroa mites: Y-axis= *Narcotic and letal effect* of propolis=degree of movment: X-axis= site; 1-5= Bako: 6&7= Eastern hararge: 8= Toke-Kutaye: 9&10= control: The bar indicate the time of propolis treatment (contact time of the propolis).

However, a contact time of 5, 10, 20, 30 and 40 seconds resulted in a mortality in case of 20, 15, 10, 7.5 and 5 % propolis respectively regardless of propolis origins. Even though all propolis concentration in 55% ethanol was used in these investigations after contact time of 60seconds no movement of the mite was observed (Fig 1).

Antivarroa actions of propolis from different geographic origins and the effect of contact time of different propolis concentrations on the mortality rate of Varroa mites. Antivarroa actions of propolis from Bako were investigated at of 5, 7.5, 10, 15 and 20%, Eastern-harage 5 and 15% and whereas propolis from Toke-kutaye at 5% propolis concentrations in 55% ethanol were investigated at contact time of 5, 10, 20, 30, 40, 60, 75 and 90 seconds (Fig 2).

Treatment with various propolis concentration of Bako at different contact times showed that both narcosis immediately after treatment and lethality was achieved after treatment regardless of both propolis concentration and contact time. Further observation of the activity of the mites displayed that with 5% propolis concentration of solution Bako narcosis lasted longer 5s to 30s after this time it was very low movement and at 40s no movement of the mites were observed and no mites were recovered after the allocated period of time.

Treatment of Varroa mites with 5% propolis concentration of solution East hararge the proportion of narcotized mites just after treatment ranged from 37.8 ± 4.41% to 30 ± 5% at and 10s contact times respectively and 100% mortality at 20s. In case of 5% propolis concentration of solution Bako the proportion of

narcotized mites just after treatment from 37.8 ± 4.41% to 22 ± 6.67% at 5s and 40s contact times respectively and 100% mortality at 60s (Table 1 and Fig 2 and 3).

Treatment time played a role in case of treatments with high concentration of propolis for both solutions (Bako and East hararge). Treatment of mites with 15%(w/v) propolis in 55% of solution East harage the proportion of narcotized mites just after treatment was 25 ± 5.48% at 5s and 100% lethal which was achieved at 10s. Even though Treatment of mites with 15% (W/V) propolis in 55% solution of Bako narcotized mites just after treatment were 25 ± 5.48% at 5s and 100% mortality were achieved at 10s as there was no activity of mites observed after this contact time (Table 1 and Fig 2 and 3).

Treatment of Varroa mites with 5% propolis concentration solution of Toke-kutaye proportion of narcotized mites range from 37.8 ± 4.41% to 30 ± 5% at 5s and 10s contact times respectively and 100% mortality at 20s. resulted in an initial narcosis at 5s which was seems similar in comparison with that of solution Bako but narcosis lasted within the first 10s of contact time and mortality achieved at 20s exactly as of East harage of the same concentration 5% (Table 1 and Fig 2 and 3).

Treatments with higher concentration of propolis (20% (W/V) propolis) in 55% of Bako resulted 100% mortality, regardless of contact time, indicating its high toxicity with the slightest contact time, as low as 5s (Fig 2:5).

The control treatments of the experiment solutions of distilled water and 55% ethanol have not shown lethality effects after treatment of specified contact time all the mite was recovered after 90s (Fig 2; 9:10). The control mites of both solutions after treatment their movement activity were not affected. Hence the experimental control used (distilled water and 55% ethanol) did not show in lethality effect with increasing contact time (Fig 2; 9:10).

DISCUSSIONS

All the surveyed areas tested positive to varroa mite with infection levels ranging 80 % to 92.3%. Which was concise much greater with recent reported in Ethiopia by Begna [27] reported that varroa mite with infestation level ranging 37.5 % to 100 % in Tigray Region, Ethiopia.

The average number of varroa mite recovered from a single bee colony through adults and brood's analyses were 7 and 16 mites, respectively observed in the current study suggesting *Varroa* mites have led to the virtual elimination of feral bee colonies and the honey bees are close to collapse concurring with the finding that established more 10 mites natural drop per day can cause colony collapse [28].

On the other hand, the wide distribution of the *varroa* mites in all the surveyed areas and in all inspected bee colonies indicates longtime introduction [29,30]. Higher infestation rate of varroa mite observed in the apiary might be associated with contact among colonies. Colonies in the apiary found close to each other, hence, facilitate transmission of varroa mite among the colonies through swarming and drifting [31]. Chance of bees in apiary to visit same flower is higher than bees in backyard. However, the causes of variations in infestation rates among the studied districts, it cannot be established whether the infestation was due to poor hive management or climatic factors or related to the geographical location and service level of the places as bee colony marketing points. On the other hand, it is not certain how and when these mites invaded the honeybee colonies of Apis mellifera in the country especially in the districts where mites were found.

