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Evaluation of Extracted Honey Bee Propolis as A Natural Preservative of þÿ R a w C o w s M i l k i n A w i Z o n e ,

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COLLEGE OF AGRICULTURE AND ENVIRONMENTAL SCIENCE

SCHOOL OF ANIMAL SCIENCE AND VETERINARY MEDICENE,

DEPARTMENT OF ANIMAL SCIENCE

GRADUATE PROGRAM

EVALUATION OF EXTRACTED HONEY BEE PROPOLIS AS A NATURAL PRESERVATIVE OF RAW COW'S MILK IN AWI ZONE, ETHIOPIA

MSc. Thesis

By

Abebe Melese Tirfie

March, 2024

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SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS OF SCIENCE (M.Sc) IN ANIMAL PRODUCTION

Major Advisor: - Tessema Aynalem (Ph.D)

Co-advisor:- Takele Ayanaw (Assistant professor)

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Bahir Dar, Ethiopia

THESIS APPROVAL SHEET

As member of the Board of Examiners of the Master of Sciences (M.Sc.) thesis open defense examination, we have read and evaluated this thesis prepared by Mr **Abebe Melese Tirfie** entitled **Evaluation of Extracted Honey Bee Propolis as Natural Preservative of Raw Cow's Milk in Awi Zone, Ethiopia.** We hereby certify that; the thesis is accepted for fulfilling the requirements for the award of the degree of Master of Sciences (M.Sc.) **in Animal production.**

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DECLARATION

This is to certify that this thesis entitled with **Evaluation of Extracted Honey Bee Propolis as Natural Preservative of Raw Cow's Milk in Awi Zone, Ethiopia** submitted in partial fulfilment of the requirements for the award of the degree of Master of Science in **Animal production** to the Graduate Program of College of Agriculture and Environmental Sciences, Bahir Dar University by Mr **Abebe Melese Tirfie (ID.NO=BDU1401238)** 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.

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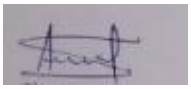
Signature  date 25/02/2024

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STATEMENT OF THE AUTHOR

First, I declare that this thesis is my bona fide work and that all sources of materials used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for an advanced M.Sc. Degree at Bahir Dar University and is deposited at the library to be made available to borrowers under rules of the library. I declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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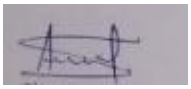
Name: **Abebe Melese Tirfie** Signature: 

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DEDICATION

This thesis is dedicated to my Father Mr, **Melese Tirfie kassa**, who saw my starting date but not the graduation day.

LIST OF ABBREVATIONS

CFU	Colony Forming Unit
SNF	Solid Non Fat
CC	Coliform Count
CSA	Central Statistical Agency
EE	Ethanol Extract
FAO	Food and Agriculture Organization
GLM	General Linear Model
GDP	Gross Domestic Product
g	gram
kg	Kilogram
L	Liter
LSD	Least Significant Difference
LDMP	Livestock Development Master Plan
ml	Milliliter
NaoH	Sodium Hydroxide
pH	Power Of Hydrogen
SAS	Statistical Analysis Software
SPSS	Statistical Package For Social Science
TS	Total Solid
VRBA	Violet Red Bile Agar
WEP	Water Extracted Propolis

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EVALUATION OF EXTRACTED HONEY BEE PROPOLIS AS A NATURAL PRESERVATIVE OF RAW COW'S MILK IN AWI ZONE, ETHIOPIA

ABSTRACT

Milk preservatives can originate from a natural or synthetic source. Incorporation of synthetic source leads to undesirable effect to consumers. In addition, the tendency of consumers to use natural preservatives has greatly influenced the food industry. Among natural preservatives, honey bee propolis was effective in preservation of food. Therefore, the aim of this research was to evaluate the effectiveness of extracted honey bee propolis as a natural preservative for raw cow's milk. The propolis was harvested from three agro ecologies of Awi zone namely Banja, Dangla and Chagni and extracted using 70 % ethanol. One hundred millilitres of raw cow's milk samples collected from injibara university dairy farm were placed into five pre-sterilized sample bottles in each study districts and ethanol extracted honey bee propolis from three sources (Banja,Dangla and Chagni) prepared with a volume of T1 (control), T2 (5ml), T3 (10ml), T4 (15ml) and T5 (20ml) was added in to 100 ml of raw cow's milk samples. All samples were analyzed for the physicochemical (protein, fat, ash, solid nonfat, pH, TA clot on boiling, alcohol, and methylene blue reduction test, microbiological (aerobic plate count (ABC), coliforms count (CC) and Yeast and mold counts (YMC), and organoleptic properties. The data was encoded and analyzed by using SAS version 9.4 software. The study shows the Fat, SNF, Ash, pH, TA and Protein content were significantly ($p<0.05$) influenced by propolis concentration and agro-ecologies of propolis harvested. The interaction has shown a significant ($p<0.05$) effect on protein and fat contents of raw cow's milk. However, a non-significant ($p>0.05$) interaction effect on SNF,pH and TA contents of raw cow's milk. In organoleptic test, both propolis concentration and agro-ecologies were a significantly ($p<0.05$) affected all the sensory values of raw cows milk. However, interaction effects of propolis concentration and agro-ecologies of propolis collected significant ($p<0.05$) only on color and flavor values of raw cow's milk. Result revealed that significantly ($p<0.05$) higher preference on sensory parameters of milk was recorded in milks treated with propolis concentration harvested from Banja than Dangla and Guangua woredas and lower preference in T4 and T5 concentration treated raw cow's milk. Agro ecologies and propolis concentration had highly significant ($P < 0.001$) effect on the main and significance ($P < 0.05$) interaction effect on clot on boiling, alcohol and methylene blue reduction tests of raw cow's milk. The shortest coagulation time (2 hour) was recorded in control group. However, the highest coagulation time was recorded in BT5 9 hour in clot on boiling, 9:50 hour/minutes in alcohol test, DT5 10 hour both in clot on boiling and alcohol test and in GT5 12 hour both in clot on boiling and alcohol test and in MBRTs in GT5 (12 hour), DT5 (10.50 hour/mitutes) and BT5 (9 hour). In microbial test, agro ecologies of propolis harvested and propolis concentration had a highly significant ($P < 0.001$) effect on ABC, CF and YM counts of sample raw cow's milk. In the main effects of agro-ecologies propolis harvested, a significant lower ABCs,TCFs and YMCs values were registered in Guangu woredas and the highest was recorded in Banja and Dangla woreds in decreasing order. Regarding, propolis concentration, the microbial counts significantly ($P<0.05$) decreased with the addition of higher concentrations of ethanol extracted propolis. Finally, it can be concluded that the use of 5ml and 10ml extracted honey bee propolis concentration harvested from Guangua Woredas (lowland) extends the shelf life of raw milk for 8–9 hours under ambient temperatures without affecting organoleptic/sensory characteristics.

Key word:-Microbiological Examination, Raw Milk, Propolis, Sensory Evaluation.

CHAPTER: INTRODUCTION

1.1. Background and Justification

Agriculture in Ethiopia remains the cornerstone of the economy and the most important source of growth. It accounts for almost 48% of the gross domestic product, and 85% of export earnings, and is also the main source of income, livelihood, and way of life for 85% of Ethiopians living in rural areas (World Bank, 2016). According to the Central Statistical Agency, livestock products and by-products in the form of meat, milk, honey, eggs, cheese, and butter supply, etc. provide the needed animal protein that contributes to the improvement of the nutritional status of the people (CSA, 2021). Regarding dairy cattle production, it plays a vital role in the production of world milk and other products that remain stable in the diet of most developed countries (Philips; 2001). The main dairy cattle-producing countries in the world are the United States, Holland, Germany, France, and India (Nigussie, 2006). Dairy cattle production is an essential economic activity in most parts of Africa's farming system for rural and urban societies (Mihret Tadesse *et al*., 2017). In Ethiopia, 15 million dairy cows are producing approximately 4.69 billion liters of milk, an average of 1.48 liters per cow per day over a lactation period of 210 days (CSA, 2021).

Milk is defined as a fresh, clean, and normal mammary secretion obtained by the milking of one or more dairy cows that are properly fed and kept (Ajogi *et al*., 2005). Milk from a healthy udder contains very few numbers of bacteria ($<3x10^4$ cfu/ml) but may become contaminated by microorganisms from the surrounding environment during milking and milk handling, from water, and milk equipment (Geta Mohammed *et al*., 2022). Natural milk (preservative-free) is perishable and is usually of a relatively short lifetime, as it offers an ideal environment for the microorganisms to grow (Geta Mohammed *et al*., 2022). Preservative-free milk can have a considerable effect in spreading certain pathogenic bacteria causing salmonellosis, brucellosis, listeriosis, and tuberculosis (Fasil Abebe *et al*., 2020). Nowadays, preservatives present in milk are considered contaminants (El-Deeb, A. 2017). Various preservatives like formalin and some antibiotics are also added to milk to increase its shelf life. This addition decreases the nutritive value of milk (Raturi *et al*., 2022). These adulterants, preservatives, and drugs in milk cause very serious health-related problems (Afzal, *et al*., 2011). To solve these effects, researchers tried a lot

to find out natural antimicrobial substitutes to prevent bacterial and fungal growth in foods and dairy products (Hadi *et al*., 2020).

Several techniques were tried in the process of milk preservation ((Kawanga, 2017). In rural settings particularly in several pastoralist communities, the use of medicinal plants is the commonly practiced technique for different food preservation including milk (Kawanga, C.2017). Several plants, herbs, and spice extracts have been reported to possess antimicrobial activity against a range of bacteria, yeast, and molds (Burt, 2004). Charcoal from *Olea europea*, *Lagenaria siceraria*, and *Olea africana* were documented to be used to coat the inside of milk storage gourds to add flavor/aroma and also to protect the milk against spoilage microorganisms (Kawanga., 2017). Therefore, it is important to seek out natural antimicrobial preservatives for food especially for milk.

Even if it needs further experiments, honey bee-extracted propolis was tried as natural milk and other animal by-product preservatives (Çelik and Aşgun., 2020). Propolis is a bee product that is a mixture of beeswax and resins prepared by the honeybee (*Apis mellifera*) (Casquete *et al*., 2016). Propolis is attracted great interest due to its functional effects such as antibacterial, antioxidant, and anticancer and it is composed of 55% resinous compounds and balms, 30% beeswax, 10% aromatic essential oils, 5% bee pollen, and about 150 other compounds but it varies due to bee forage source(Melliou and Chinou, 2004).