The presence of *Varroa destructor,* a parasitic mite could major honey bee health problem cause the decline in colony establishment and are a major problem for kept bees in apiaries [32-35].

In the present study *Nosema* spores were found in five sampled sites with a total of 39% infestation rate observed in the current study was much lower than other previous reports in Ethiopia. Diagnosis made on 152 colony bees in field and laboratory at Addis Abeba reported prevalence rate of 53.3% [36]. nosema was also reported from different regions with varying prevalence rate such as 58% in Oromia, 60% Benishangul-Gumuz and 47% in Amhara regions [37]. However higher than when compared with survey conducted in Ethiopia regional state of Oromia, Amhara, Southern Nations and Nationality and Peoples (SNNP), Tigray, Gambella, Benishangul–gumuz, Somale with 37.3% of infection rate [38]. While in the present diagnostic survey of *Amoeba* cyst were found with total of 51(62.2%) colonies tested it is unequal when compared with diagnosis made on honey bees in field and laboratory at Addis Abeba which reported a prevalence rate of 73% of amoeba infestation [36]. it was also lower than Diagnosis made in Ethiopia regional state such as; Oromia region with prevalence rate (88%), Amhara region (95%) and Benishangul- Gumuz with 60% [37], this suggest the unequal distribution of the disease. Study on annual cycle and seasonal dynamics of amoeba from the Holeta research center apiary [39] reported, amoeba cysts were reported throughout the year regardless of hive type.

The infestation of Nosema spore and amoeba cyst observed in the area pointed out that the late detections of adult honeybee diseases Nosema and Amoeba in 1989 in the country and the follow-up investigations on its wide distribution suggests, less attention given to monitoring of local honeybee diseases [38].

The presence of pests on the colonies confirmed that pest infestation is a major honey bee health problem in beekeeping in the tropics [40-43]. The pests are responsible for the destruction of the colony and decline in its establishment [40].

The wax moth that is one of the most important pests of honeybee colony with worldwide distributions is also identified as one of the serious local honeybee pest in the country [44]. The infestation of wax moth in the studied district 61% was higher than the previous studies The study that was conducted in three zones of the country investigated wax moth prevalence variations from zone to zones with South west Shoa zone having high infestation level (26.66%) followed by West and East shoa zones 22.85% and 26.66%, respectively [45].

Also, the same study indicated that about 56%-75% of the wax moth infected honeybee colonies absconded and the remaining dwindled. So this pointed out the presence of wax moth one of major honeybee's health problem in the studied localities district. In the considerations of its widely distributions and serious effects, practical experiment was designed and identified effective preventive and/or control management practice [46]. According to the results of this experiment, 82.3% effective measures that restrain the pest entry into beehives have been developed. The study recommended management techniques of strengthening honeybee colonies via feeding, removing unoccupied suppers and combs, combination of these practices and trapping adult wax.

Proportion of narcotized mite just after treatment and mortality rate and effect of different concentration of propolis from the Petri dish assay in this experimental study displayed propolis concentrations that showed considerable effects on the mortality rate of varroa mites and propolis from diverse geographical origins displayed the differences in the antivarroal activities (Table 1, fig 3). Accordingly proportion of narcotized mite just after treatment decreases and mortality rate increased with increasing contact time for propolis concentrations of 5, 7.5, 10, 15 and 20% in 55% ethanol used in this experiment.

Remarkably propolis in previous experimental laboratory by the work of Garedewa *et al*. [26] proved that propolis has a strong narcotic and lethal effect on V. destructor mites that the length of narcosis and mortality rate depended on propolis concentration, solvent of extraction and length of contact time.

As seen from the present experimental results, treatment of mites with 5% of propolis (from Eastern-hararge) and (from Toke-kutaye) in 55% ethanol (w/v %) concentration was more effective than the corresponding treatment with 5% of propolis (from Bako). The most plausible explanation for these differences that origin of propolis from diverse agro-ecolological zones affected by temperature. Most of the biological active hydrophopic components of propolis might differ from area to area. This means that propolis from Eastern-harage and Toke-kutaye was quantitate component of biological soluble active ingredient possibly higher than propolis from Bako. Even though propolis in general and most of its components in particular, are water insoluble. The water soluble components of propolis comprise about 2.5–6.5% only depending on the origin of propolis [47]. This is also possible explanation why propolis does not kill Varroa mites in the beehive while mite walking on thin propolis layers throughout the hive in the beehive's interior. Since most of its components in particular are water insoluble in the beehive's interior [47]. The mortality rate of Varroa mites with propolis, as seen from present result depended on concentration, contact time and origins of propolis. The 20% Propolis extracted in 55% ethanol was superior to the 5% Propolis extracted in 55% ethanol. The 5s contact time of Varroa mites with 20% propolis in 55% ethanol resulted in 100% mortality indicating that it is highly toxic. In the case of treatments with propolis 5, 7.5, 10 and 15 % propolis in 55% ethanol also the mortality rate of Varroa mites rose with increasing contact time. But in comparison of mortality rate of varroa mite with experimental work of Garedewa *et al*. [26] the treatment with 10% propolis in 55% ethanol resulted in 100% mortality, regardless of contact time but in the case of treatments with propolis 5 and 7.5% propolis in 55% ethanol the mortality rate Varroa mites rose with increasing contact time seems in agreement with present result. Hence length of mortality depended on propolis concentration, solvent of extraction and length of contact time [26].

The control experiments did not have lethal effects at all. This may show that, in order to detect the influence of propolis concentrations of varroacidal agents on the narcotic and mortality of Varroa mites. One needs a higher concentration of propolis to observe a remarkable varroacidal effect when propolis was extracted and used in 55% ethanol, where most of the water-soluble and some

water insoluble components were extracted. This shows that even if some of the components of propolis are solubilised in the high humidity of the hive's interior, their concentration is too weak to kill the mites [26]. Most of the varroacidal agents of propolis are water insoluble leading to the dominance of propolis extracted in 55% ethanol.

CONCLUSIONS AND RECOMMENDATIONS

This preliminary work was focused on the narcotic and lethality efficacy of propolis of different origins and the pest's diseases affecting bee health. To gain a better view of what is affecting the managed honey bee health, it is important to understand the key pests and diseases affecting bee health. The world distribution of honeybee disease is a great importance to bee keepers as there is a significant relationship between the success of apiculture industry and the control of honey bee health problems. This is because if it once occurs in the colony, they cause partial or total loss of colonies and most of them spread very quickly and difficult to treat. Varroa, nosema, amoeba, pests like wax moth and spider could be the major honey bee health problems responsible for endanger honey bee health within the hive in Toke-kutaye districts. it is difficult to trace how and when varroa mite was introduced, Ethiopia has been tested positive to *varroa* mite. The presence of *varroa* mite in the country is highly significant and market oriented might be due to high bee colony mobility coupled with lack of awareness neither from the beekeepers nor from the experts enhanced its high rate of distributions. This study for second time reported the presence of *varroa* mite in Ethiopia and the first finding in the study area. Furthermore, it advanced understanding of the existences of *varroa* mite as implicated with honeybee colony losses and economic significance to the countries in general. Even though Nosema and amoeba their presence in honeybee colonies could result in shortening of honey bee live and determinately reduces honeybee colonies strength predispose bees to pest and predators which directly and indirectly affecting the beekeeping sector and honey production in the woreda. Associated with the current problematic varroa mite in beekeeping subsector and propolis has already investigated and proved to be varroacidal in vitro. Information on the effect of propolis from different geographic origins differs in varroa narcotizing effect and varroacidal actions. If to be recommended used as a varroacidal agent it may minimize the contamination of hive products by synthetic acaricides and if it is effective in the field experiment and if it has no negative effects on bee themselves it may has the possibility to shed light on the use of propolis as in hive treatment with possible natural benefit of propolis used by honeybees and minimize the cost of beekeeping.

Based on the above conclusions the following recommendations forwarded.

- Further research in this field needs to be encouraged, as this will help to sustain the health of honey bee (*Apis mellifera*) already established in the bee keeper's apiary in the district.
- International trade in these commodities has been mainly responsible for distributing most of the known pests and diseases of bees. Authorities, should responsible for monitoring the spread of animal diseases and for regulating international trade in bees and bee products.
- To fully exploit opportunities in beekeeping subsector, interventions to address constraints and detecting the occurrence of honey bee diseases and health problem at apiaries level is the key step to prevent their harmful effects as early as possible.
- Nosema and Amoeba and require careful monitoring and management to ensure losses are kept to a minimum.
- Authorities are advised to come up with an urgent monitoring programme to determine mite infestation levels and its effects to honeybee colonies
- It is therefore very important that the existing problems are well managed to maintain bee health and that the risks and consequences of pests and diseases are well understood and appropriate plans in place to deal with any such honey bee health problem.
- Propolis from different geographic origins of the country should further investigated effect of different concentration of propolis extracted in different level of ethanol and type of effective propolis identified
- The varroacidal component of propolis should extracted and field trial in the beehives as soon as possible

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