Propolis gained popularity as an alternative medicine and as a substitute for antimicrobial substances used in the preservation of food and dairy products (El-Deeb. 2017). Preservatives present in milk are considered as contaminants (Shaban *et al*., 2021). With the increasing demand for dairy products and the necessity for reducing losses in industrial production due to poor quality, the requirement for high quality milk has increased (El-Deeb, 2017). Numerous efforts were conducted to find out natural antimicrobial substitutes to prevent bacterial and fungal growth in foods and dairy products (Willis, *et al.* 2017). Recently, due to great consumer awareness, using natural preservatives has become very popular to inhibit the growth of undesirable microorganisms in food (Schiano *et al*., 2020.) Such antimicrobials could be directly added to the product formulation, coated on its surface, or incorporated into the packaging material (Batiha *et al*., 2021). Some researchers across the world have tried a lot to seek neutral food preservatives that do not cause contamination of products and harm consumers (Hadi *et al*., 2020). In the case of milk,

propolis was used to reduce the microbial load of milk and preserve the milk without health risk factors for consumers (Elkassas *et al*., 2023). But it needs further evaluation level of concentrations, types of propolis extract, and the effect of the source of propolis extract agro ecologies (feed source of honey bee) (Burgut, 2020).

1.2. Statements of the Problem

Milk is an essential source of nutrition and thus its consumption is increasing in parallel with the continuous increase in the human population (Adisie Kassa *et al*., 2020). In Ethiopia in general and in the Awi zone in particular, milk and milk products represent an important share in the nutrition of consumers as well as the nutrition and income of producers. Nowadays, the estimated annual total national milk production are 4,692,993,642 national, 820,094,328 Amhara region and 65,557,551 in the Awi zone (CSA, 2021). Even if the study area in particular and the country, in general, produce a high amount of milk, due to the favorable nature of milk for the growth of different microbes, it is easily spoiled (Adugna Chala and Mitiku Eshetu, 2021). Milk and dairy products are excellent mediums for microbial growth and thus may play a role as a vector of spoilage microorganisms and foodborne pathogens. Post-harvest spoilage losses of about 40% have been reported from milking to consumption (Hiwot, 2016). So to protect the milk from spoilage and foodborne health risks to consumers, using different preservative methods such as smoking with herb plants and using different chemicals like Sorbic acid (E200), benzoic acid (E210), sodium benzoate and potassium sorbate was applicable throughout the country. This allows adequate time for the milk to be transported from the collection point to a processing center without refrigeration (FAO/WHO, 2005). But synthetic (chemical) source leads to undesirable side effects for consumers. In addition, the tendency of consumers to prefer natural preservatives has greatly influenced the food industry. Among the natural preservatives honey bee propolis is effective in the preservation of different foods like milk and milk products.

The antimicrobial and antioxidant properties of propolis suggest its use as a preservative in the food industry (Essawy, 2007). Therefore, this research aimed to evaluate the effects of adding different volumes of ethanol-extracted honey bee propolis collected from different agro-ecologies on the microbial test, physiochemical, organoleptic, and shelf life of raw cow's milk in a zone.

1.3. Objective

1.3.1. General objective

- To evaluate the effectiveness of honey bee propolis extract from different agro-ecological zones in the Awi zone as a natural raw milk preservative by applying different volumes to the raw cow's milk.

1.3.2. Specific objectives

- To evaluate the effect of honey bee propolis extract from different agro-ecologies on microbial, physicochemical, and organoleptic characteristics of milk.
- To evaluate the effect of volume of honey bee propolis extract on microbial, physicochemical, and organoleptic characteristics of milk.
- To evaluate the shelf life of raw cow's milk treated with different volumes of extracted honey bee propolis harvested from different agro-ecologies.

1.4. Hypothesis Test

1.4.1. Null hypothesis (H0)

- There is no difference in organoleptic parameters of milk, which supplement different levels of ethanol extracted honey bee propolis from three agro-ecologies.
- There is no difference in microbial load of milk between normal raw milk and milk treated with propolis collected from different agro-ecologies.
- There is no difference in microbial load of milk treated at different levels of extract propolis volume (0ml=5ml=10ml=15ml=20ml).
- There is no difference in fresh shelf life of raw cow’s milk between normal raw cow’s milk and milk treated with propolis collected from different agro-ecologies.

CHAPTER 2. LITERATURE REVIEW

2.1. Milk production system in Ethiopia

Dairy is one of the livestock productions practice almost all over the World including Ethiopia, including small to large size farms or subsistence to market oriented farms (Kelay *et al*., 2002). Dairy production is traditional in most parts of Ethiopia (Dereje Tadesse *et al*., 2005). Depending on the area under consideration cattle, goats, camels and sheep all provide milk for human consumption (Zegeye yigezu, 2002). However, cattle are the main source of milk even though they are primarily kept as a draught power sources with very little or no consideration given to improving their milk production capabilities in the country (Zegeye yigezu, 2002). According to Guadu Tadesse, and Mengistie Abebaw(2016), dairy production constitutes one of the principal means of achieving improved living standard in many regions of developing World.

Ethiopia holds large population for dairy development due to its large numbers of livestock population, the favourable climate for improved, emerging market opportunity, improved policy environment for involvement of private sector and relatively disease free environment for livestock(Getachew Feleke,2003). Dairy production is a critical issues in Ethiopia's livestock based society where livestock and its product are more important sources of food and income and dairying has not been fully exploited(Yigrem Sintayehu *et al.*, 2008). Like most dairy production systems found in the tropics, the Ethiopian dairy production system includes large number from small to large sized and subsistence to market oriented farms (Bereda Abebe *et al*., 2014). Based on climate, landholdings and integration with crop production criteria, three production systems are recognized in Ethiopia (Yigrem Sintayehu *et al.*, 2008). In Ethiopia, there are three identified major dairy production systems including the rural, peri-urban, and urban dairy production systems (Redda Tsehaye, 2002).

2.1.1. Rural household milk production system

The traditional smallholder milk production system is practiced in rural areas that account for about 97% of the total national milk production of the country (ZelalemYilma *et al*., 2011). It is part of the subsistence farming system that includes pastoralists, agro-pastoralists, and mixed crop-livestock producers (Taddesee Dereje *et al*., 2005, Ketema Hizkias, 2000). The system is not market oriented and most of the milk produced in this system is only for home consumption (Ahmed Mohommad *et al*., 2003). The level of

surplus milk is determined by the demand by the milk demand, household, its neighbors, herd size, production season, and access to a nearby market. The surplus is mainly processed using traditional technologies and the processed milk products such as butter, ghee, *Ayib* and sour milk are usually marketed through the informal market after the households need satisfaction (Ahmed Mohommad *et al*., 2003).

2.1.2. Per-urban or small scale milk production system

Per-urban milk production system is practiced in areas where the population density is high and agricultural land is shrinking due to urbanization. It possesses animal types ranging from 50% crosses to high grade Friesian in small to large sized farms and contributed only 2% of the total milk production of Ethiopia. This sector owns most of the country‘s improved dairy stock (Gebre *et al*., 2000). The main source of feed is both home produced and purchased hay and the primary objective is to get additional cash income from milk sale (Emebet and Zeleke, 2008). This production system is now expanding in the highlands among mixed crop–livestock farmers and serves as the major milk supplier to the urban market (Azage Tegegn *et al*., 2013).

2.1.3. Urban (commercial) milk production systems

Urban dairy farming is a more specialized farming practiced in state sector and very few individuals on commercial basis. These farming systems with combination to per urban and urban small scale dairy farmers produce 2% of the total milk production of the country (Bereda Abebe *et al*., 2014). Farmers use part or all of their land to grow fodder crops for their dairy cattle. The dairy animals do not provide draft, but their manure is used as fertilizer on crops, milk is the main source of farm income. It is mainly under taken by small farmers using family labor, but commercial farmers using herd labor also practices this system on a large scale. The herd is dominated with improved/cross breed dairy cattle and the production system is market oriented and milk production is for sales(Emebet and Zelek., 2008). As compared to other systems they have relatively better access to inputs (e.g. feeds) and services (e.g. artificial insemination) provided by the public and private sectors and use intensive management system(Gobena,Matawork, 2016; Gebreselassie Lidetu, 2019). Marketing of fluid milk is arranged through direct contact between producers and consumers, and/or involves wholesalers/ processors, cooperatives, and retailers (Siegefreid and Berhanu, 1991).

2.2. Milk Consumption in Ethiopia

Ethiopia has a low level of milk consumption compared to other African countries; - Kenya 90 liter; Uganda 50 liter (Benyam Tadesse, 2016). Even though Ethiopia has the huge inventory of milk producing animals, (cattle, sheep, goats and camels), per capita consumption of milk is low compared to Kenya and Sudan with fewer livestock (Benyam Tadesse, 2016). The national per capita consumption of milk and milk products is estimated at 17 kg (Ahmed Mohamed *et al*., 2004). Ethiopia's annual per capita milk consumption is less than 20 kg, placing it in the same range as Tanzania and Rwanda (Mebrate Getabalew *et al*., 2019). Average expenditures by households on milk and milk products were only 4% of the total household food budget (LDMP, 2007).

In rural areas of Ethiopia, consumption of milk is determined by livestock ownership and season (LMP, 2007, Bachewe Fantu, 2017). The demand for milk is mainly for fresh whole milk which is satisfied by own production or purchased from neighbors. Processed milk is currently not sold in rural markets. In the rural areas producers consume fresh milk and convert their surplus milk to butter and cheese. It is estimated that about 40% of the milk produced is converted to butter, while only 9% is converted to cheese (LMP, 2007). Traditional butter ferments slowly at room temperature and can be kept for a year or longer, offering rural consumers a readily storable and durable (Sadler Kate and Catley Andrew, 2009).

In pastoral communities of Ethiopia like Somali region, Women perceive milk from camels and goats to be the most beneficial for children's overall health, strength, and growth ((Sadler and Catley, 2009). In the wet season, milk consumed by pastoral children can accounts about 67% of the mean daily energy they require and 100 % of their protein requirements (Staal, *et al*., 2008). Lack of availability and access to milk in the dry season decreased daily consumption amounts by almost 25% with milk contributing only 16% and 50% of energy and protein requirements respectively. In drought years, children's milk consumption will drop an average of 50% in surveyed communities (Staal, *et al*., 2008).

2.3. Chemical Composition of Milk

Chemical composition, particularly milk fat content is used as quality test (Larsen, *et al.,* 2013). The solid constituents of milk make an important food item from both nutritional as well as processing point of view (Manay, 2001). Milk fat and protein are most important components of different varieties of most shelf stable milk products. It is therefore very

important to determine the major chemical compositions of milk as it is the basis of further processing into more shelf stable products. Moreover, knowledge of the total solids and solids-not-fat (SNF) content of milk is necessary when it is sold for liquid consumption. In most countries, milk offered for sale for liquid consumption must conform to certain legal standards with regard to its total solids content, for example the minimum 3% fat and 8.5% solids-not-fat (EAC, 2006). The yield of dairy products obtained from milk will depend on the amount of constituents (total solids) present. The greater the amount of fat and protein in milk, the greater is the yield of cheese and milk with a high fat content gives more butter than milk with a lower fat content (O'Connor, 1994). Normal cow's milk contains approximately 87.4% water and 12.6% milk solids (Goff, 2010). The solids comprises 3.9% fat, 3.2% protein, 4.6% lactose and 0.9% others like minerals and vitamins (FAO, 1986). The composition of milk is affected by a number of factors including genetic and environmental factors (O'Connor, 1994). The factors responsible for variations in milk composition include breed and individuality of the cow, interval between milking, stage of lactation, age and health status of the cow, feeding regime and completeness of milking (O'Connor, 1994).

The natural composition and Physico-chemical properties of raw milk may change by Adulteration of milk by intentional addition of water or other substances are a common problem in many developing countries. Adulteration is illegal because it alters the natural composition of milk and can introduce harmful bacteria and other dangerous substances into milk. Water adulteration lowers the specific gravity and increases the freezing point of milk. The Normal whole milk specific gravity ranges 1.026 to 1.032 at milk collection centers and processors routinely determine the specific gravity of raw milk and reject milk suspected of having been adulterated (Kurwijila, 2006).

Table 2.1. Average milk chemical composition (%) of different cattle breeds

Breed	Fat	Protein	Lactose	Ash
Zebu	5.6	3.1	4.6	0.71
Ayrshire	3.8	3.4	4.8	0.70
Friesian	3.4	3.2	4.6	0.74
Guernsey	4.9	3.8	4.8	0.75
Jersey	5.1	3.8	4.9	0.75
Shorthorn	3.6	3.4	4.8	0.75

Source (O' Connor, 1995).

2.4. Milk Microbial Quality

The microbial content of milk is a major feature in determining the quality milk (Rogelj, 2003). Milk quality is one factor that affects child milk consumption (Yimer, G. 2000). The number of bacteria in aseptically drawn milk varies from animal to animal and even from different quarters of the same animal (O'Connor, 1994). In proportion to the numbers present, existence of coliform bacteria in milk is suggestive of fecal contamination and unsanitary practices during production (Richardson, 1985). According to Alganesh (2002), the minimum and maximum values for microbiological quality of raw cows' whole milk produced in two woredas of Eastern Wollega to be 9.0 and 1.4 x 10^9 CFU/ml. The Total bacterial count and Coliform count of milk produced in Bahir Dar zuria was 7.61 ± 0.12, 4.41 ± 0.16 cfu/ml in mecha district was 7.56 ± 0.13 and 4.55 ± 0.15 cfu/ml respectively (Asaminew Tassew and Eyassu Seifu, 2007).

2.5. Microbial Tests of Raw Milk

Microbial analysis of milk and milk products includes tests such as total bacterial count, yeast and molds and coliform estimations (Abebe Bereda *et al*., 2014).

2.5.1. Standard plate count

The standard plate count is generally accepted as the most accurate and informative method of testing bacteriological quality of milk (Godefay and Molla, 2000). The total plate count of microbes in milk provides useful general information on the microbiological quality of milk. Total or aerobic plate count shows only the mesophillic aerobic organisms as incubation is done under normal atmospheric conditions at 35°C for 48 hours (Pothakos, 2012). The number of bacteria in aseptically drawn milk varies from animal to animal and even from different breasts of the same animal. On average, aseptically drawn milk from healthy udders contains between 500 and 1000 bacteria ml/l. High initial counts (more than 105 bacteria ml/1) are evidence of poor production hygiene (O'Connor, 1994).

2.5.2. Coli form bacteria

Coliforms are aerobic or facultative anaerobic, Gram-negative, no spore forming rods that ferment lactose to produce gas when incubated on agar for 48 hours at 35°C (Suraj., 2022). Coli forms are important mastitis pathogens (Hogan and Smith, 2003) and are widely distributed in the farm environment (Sanderson *et al*., 2005). Coliform count (CC) is a non-regulated test that has been used historically to assess milk production practices such as milk refrigeration, milking machine sanitation, and pre milking udder hygiene

(Davidson *et al*., 2004). Coli form organisms contaminate raw milk from unclean milker's hands, improperly cleaned and un sanitized or faulty sterilization of raw milk utensils especially churns, milking machines, improper preparation of the cow's flecks or dirt, manure, hair dropping in to milk during milking, udder washed with unclean water, dirty towels and udder not dried before milking (Mbabazi, 2005). The presence of coliform organisms in milk indicates unsanitary conditions of production, processing or storage. Hence their presence in large number in dairy products is an indication that the products are potentially hazardous to the consumers' health (Godefay and Molla, 2000). Coliform count provides an indication of unsanitary production practices and/or mastitis infection. A count less than 100 Colony Forming Units (CFU)/ml are considered acceptable for milk intended to be pasteurized before consumption. Counts of 10 CFU/ml or less are achievable and desirable if raw milk will be consumed directly (Ruegg, 2003).

2.5.3. Enumeration of yeast and mold

Yeasts and molds counts are used as an index of the proper sanitation quality. Defects such as rancidity**,** softness and color defects arise mainly from contamination by yeasts and molds. Moreover, in view of the potential ability of some molds to produce mycotoxins during their growth, thus their presences possess potential hazards to food safety and human health (Riesute et al., 2021). Molds and yeasts are a widely diverse group of microorganisms, with the majority emerging from soil or the air. The majority of species of molds are incredibly adaptable, with the ability to absorb any source of carbon contained in meals (Da Silva *et al*., 2012). Most species can also employ various sources of nitrogen, such as organic nitrogen, nitrate, and ammonia ions. Yet, some species will exhibit restricted growth when the utilization of proteins or amino acids as sources of nitrogen or carbon is necessary (Cisse *et al*., 2019).

According to (Birhanu yeserah *et al*., 2019), the overall mean yeast and mold count of raw cow's milk produced in and around Bahir dar city was 4.12±0.14 log 10 CFU/ml. In Amhara regional state Dangla town the overall mean YMC of cows' milk produced was 0.68±0.41 log 10 CFU/ml (Bekele, *et al*., 2015). Additionally, milk samples from Shashemene town were found to have higher yeast and mold count (4.2060.082 log 10 CFU/ml (Teshome, *et al*., 2014).

2.6. Source of Microbial Contamination of Raw Milk

The common predisposing factors of milk contamination by microorganisms are milking environment, cows, milking personnel, milking equipment's, and water (Mbabazi, 2005).

2.6.1. Milking environments

Maintaining the sanitary condition of the milking area is important for the production of good quality milk (Zelalem yilma, 2012). Dirty milking places tend to breed flies, which may fall in milk causing contamination and thus spoilage may occur (Mbabazi, 2005). When a cow urinates or defecates in the course of milking some of its urine or dung particles may drop into the milk (Mbabazi, 2005).

2.6.2. Cow udder

Cleaning the udder of cows before milking is one of the most important hygienic practices required to ensure clean milk production (Zelalem Yilma, 2012). This is important since the udder of the milking cows could have direct contact with the ground, urine, dung and feed refusals. Cleaning and removal of soil particles, bedding material and manure from the udder and flanks is necessary to prevent the entry of many types of bacteria into the milk (O'Connor, 1995). Udder washing with clean water and drying using hand towels reduces milk contamination by transient bacteria located on the udder. Special care must be given to the cloths used for cleaning the udder (O'Connor, 1995). The re-use of cloths for cleaning and sanitizing may result in re-contamination of the udder. It is therefore recommended that separate cloths be used for cleaning and sanitizing and, if possible, each cloth should be used for one cow only (O'Connor, 1995). Not washing the udder before milking can impart possible contaminants into the milk. A maximum reduction of teat contamination of 90 % can be achieved with good udder preparation before milking. This depends on the initial level of contamination and the way of udder preparation. So with high initial contamination levels this 90 % reduction might not be reached (Murphy, 1996).

2.6.3. Personnel (milker)

Milk handling personnel (milker) may contribute various organisms including pathogens especially when they are careless, uninformed, or willfully negligent, directly to milk (Kathiriya ., 2024). Organisms may drop from hands, clothing, nose, and mouth and from sneezing and coughing (Ashenafi Mengstu, 1994; Kathiriya., 2024). It is important for milk men to be in good health so that they can be a source of infectious diseases such as tuberculosis (Kathiriya., 2024).

2.6.4. Milking equipment's

Poorly cleaned and sanitized milking utensils may be the source of many microorganisms (Kathiriya., 2024). Milk drops left on the surface of milking equipment's act as excellent media for the growth of a variety of bacteria (Bekuma Amanuel and Ulfina Galmessa, 2018). Milk equipment is not properly cleaned and sanitized after use. Milk residues left on equipment and utensil surfaces provide nutrients to support the growth of many microorganisms, including pathogens (Bekuma Amanuel and Ulfina Galmessa, 2018). In case cracked milking equipments large number of bacteria enter and grow in the cracks, are difficult to clean (Haile Saba, 2015). The bacterial load of milk increases during transportation and if the transportation equipment is not appropriate the bacterial counts increase causing spoilage before milk reaches its destination (Grillet *et al*., 2007). Milking equipment should be easy to clean. Aluminum and stainless steel equipment is mostly preferred (Zelalem Yilma, 2012).

2.7. Use of Propolis in Foods Preservative

The consumption of propolis should be increased in the human diet in order to provide the positive effects of its biological activities in human health (Lotfy., 2006). According to Pobiega *et al*., (2018), enrichment of food products with propolis as a natural food additive and improving food quality has become an interesting subject in recent years. Also using propolis meets the consumer demand for the use of natural antioxidants and antimicrobials instead of synthetic food additives (Negi, 2012; Soliman and Badeaa., 2002).

According to Silva-Carvalho *et al*., (2015), many of the compounds in propolis have been used as food additives and these are generally recognized as safe (GRAS) substances. Humans can consume 1.4 mg/kg body weight/day or approximately 70 mg/day as a safe dose (Burdock, 1998). propolis used in various food formulations such as meat, dairy, juice, fruits, oils and seafood to improve shelf-life, prevent lipid oxidation and provide health benefits for consumers(Ali *et al*., 2010).

The addition of ethanol and water extracts of propolis in dairy beverages showed the highest antioxidant capacity and the lowest aldehyde production during storage in light (Cottica *et al*., 2015). Propolis at certain doses significantly inhibited Streptococcus salivarius subs. thermophilus bacteria and therefore, yogurt production may be negatively affected (Çifci, 2015). Similarly, the count of methicillin-resistant Staphylococcus aureus was reduced by using ethanol extract of propolis in ice cream (El-Bassiony *et al*., 2012).

2.8. Physicochemical Composition of Propolis

Propolis (bee glue) is the generic name for the resinous substance collected by honeybees from various plant sources (Siheri et al., 2017). The word propolis is derived from the Greek pro-, for or in defense, and polis-, the city, that is defense of the city (or the hive) (Siheri et al., 2017). The source of propolis composition originates from plants, bee metabolites and propolis construction materials (Bankova *et al*., 2016). However, the composition of propolis mainly depends on plant materials, plant species, season, climate, genetic diversity in the queen bee (Toreti *et al*., 2013). Therefore, the chemical composition of propolis is highly variable and affects the variability of plant species growing around the hive and geographic location (Russo *et al.,* 2002, de Groot, 2013).

propolis is the major source of phenolic acids and its esters, flavonoid aglycones, amino acids, ketones, phenolic aldehydes, alcohols, sesquiterpenes, steroids, coumarins, and the inorganic compound(Burdock, 1998). However, according to Noori *et al*., (2012), propolis is also composed of resins (50%), wax and fatty acids (30%), essential and aromatic oils (10%), polyphenols and flavonoids (10%), pollen (5%), vitamins and minerals (5%) approximately.

2.9. Anti-Microbial Activities of Propolis

Propolis extracts have antibacterial, antifungal and antiviral activity (Valente *et al*., 2011, Silva *et al*., 2012). One of the most widely known and extensively tested properties of propolis is its antibacterial activity. Propolis has greater activity against Gram-positive bacteria than Gram-negative bacteria (Przybyłek and Karpiński., 2019). This may be related to the differences in the cell wall of gram-negative and positive bacteria (Silva *et al*., 2012, Vardar-Unlu *et al*., 2008). Propolis exhibits antibacterial effects by protein synthesis, cell division and bacterial growth inhibition (Sforcin and Bankova ., 2011; Kędzia and Hołderna ., 2013). According to Ugur and Arslan, (2004), antibacterial properties of propolis increase in a dose-dependent. The bactericidal effect of propolis was tested and the result showed that it had a bactericidal effect on Staphylococcus and E. coli strains at concentrations of 1.5-3 mg/ml, on Shiga Bacillus and Pyoceanic Bacillus at 6 mg/ml; on Sonne Bacillus at 1.5 mg/ml and on Salmonella strains at 3-5 mg/ml(Malimon *et al* .,1980).

CHAPTER 3. MATERIALS AND METHODS

3.1. Propolis Sample collection site

Propolis was collected from different agro-ecologies of the Awi zone. From a total of 12 districts in the zone, it was stratified into 3 categories based on the agro-ecologies of administrative woredas, which are high, medium, and low land districts to harvest propolis from different botanical sources. From each agro-ecology, one district was selected purposively based on the potential of beekeeping to collect enough honey bee propolis. Then Banja, Dangla, and Guangua woreda were selected from high, middle, and low lands, respectively.

Banja district is bordered on the south by Ankesha, on the West by Guangua, on the North by Fageta Lekoma, and on the East by the West Gojam zone. The district consists of 26 kebeles, of which 25 are rural kebeles and one urban kebele. One urban kebele(02) was selected to harvested honey bee propolis. The Apiary site of which propolis collected was established by Injibara University in Liwi Mountain for demonstration purpose in injibara town. The town is both the capital of Awi Zone and Banja district, which is located about 450 km northwest of Addis Ababa and 118 km south of Bahir Dar. The district is comprised of Dega (80 percent) and Winadega (20 percent) agroecologies, and the altitude ranges from 1900 to 2750 meters above sea level. The annual temperature is 26°C at the maximum and 16°C at the minimum. It has an unimodal rainfall distribution pattern.

Dangila district, which is found in Awi zone, Amhara Regional State, in north-western Ethiopia, along the main road between the cities of Addis Ababa and Bahir Dar, is about 472 km from Addis Ababa, the capital of Ethiopia. From the regional capital, Bahir Dar, Dangila district is about 72 km. astronomically; Dangila is located at 11° 18' N latitude and 36° 57' E longitude. Geographically, it is located at an elevation of 2200 m above sea level with area coverage of 9486.4 hectares and a Woina Dega (temperature) climate. The annual average rainfall and temperature are 1576 mm and 17 °C, respectively. From the total 29 kebeles of Dangla Woreda, East Zelesa was selected based on bee keeping production potential to harvest honey bee propolis for extraction.

Guangua district is a town in north-western Ethiopia. Located in the Agew Awi Zone of the Amhara Region, this town has a longitude and latitude of 10°57′N, 36°30′E and an elevation of 1583 meters above sea level. The average annual temperature of Guangua district is 36^0 °C, and the rainfall is about 950 mm (Agricultural Office of chagni, 2018).

Semen Degela Kebele was selected purposively based on beekeeping production potential of total 27 kebeles existed in Woredas to harvest honey bee propolis.

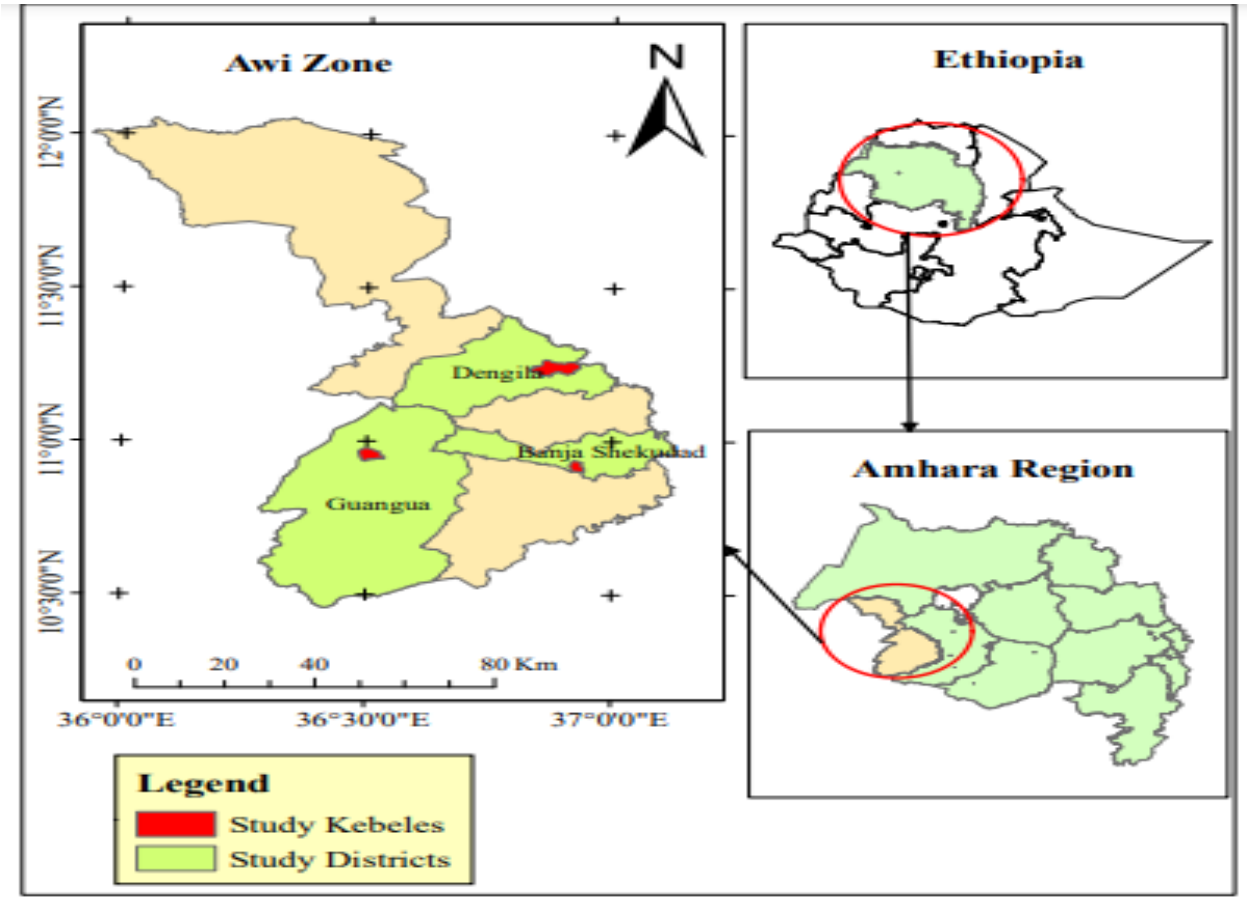

Figure 1. Map of the study area

3.2. Experimental Design

A study was conducted from September 2022 to May 2023 to evaluate the effect of honey bee propolis collected from different agroecologies for the preservation of raw cow's milk. A Factorial Completely Randomized Design was used involving two factors. Agro-ecologies effect for the source of propolis (dega, wyna dega, and low land) and volumes of the propolis extract (T1=control, T2=5ml, T3=10ml, T4=15ml, T5=20ml) and each observation is done in three replication. So the total sample size consists of N= 3*5= 15*3=45 the outcome variables. Samples were examined for, chemical composition, Sensory evaluation(color, flavor, taste, texture, and overall acceptability) of milk, clot on boiling test, alcohol test, methylene blue reduction test, total aerobic bacterial count, total coliform count, and total yeast and mold count. The laboratory analysis was done in Injibara University microbiology laboratory, Injibara University, Ethiopia.

Table 3.1. Lay out of the Experimental design

Factors	**Propolis concentration**				
Agro ecologies	T1	T2	T3	T4	T5
Banja(B)	BT1	BT2	BT3	BT4	BT5
Dangla(D)	DT1	DT2	DT3	DT4	DT5
Guangua(G)	GT1	GT2	GT3	GT4	GT5

3.3. Harvesting and Preparation of Propolis Extract

Propolis was collected using plastic propolis traps which were placed on top of the hive for up to four weeks, and then the plastic propolis trap trapped enough amount of propolis.. The propolis sample was cut into small pieces, and to prepare aqueous propolis extract, each 20 grams of propolis was mixed with 200 ml solution of proof 70% ethanol for every three agro-ecologies and shaking it every day. Then, the extract was filtered with filter paper using the conical flasks. The purified extract was stored in a dark place at 4 °C in a dark bottle until used (Said *et al*., 2006).

3.4. Milk Sample Collection and Analysis

The milk sample was collected from Injibara University dairy farms from the morning milking for preservation by adding honey bee propolis extract. The milk was collected in pre sterilized bottle, labeled, and transported to the microbiology laboratory of Injibara University in an ice-packed cooler box. Propolis collected from high land, midland, and low land agro-ecologies and extracted by 70% ethanol solution in one to ten ratio of propolis to ethanol solution was added at various volumes (0ml, 5ml, 10ml, 15ml and 20ml) to 100 ml of milk and homogenized. The tested parameters were Microbiological and physicochemical; milk fresh shelf life and sensory analysis were conducted for each treatment. The laboratory test parameters were done in a controlled environment.

3.4.1. Microbiological tests

The determination of microbial load was performed at the initial time, 24- and 72-hours storage period.

Aerobic Total Bacterial Count (ABC):- was determined using plate count agar (PCA) following the ISO standard (ISO 17025:1999). 10 ml of the sample was homogenized in 100 ml of peptone water which gave the first dilution (10^{-1} dilution) and up to 10^{-5} serial

dilutions were prepared in a sterile test tube containing 9 ml of peptone water. 1 ml of each serial diluted sample was poured into sterile Petri dishes in triplicate and mixed thoroughly by adding 15-20 mL of molten PCA (Oxoid, UK) tempered to 44 0C. The plates were allowed to solidify at room temperature and then incubated at 32 ºC for 48 hours (Murphy, 1996). The plates with colonies ranging from 30-300 colony forming units (cfu) per plate were selected and counted for determination of TABC (Kiiyukia, 2003). The TABC was determined as cfu per ml of milk sample calculated by the formula provided by IDF (2004).

$$N = \frac{\sum C}{[(1xn1) + (0.1xn2)]d} \qquad (1)$$

Where=N is the number of cfu per milliliter of milk sample

ΣC= is the sum of colonies on all plates counted;

n1 =is the number of plates in the first dilution counted;

n2 =is the number of plates in the second dilution counted; and

d =is the dilution from which the first counts were obtained.

Coliform Counts (CC):- The coliform was determined using the ISO standard by plate count method (ISO 17025:1999). 1 ml of each serially diluted sample was poured into sterile Petri dishes in duplicates and mixed thoroughly by adding 15-20 ml of molten Violet red bile agar (VRBA, Oxoid, UK) tempered to 44 0C. The plates were allowed to solidify at room temperature and then incubated at 32 ºC for 24-48 hours (Murphy, 1996). The plates containing red or violet colonies ranging from 15-150 colony-forming units (cfu) were considered coliform and counted using colony counters (Marth, 1978). The coliform was calculated as cfu per ml which is mentioned earlier formula IDF (1991).

Determination of Yeast and Mold Counts: - Yeast and mold count (YMC) were determined using sterile Potato Dextrose Agar (PDA)(Oxoid, UK). Serial diluted up to 10^{-7} samples of 0.1ml, each serial diluted sample was added on to sulfurized PDA plates in triplicate and spread- plate using sterile bent-glass rod. The plates were incubated at 25 ^{0} C for 5 days (Andrews, 1992; Roberts and Greenwood, 2003). Creamy to white/gray colonies were counted as yeasts whereas, filamentous (fuzzy) colonies of various colors (yellow, green, light brown) were counted as molds (Yousef and Carlstrom, 2003). Plates with 10 - 150 colonies were used for determining yeast and mold counts (IDF, 2004).

3.4.2. Chemical composition of milk

Physico-chemical properties determined for the milk include fat content, total solid (TS), protein, and pH determined with calibrated milk analyzer (lactoscan).

Titratable acidity test: - Ten ml of milk sample was pipetted into a beaker and then 3-5 drops of 0.5 % phenolphthalein indicator was added into the milk samples. Then the milk samples were titrated with 0.1N NaOH solution until a definite pink color persisted (O'Connor 1994).

3.4.3. Milk shelf life test

Clot-On-Boiling Test: - Five ml of milk in a sterile test tube was placed in a boiling water bath for five minutes. Then, the test tube was carefully removed from the water bath and examined for the presence of curds for the freshness of milk. This was done in one hour of time intervals and the hours sample milk started to form curd was scored as a shelf life of sample raw cow's milk.

Alcohol Test:- Milk was sampled at the interval of an hour; five ml of milk and 5 ml of 68 percent ethanol were placed in a test tube. The test tube was inverted several times with the thumb held tightly over the open end of the tube. Then the tube was examined for the formation of curd particles for a freshness checkup of the milk (O'Connor, 1994). An hour's sample milk started to form curd and was scored as a shelf life of sample raw cow's milk.

Methylene Blue Reduction Test: The methylene blue test was performed according to the method described by C.B. O'Connor (1995). The methylene blue decolonization test is a good measure of its bacterial content and hence of its hygienic quality. 1 ml of methylene blue was added to 10 ml of milk in test tubes. The test tube was inverted 4-5 times tightening the mouth with stoppers, and then putting it in a water bath at $37C^{o.}$ The quality of the milk is determined by observing the color. The time taken by the dye to get reduced determines the number of microbes in the milk and the quality of the milk (Mayra *et al*., 2020).

$\leq$ 30 minutes = very poor quality milk

30 minutes -2 hours poor quality milk

2–6 hours = fair-quality milk

6-8 hours then it is good quality milk and finally if not reduced to 8 hours then it is considered excellent quality milk (Mayra.P.*et al*., 2020).

3.4.4. Organoleptic characteristics of milk

Organoleptic parameters of raw milk including color, flavor, taste, texture, and overall acceptability were evaluated by a testing group of panelists consisting of 10 persons. The panelists were selected from Injibara University animal science Department staff members taking into account their specializations. Before evaluating, the panelists were trained in the sensorial attributes related to milk using a 9-point Hedonic rating scale. Thirty beakers containing 100 ml sample raw cow's milk treated with propolis were prepared that were categorized into three levels of agro-ecologies, 5 levels of concentration extract via ethanol solution used. Each milk sample was coded and the sensory evaluation was conducted based on a 9-point Hedonic scale method (9= Like extremely; 8=Like very much; 7=Like moderately; 6= Like slightly; 5=neither like nor dislike; 4= Dislike slightly; 3=Dislike moderately 2= dislike very much; 1= dislike extremely) for each sensory attribute of milk (Das PC *et al*., 2018; Sarker A *et al*., 2012).

3.4. Method of Data Analysis

The raw data was summarized using Microsoft Excel and analyzed using Statistical Analysis Software (SAS) version 9.4. The data for aerobic bacteria, coliform, yeast, and mold count data were transformed into log10 in Excel before being subjected to statistical analysis. Both descriptive and exploratory analysis was performed using the General Linear Model (GLM) with least square mean using least significance difference(LSD) tests to see the effects of agro-ecologies and concentration level of propolis on the parameters of physiochemical, microbial test, sensory attribute, and organoleptic of raw cow's milk.

The following model was used to analyze the microbiological quality of milk:

$Yij = \mu + Ai + Bj + ABij + Eijk$

Where μ= is the overall mean,

Ai= agro-ecologies effect of propolis harvested

Bj= concentration of propolis added per 100ml of milk

ABij=interaction effect of i level agro-ecologies and j level propolis concentration

Eijk=random error

CHAPTER 4. RESULTS AND DISCUSSION

4.1. Effects of Honey Bee Propolis on Physicochemical Quality of Raw Cow's Milk

The physicochemical quality of the raw cow's milk from the Injibara University dairy cattle farm treated with different volumes of 70% ethanol-extracted honey bee propolis harvested from Banja, Dangla, and Guangua districts is presented in Tables 4.1 and 4.2 below.

4.1.1. pH and titratable acidity of raw cow's milk

The analysis of variance showed that the main effects of agro-ecology and propolis volumes did not have a significant ($P > 0.05$) effect on the pH and TA of raw cow's milk at the initial evaluated time and at interaction effects at all evaluated times. However, at 24 and 72 hours of examination, both agro-ecologies of propolis harvested and propolis concentrations had a significant($P > 0.05$) effect on the pH and TA of raw cow's milk, as indicated in Tables 4.1 and 4.2 below.

The main effects of ethanol-extracted honey bee propolis collected from Banja, Dangla, and Guangua Woredas at the initial time were 6.67, 6.71, and 6.62 pH values of raw cow's milk, respectively. This result meets the acceptable range between 6.3 and 6.8 of normal raw cow's milk. However, the high reduction in pH values of raw cow's milk was observed at 24 and 72 evaluated hours. Especially significantly lowest pH values of raw cow's milk were recorded in Guangua and Dangla woredas than in Banja Woredas. This variation of pH in raw cow's milk might be due to the difference in the chemical composition of propolis in different agroecologies and the nature of extracted honey bee propolis needs time to start antimicrobial activities. The current finding agrees with Hadi Koohsari *et al*., (2019), who concluded that the antimicrobial activity of the propolis extracts is more observed after 24, 48, and 72 hours, and no changes are observed at the initial times.

Titratable acidity (TA) of raw cow's milk treated with ethanol extracted honey bee propilis harvested from Banja was 0.15, 0.21, 0.31, Dangla 0.15, 0.28, 0.38, and Guangua 0.16, 0.29, and 0.40 at initial, 24 and 72 hours in each Woreda, respectively. At the initial time, the result showed that agroecologies of propolis harvested had no significant ($p > 0.05$) influence on the TA of raw cow's milk. However, a significant ($p<0.05$) effect was observed at 24 and 72 hours of evaluated time in all agro-ecologies of propolis harvested. A significantly higher increment in TA of raw cow's milk was recorded in milk treated with Guangua (low land) and Dangla (midland) harvested extracted honey bee propolis

than Banja Woreda(high land). The variation in the increment of TA in raw cow's milk might be due to the variation of the propolis sources, which plays a role in inhibiting the microbes in milk. This result agrees with Bankova *et al*., (2016), who reported that the chemical composition of propolis strictly depends on the plant sources and is a result of geographical location, climate conditions, and environmental factors.

The main effects of extracted honey bee propolis concentration on pH and TA of raw cow's milk, is given in Table 4.2 below. The result has clearly showed that propolis concentration had a significant ($p<0.05$) effect on pH and TA of raw cow's milk at initial, 24 and 72 evaluated hours in all treatment groups. From the initial time to 72 hours of the storage period, the pH levels of the raw cow's milk significantly declined from T1 (control) (6.73-4.2), T2 (6.69-4.63), T3(6.67-4.80), T4(6.68-5.66) and T5(6.57-5.90) after supplemented extracted honey bee propolis. However, the TA of raw cow's milk significantly increased from the initial time to 72 hours of the evaluation hours ranging from 0.17-0.43, 0.16-0.38, 0.156-0.37, 0.154-0.34 and 0.145-0.30 in T1, T2, T3, T4 and T5 treatment groups respectively. The result indicated that the lowest pH value reduction and the lowest increment on TA values of raw cow's milk treated with a higher amount of propolis extracted concentration than lower concentrations of extracted honey bee propolis. At initial evaluated hours the pH and TA of raw cow's milk meets the acceptable ranges of raw cow's milk from 6.3-6.8 pH and 0.14-0.17 TA of raw cow's milk (Caprita *et al*, 2014). However, at 24 and 72 evaluated hours, the pH of the control group had the lowest pH value and highest TA of raw cow’s milk compared to the treated groups.

The variation in pH and TA of raw cow's milk might be due to the impact of propolis which is supplemented to raw cow's milk. The current finding is in agreement with El-Deeb, (2017), who reported, that the pH of control milk decreased from 6.72 to 4.32. While treatment concentrations of water extract of propolis (WEP) at (5, 10, and 20%), the pH decreased from 6.79 to 4.53, 6.77 to 4.74, and 6.77 to 6.37 and also acidity of the sample raw cow's milk increased from 0.153 to 0.762%, 0.153 to 0.680%, 0.155 to 0.464% and 0.156 to 0.275% after 24h and 72h of supplemented water extracted honey bee propolis of 0, 5, 10 and 20% respectively. Similarly, Elkassas *et al*, (2023) who reported that the pH levels of the treated groups declined significantly, where EEP 5 % had the lowest pH in the treated groups and EEP 20% had the highest rate. Additionally, the result is in line with Sivakumar, (2017) who stated that, an increase in the titratable acidity and a decrease in the pH of the milk samples with added 0.5%, 0.75% and 1% levels of tulsi

leaves extract. However, the current finding does not agree with Chon *et al*, (2020), who reported that the pH of market milk, supplemented with different concentrations of 0%(6.68), 0.5%(6.68), 1.0%(6.63), 1.5%(6.52), and 2.0%(6.51) of water extracted honeybee propolis, has no significant difference between pH values of the treated and control groups at initial evaluated hours.

4.1.2. Protein

The analysis of variance showed that agro-ecologies of propolis harvested and propolis concentration had a significant ($P < 0.05$) effect on the protein of raw cow's milk. Similarly, the interaction effect (Agro ecologies*propolis concentration) had a highly significant ($P < 0.01$) influence on the protein of raw cow's milk. In the main effect, the significantly the highest protein values were recorded in raw cow's milk treated with Guangua woredas(3.71) than in Dangla(3.59) and Banja woredas(3.49) harvested extracted honey bee propolis. On the other hand, the highest protein values were recorded in a high amount of propolis concentration-supplemented treatment groups of raw cow's milk.

In the interaction effects, the result indicated that the highest protein values were recorded from GT5 (4.15). While, the lowest protein values (3.15) were recorded from control groups of both in BT1, DT1, and GT1 treatment combination of raw cow's milk as showed (Table 4.3). This might be due to environmental factors of propolis harvested interacting with a dose of propolis concentration supplemented with raw cow's milk. As it was recorded, significantly the highest protein values were recorded in Guangua woredas .While significantly the lowest values were recorded in Dangla and Banja woreda extracted honey bee propolis concentration treated raw cow's milk (Table 4.3). The control (T1) group of raw cow's milk had lower protein values than propolis concentration-supplemented groups. This high protein values in propolis supplemented raw cow’s milk might be due to the high protein values of supplemented extracted honey bee propolis. Some research results on the physiochemical quality of raw propolis, the result showed that propolis had high protein values. According to Afata *et al.*, (2022), who reported that propolis collected from Boji Dirmaji and Fincha'a districts of western Ethiopia, minimum and maximum protein values of propolis produced from Fincha'a were 4.1, 5.5, and 4.2, 5.6 in Boji Dirmaji district in (g/100 g) protein values of propolis in each study districts respectively.

4.1.3. Ash

The analysis of variance revealed that agro-ecologies of propolis harvested and propolis concentration as well as, the interaction effect of agro-ecologies of propolis harvested and honey bee propolis concentration had a significant ($P < 0.05$) effect on ash contents of raw cow's milk as indicated in Table (4.1, 4.2 and 4.3) below respectively. A significantly higher ash content of 0.68% was observed in raw milk samples treated by propolis from Dangla woredas and a lower ash content of 0.61%in Banja and 0.64 % in Guangua woredas was recorded. The current finding meets within the Ethiopia standard range of ash content 0.6-0.8 percent (ES, 2009). And also normal ash contents of cows raw milk approximately ranges from 0.7% to 0.9% (Walstra *et al*., 2015). The effects of extracted honey bee propolis concentration on ash contents of raw cow's milk, the result revealed that the highest ash values were registered in milk treated with high amounts of propolis than those treated with lower amounts of propolis and control groups. This might be due to propolis had high ash content.

In the interaction effects of agro-ecologies of propolis harvested and propolis concentration, the ash contents of raw cow's milk were significantly higher at DT5 and GT5 (0.77) than control and other treatment groups of raw cow's milk supplemented extracted honey bee propolis concentration. This might be due to the agro-ecologies difference in propolis harvested and amounts added to raw cow's milk influence the ash contents of milk besides too high ash values of propolis. according to (Afata *et al.*, 2022) who reported that propolis collected from Boji Dirmaji and Fincha'a districts of western Ethiopia, minimum and maximum ash values of propolis produced from Fincha'a were 2.74, 2.84, and 9.6, 9.71 in Boji Dirmaji district in (g/100 g) ash values of propolis in each study districts respectively.

4.1.4. Fat

The analysis of variance showed that agro-ecologies of propolis harvested and extracted honey bee propolis concentration had a significant effect ($P < 0.05$) on the fat contents of raw cow's milk. However, the interaction effect (agro ecologies of propolis harvested*propolis concentration) did not significantly ($P > 0.05$) influence the fat contents of raw cow's milk. In the main effect of propolis by agro-ecologies harvested, significantly higher values of fat were recorded in raw cow's milk treated with extracted honey bee propolis harvested from Banja woredas. However, significantly lower fat content was

recorded in raw cow's milk treated with Dangla and Guangu woreda harvested extracted honey bee propolis. The variation in fat contents of raw cow's milk might be due to supplemented propolis to raw cow's milk from different environments may have different fat contents/ composition.

The effects of extracted honey bee propolis concentration on fat contents of raw cow's milk have showed a significant difference in fat contents of raw cow's milk. As indicated in Table (4.2) below, higher fat contents of raw cow's milk were recorded (3.55) in T5 treatment levels of supplemented extracted honey bee propolis. However, control (T1) groups had significantly lower fat values than raw cow's milk treated with extracted honey bee propolis concentration. As shown in the result of the fat contents of raw cow's milk, the high-fat content values were recorded in milk treated with high treatment groups of extracted honey bee propolis concentration than lower concentration. The result might be due to the high-fat contents of propolis supplemented with raw cow's milk. The current finding of milk fat content of raw cow's milk meets the standard values of worlds raw cow milk contains 3-4%, although values high 5.5% have been reported in raw cow's milk (FAO, 2013).

In control raw cow's milk, the fat contents of raw cow's milk was 3.06%. This finding is lower than 4.14% of crossbred cows' milk (Asamnew Tassew, 2007) and 3.4% Derese Teressa, (2008) in the west shoa zone of the Oromia region.

4.1.5. Solid non-fat (SNF)

The main effects of Agro-ecologies of propolis harvested and extracted honey bee propolis concentration had a significant ($P < 0.05$) effect on solid non-fat contents of raw cow's milk. However, the interaction effect of the factors did not significantly influence the solid non-fat contents of raw cow's milk.

As indicated in table (4.1) below, the main effects of agro-ecologies of propolis harvested on solid non-fat contents of raw cow's milk, significantly higher solid non-fat contents of raw cow's milk was recorded in milk treated with Guangu(8.77%) and Dangla (8.73%) Woredas harvested and extracted honey bee propolis concentration. However, significantly the lower solid non-fat value in raw cow's milk was registered in milks treated with Banja Woredas (8.64) harvested and extracted honey bee propolis concentration. The variation in solid non-fat contents of raw cow's milk which supplemented extracted propolis from different source might be due to the difference in propolis chemical composition.

The effects of propolis concentration on raw cow's milk solid non-fat contents as indicated in Table (4.2) below, the result revealed that significantly higher solid nonfat contents were recorded in raw cow's milk treated with T5 treatment levels of extracted honey bee propolis concentration than control and other treatment groups treated with lower amounts of extracted honey beee propolis concentration. This might be due to extracted honey bee propolis concentration improve the solid nonfat contents of raw cow's milk. The finding meets the standard of the east African raw milk chemical compositions of solid nonfat contents of raw cow's milk not less than 8.50% (EAC, 2006).

In control raw cow's milk the solid nonfat contents of raw cow's milk was 8.40%. This finding is lower than than Asamnew Tassew, (2007), who reported that the SNF contents of crossbred cows' milk were 8.96%. and Bekele Aysheshim *et al* (2015) who reported 9.49% for the urban and peri-urban area of Dangila in the western Amhara Region and Gurmessa Terfa *et al*.,(2015) who reported that 9.47 ±0.17% in case of Borena zone Yabello district. Additionally, similar with the finding of DereseTeressa, (2008) who reported that 8.4% SNF in the west shoa zone of the Oromia region.

Table 4.1. Main effects of extracted honeybee propolis on physiochemical parameters of raw cow's milk by agro ecologies

	Parameters									
Agro ecologies	pH	PH_{24hr}	PH_{72hr}	Protein	Ash	Fat	SNF	TA	$TA24_{hr}$	$TA72_{hr}$
Banja	6.67^{a}	5.61a	5.41^{a}	3.49^{b}	0.61^{b}	3.34^{a}	8.64^{b}	0.15^{a}	0.21^{b}	0.31^{b}
Dangla	6.71^{a}	5.57^{ab}	4.99^{b}	3.59^{b}	0.68^{a}	3.22^{b}	8.73^{a}	0.15^{a}	0.28^{a}	0.38^{a}
Guangua	6.62^{a}	5.4^{b}	4.81^{b}	3.71^{a}	0.64^{b}	3.23^{b}	8.77^{a}	0.16^{a}	0.29^{a}	0.40^{a}
P-values	ns	*	*	*	*	*	*	*ns*	*	*
LSD(0.05)	0.11	0.15	0.35	0.11	0.03	0.09	0.08	0.008	0.001	0.05

Table 4. 2. Main effects of honey bee propolis concentration on physiochemical parameters of raw cow's milk by treatment

	Parameters									
Concentration	pH	PH_{24hr}	PH_{72hr}	Protein	Ash	Fat	SNF	TA	$TA24_{hr}$	$TA72_{hr}$
T1	6.73^{a}	4.8^{c}	4.2^{c}	3.15^{e}	0.57^{d}	3.06^{d}	8.40^{d}	0.17^{a}	0.29^{a}	0.43^{a}
T2	6.69^{ab}	5.05^{b}	4.63^{b}	3.38^{d}	0.62^{cd}	3.11^{cd}	8.58^{c}	0.16^{ab}	0.27^{a}	0.38^{ab}
T3	6.67^{ab}	5.18^{b}	4.80^{b}	3.61^{c}	0.65^{cd}	3.20^{c}	8.78^{b}	0.156^{b}	0.26^{a}	0.37^{ab}
T4	6.68^{ab}	6.13^{a}	5.66^{a}	3.83^{b}	0.68^{ab}	3.38^{b}	8.86^{ab}	0.154^{cd}	0.23^{b}	0.34^{bc}
T5	6.57^{b}	6.25^{a}	5.90^{a}	4^{a}	0.70^{a}	3.55^{a}	8.93^{a}	0.145^{c}	0.22^{c}	0.30^{c}
P values	*	*	*	**	*	*	*	*	*	*
LSD(0.05)	0.15	0.2	0.4	0.15	0.04	0.12	0.11	0.0099	0.05	0.05

Where; * = Significant ($P < 0.05$); ns= non-significant; T1= control sample raw milk, T2= sample raw cow's milk supplemented 5 milliliter propolis; T3=sample raw cow's milk supplemented 10 milliliter propolis; T4=sample raw cow's milk supplemented 15 milliliter propolis; T5=sample raw cow's milk supplemented 20 milliliter propolis; and; means followed by the different letter (s) within a column are significantly different.

Table 4.3.Interaction effect of agro-ecologies of propolis harvested and propolis concentration on chemical composition of milk

Ag*co	Protein	Ash	Fat	SNF	TA_0	TA_{24}	TA_{72}	pH_0	pH_{24}	pH_{72}
BT1	3.15^{f}	0.56^{ef}	3.6^{a}	8.40^{h}	0.165^{abc}	0.25^{abcde}	0.43^{ab}	6.74^{a}	4.9^{cd}	4.32^{d}
BT2	3.35^{def}	0.62^{de}	3.5^{ab}	8.50^{h}	0.16^{abcd}	0.19^{ed}	0.29^{cd}	6.69^{a}	5.31^{b}	4.87^{bcd}
BT3	3.45^{de}	0.65^{cd}	3.25^{cdef}	8.70^{def}	0.157^{abcd}	0.18^{e}	0.28^{d}	6.63^{a}	5.30^{b}	5.66^{ab}
BT4	3.6^{cd}	0.73^{ab}	3.20^{defg}	8.75^{cdef}	0.15^{cd}	0.21^{bcde}	0.27^{d}	6.67^{a}	6.22^{a}	6.10^{a}
BT5	3.9^{ab}	0.50^{f}	3.15^{defg}	8.85^{abcd}	0.145^{d}	0.20^{cde}	0.27^{d}	6.65^{a}	6.35^{a}	6.13^{a}
DT1	3.15^{f}	0.61^{de}	3.45^{abc}	8.40^{h}	0.17^{ab}	0.25^{abcde}	0.43^{ab}	6.73^{a}	5.23^{bc}	4.33^{d}
DT2	3.30^{ef}	0.67^{bcd}	3.30^{bcde}	8.60^{fg}	0.165^{abc}	0.30^{abc}	0.40^{ab}	6.72^{a}	5.07^{bcd}	4.50^{cd}
DT3	3.60^{cd}	0.67^{bcd}	3.20^{defg}	8.80^{bcde}	0.155^{bcd}	0.33^{a}	0.39^{ab}	6.66^{a}	5.15^{bcd}	4.67^{cd}
DT4	3.95^{ab}	0.70^{abc}	3.10^{efg}	8.90^{abc}	0.153^{bcd}	0.31^{ab}	0.38^{abcd}	6.71^{a}	6.07a	5.60^{ab}
DT5	3.95^{ab}	0.77^{a}	3.05^{fg}	8.95^{ab}	0.145^{d}	0.27^{abcde}	0.32^{bcd}	6.79^{a}	6.35^{a}	5.85^{a}
GT1	3.15^{f}	0.56^{ef}	3.60^{a}	8.40^{h}	0.175^{a}	0.25^{abcde}	0.43^{ab}	6.74^{a}	4.97^{bcd}	4.38^{d}
GT2	3.50^{de}	0.56^{ef}	3.35^{bcd}	8.65^{efg}	0.165^{abc}	0.29^{abcd}	0.42^{ab}	6.67^{a}	4.83^{d}	4.26^{d}
GT3	3.80^{bc}	0.63^{cde}	3.15^{defg}	8.85^{abcd}	0.157^{abcd}	0.35^{a}	0.38^{abcd}	6.74^{a}	5.12^{bcd}	4.36^{d}
GT4	3.95^{ab}	0.66^{bcd}	3.05^{fg}	8.95^{ab}	0.160^{abcd}	0.29^{abcd}	0.38^{abcd}	6.67^{a}	6.13^{a}	5.30^{abc}
GT5	4.15^{a}	0.77^{a}	3.0^{g}	9.0^{a}	0.145^{d}	0.25^{abcde}	0.31^{cd}	6.30^{b}	6.10^{a}	5.75^{a}
P- value	0.0544	<.0001	0.8536	0.9393	0.9531	0.3310	0.2848	0.1836	0.2805	0.3410

Where; T1= control sample raw milk, T2= sample raw cow's milk supplemented 5-milliliter propolis; T3=sample raw cow's milk supplemented 10-milliliter propolis; T4=sample raw cow's milk supplemented 15-milliliter propolis; T5=sample raw cow's milk supplemented 20-milliliter propolis; and; means followed by the different letter (s) within a column are significantly different.

4.2. Effects of Honey Bee Propolis on sensory evaluation of raw cow's milk

The panelists' preference results of organoleptic characteristics of raw cow's milk which supplemented different amounts of 70 % ethanol extracted honey bee propolis collected from different agroecologies were presented in table (4.4) main and (4.5) interaction effects below. The analysis of variance showed that agroecologies of propolis harvested and propolis concentration had very highly significant ($p<0.001$) main effects and **very** highly significant ($P < 0.001$**)** interaction effects on color and flavor sensory characteristics of raw cow's milk as indicated in table (4.4) below. However, the interaction effects of agro-ecologies of propolis harvested and propolis concentration had non-significant ($p>0.05$) effects on the texture and overall acceptability of sensory characteristics of raw cow's milk (appendix table 4).

4.2.1. Colour

The interaction effect of agro-ecologies of propolis harvested and propolis concentration had a highly significant ($p<0.05$) effect on the color of raw cow's milk. As shown in the interaction (Table 4.5) below, the panelists' preference for the color of raw cow's milk, values ranged from like extremely to neither like nor dislike (9 to 5.4) 9 points hedonic rating scale. The highest panelists' preference was recorded in BT1, DT1, and GTI with like extremely criteria. However, the lowest panelists' preferences were recorded in DT5 (5.4), GT5 (6) and BT5 (6.7). The panelists' preference result showed that the higher propolis concentration supplemented with raw cow's milk, gradually decreased the panelists' preference score on the color of raw cow's milk because the resulting color is less attractive. Especially, in DT5 the panelists' had neither like nor dislike preference.

The result might be due to botanical source propolis affecting the color of the milk and supplemented amounts of propolis used to treat raw cow's milk. The result determined in this study agreed with the results reported by Walaa Mohamed et *al*. (2023) the concentrations of WEP increased, and the sensory score of the produced yogurt groups decreased. The result is also in line with El-Deeb, (2017), who reported that propolis use in milk and yogurt at higher concentrations (3%) had a negative impact on acceptance and the highest sensory scores were obtained at concentrations of 1 and 2% WEP (20% extract). Similarly, Jansen-Alves *et al*. (2019) who reported that Propolis inclusion in food formulas may cause a change in color and, more importantly, a disagreeable odor.

4.2.2. Flavour

In the present study, the main effects of agroecologies of propolis harvested and propolis concentration, as well as the interaction effects, had very highly significant (*p<0.001*) effects on the flavor of raw cow's milk. The level of preference for the panelists towards the flavor of raw cow's milk, values ranged from 8-7.12(like very much to like moderately) in the main effects of propolis concentration by agro-ecologies of propolis harvested. The main effects of propolis concentration ranged from 9-6.02 (like extremely to like slightly). The result showed that the panelists' preference score on the flavor of raw cow's milk was higher in control (T1) than supplemented with different concentrations of T2, T3, T4, and T5 treatment levels of ethanol-extracted honey bee propolis. Furthermore, as the supplemented extracted honey bee propolis increased, the panelist preference regarding to flavor of raw cow's milk decreased gradually as indicated in table (4.4) below.

The interaction effect of propolis by agroecologies of propolis harvested and propolis concentration as shown in Table (4.5) below, the panelist preference score was higher at GT1(9), DT1(9), and BT1(8.9). However, the lowest score was registered at DT5 (5.4), GT5 (6), and BT5 (6.7) meaning that the control group in all agro-ecologies got the highest score; on the other hand, as the concentrations of propolis extracted increased, the sensory score of the raw cow's milk preferred by panelists decreased. The result might be due to the high concentration of extracted honey bee propolis added to raw cow's milk changing the flavor of the milk. This reduces the acceptance values of panelist preference for the flavor of raw cow's milk. Especially, neither like nor dislike penalise preference was observed in the treatment combination of DT5 than others. This result also might be due to the role of propolis on the flavor of the milk varied in propolis harvested from different areas.

The current finding is similar to that Chon, *et al*., (2020), who reported that The flavor value of market milk supplemented with different concentrations (0%, 0.5%, 1.0%, 1.5%, and 2.0%) of honey bee propolis was ranges from 3.18 to 2.45, which was lower than 4.0 of the control group. The result is also in line with Kim *et al*., (2008), who reported that the supplement of propolis increased, preference decreased due to the propolis-specific flavor. The result is similar to previous research findings Chon *et al*., (2020), who reported the tendency for preference for milk to decrease as the amount of extracted honey bee propolis supplement increased. The finding is also in line with El-Deeb, (2017), who found that propolis use in milk at higher

concentrations had a negative impact on acceptance and the highest sensory scores were obtained at low concentrations of water-extracted honey bee propolis. Similarly, Jansen-Alves *et al*. (2019), who reported that Propolis had a distinct and pungent odor, as well as its inclusion in food formulas, may cause a change in color and, more importantly, a disagreeable odor.

4.2.3. Texture

The analysis of variance showed that agro ecologies of propolis harvested and treatment levels of extracted honey bee propolis had a very highly significant effect ($P < 0.001$) on the texture of raw cow's milk. However, the interaction effect (agro ecologies*propolis concentration) did not significantly ($P > 0.05$) influence on the texture of raw cow's milk.

The main effects of Agro ecologies of propolis harvested on the texture of raw cow's milk the panelist preference hedonic rating scale values ranged from 8.1 to 7.12 (like very much to like moderately) assessment criteria. The result indicated that the highest hedonic rating scale values (like very much) were recorded in raw cow's milk treated with propolis harvested with Banja (high land) woredas. However, the lowest values were recorded at Dangla(7.34) and Guangua(7.12) woredas with the assessment criteria being like moderately by panelist preferences. The result showed that milk treated with high land collected water-extracted propolis was more accepted than midland and low-land collected water-extracted honey bee propolis on the texture of raw cow's milk. This might be due to the function of propolis varied at different conditions of the environment in propolis collection season. The current finding is similar with (Bueno-Silva *et al*., 2017), who reported that plant sources surrounding beehives, honeybee species, method of collection, geographical and climatic variation, collecting seasons, altitudes and adequate lighting have influences on propolis functions and texture.

The main effects of treatment levels of extracted honey bee propolis concentration as indicated in (Table 4.4) below, the panelist preference hedonic rating scale values ranged from 9 to 5.93(like extremely to neither like nor dislike) assessment criteria. The control (T1) raw cow's milk got the highest hedonic rating scores values of 9 than T2 (8.03), T3 (7.33), T4 (6.76) and T5 (5.93). The panelist's preference for the texture of the raw cow's milk showed acceptable ranges in the treatment levels of control (T1), T2 and T3. However, the panelist's preference in T4 and T5 treatment levels of extracted honey bee propolis concentration had a neutral assessment hedonic rating scale values. This suggests that the amount of extracted honey bee propolis which

is supplemented in raw cow's milk not more than 10 %(T3), the raw cow's milk has good sensory quality on color, flavor, texture, and overall acceptability of raw cow's milk.

The result indicated that the concentrations of extracted honey bee propolis increased, and the panelist preference for hedonic rating score values of T2, T3, T4, and T5 propolis-treated raw cow's milk was decreased. The result in varied panelist preferences on the texture of raw cow's milk could be the high amount of propolis concentration. The current result agreed with Elkassas *et al*. (2023), who reported that concentrations of extracted propolis increased, the sensory score of the milk texture decreased. Similarly, the concentrations of extracted propolis increased the texture sensory score of the produced yoghurt groups decreased. Lower propolis concentration treatment levels than our findings were reported by El-Deeb, (2017), propolis (1% and 2% in ethanolic extract) mixed with raw milk produced better characteristics in terms of aroma, body, texture, flavor, and general acceptability.

4.2.4. Overall acceptability

The analysis of variance showed that the main effects of propolis concentration had very highly significant *($P < 0.001$)* effects on the acceptability of raw cow's milk. However, agro-ecologies of propolis harvested and interaction effect (agro ecologies*propolis concentration) did not have a significant ($P > 0.05$) influence on the acceptability of raw cow's milk.

The panelist preferences on the acceptability of raw cow's milk treated with different concentrations of honey bee propolis, hedonic rating scale values ranged from 9-5.53(like extremely to neither like nor dislike) assessment criteria. The sample raw cow's milk containing T1(control) ethanol extracted honey bee propolis recorded the highest values for overall sensory attributes as compared to other treatments followed by T2 8.33(like very much), T3 7.40(like moderately), T4 6.73(like slightly) and T5 5.53(neither like nor dislike). The current result indicated that the higher the amount of the extracted honey bee propolis supplemented the lower (low hedonic rating values) preference by panelists for overall acceptability of raw cow's milk. The current finding suggests that the amount of extracted honey bee propolis up to T3 (10%) treatment level had highly prefered. However, at treatment levels of T4 and T5 propolis concentration the panelist's preference had a lower preference. These results were similar to Walaa Mohamed et *al*. (2023) the concentrations of WEP increased, and the sensory score of the produced yoghurt groups decreased. The finding also matches with Chon *et a*l. (2020), who

reported that the supplemented of propolis increased, the category of overall acceptability tended to decrease.

A B

Figure 4.2. Titrable acidity of milk (A) and organoleptic test of milk (B)

Table 4.4. Main effects of agro-ecologies of propolis harvested and propolis concentration on sensory parameters of raw cow's milk

Factor	**parameters**			
Agro ecologies	Colour	Flavour	Texture	Overall acceptability
Banja	7.40^{a}	8^{a}	8.1^{a}	7.52^{a}
Dangla	7.24^{ab}	7.24^{ab}	7.34^{b}	7.38^{a}
Guangua	7.12^{b}	7.12^{b}	7.12^{b}	7.30^{a}
P-values	*	*	*	*ns*
LSD(0.05)	0.21	0.21	0.2564	0.2596
Concentration				
T1	9.0^{a}	9.0^{a}	9.0^{a}	9.0^{a}
T2	7.47^{b}	7.46^{b}	8.03^{b}	8.33^{b}
T3	7.23^{b}	7.22^{b}	7.33^{c}	7.40^{c}
T4	6.53^{c}	6.53^{c}	6.76^{d}	6.73^{d}
T5	6.03^{d}	6.02^{d}	5.93^{e}	5.53^{e}
P-values	***	***	***	***
LSD(0.05)	0.273	0.272	0.3311	0.3351

*Where; * = Significant (P < 0.05); ** = highly significant (P < 0.01); *** = very highly significance (P <0.001); T1= control sample raw milk, T2= sample raw cow's milk supplemented 5 milileter propolis; T3=sample raw cow's milk supplemented 10 milileter propolis; T4=sample raw cow's milk supplemented 15 milileter propolis; T5=sample raw cow's milk supplemented 20 milileter propolis; and; means followed by the different letter (s) within a column are significantly different.*

Table 4.5. Interaction effects of agro ecologies of propolis harvested and propolis concentration on sensory parameters of raw cow's milk

Ag*con	**parameters**			
	Color	Flavor	Texture	overallacceptablity
BT1	9^{a}	8.9^{a}	9^{a}	9^{a}
BT2	7.40^{c}	7.41^{c}	8.2^{b}	8.6^{a}
BT3	7.1^{cd}	7.1^{cd}	7.3^{cd}	7.3^{bcd}
BT4	6.8^{de}	6.8^{de}	7.2^{cd}	7^{cde}
BT5	6.7^{def}	6.7^{def}	6.3^{efg}	5.7^{f}
DT1	9^{a}	9^{a}	9^{a}	9^{a}
DT2	8.2^{b}	8.2^{b}	8.2^{b}	7.8^{b}
DT3	7.3^{c}	7.3^{c}	7.4^{c}	7.6^{bc}
DT4	6.3^{fg}	6.3^{fg}	6.4^{ef}	6.5^{e}
DT5	5.4^{h}	5.4^{h}	5.7^{g}	5.6^{f}
GT1	9^{a}	9^{a}	9^{a}	9^{a}
GT2	6.8^{de}	6.8^{de}	7.7^{bc}	8.6^{a}
GT3	7.3^{c}	7.3^{c}	7.3^{cd}	7.3^{bcd}
GT4	6.5^{ef}	6.5^{ef}	6.7^{ed}	6.7^{de}
GT5	6^{g}	6^{g}	5.8^{fg}	5.3^{f}
P-values	*<.0001*	*<.0001*	*0.2423*	*0.1110*

Where; T1= control sample raw milk, T2= sample raw cow's milk supplemented 5 milileter propolis; T3=sample raw cow's milk supplemented 10 milileter propolis; T4=sample raw cow's milk supplemented 15 milileter propolis; T5=sample raw cow's milk supplemented 20 milileter propolis; and; means followed by the different letter (s) within a column are significantly different.

4.3. Effects of Honey Bee Propolis on Milk Quality Test

4.3.1. Effects of honey bee propolis on alcohol and clot on boiling test

The main and interaction effects of agro ecologies and propolis concentration on clot on boiling, alcohol, and methylene blue reduction test of raw cow's milk is showed in Table 4.6 and 4.7 respectively. The main effects of agro ecologies and propolis concentration had a highly significant ($P < 0.001$) effect on the main and significant ($P < 0.05$) interaction effect on clot on boiling, alcohol and methylene blue reduction tests of raw cow's milk.

The main effect of agro-ecologies was highly significant (*p <.0001*) on clot on boiling and alcohol test of raw cow's milk. As shown in Table 4.5 mean comparison tests indicated that significantly highest negative result of 12hr both on clot on boiling and alcohol test of raw cow's milk was registered in Guangua/low land woreda/ treated with T5 treatment leveles. However significantly a short negative result was recorded (2hrs) in control treatment (T1) of all agro ecologies of study district both on clot on boiling and alcohol test of raw cow's milk. This variance might be related to the influence of propolis chemical composition due to botanical sources of agroecology in which the propolis is harvested. This line with the finding of (Bankova *et al*., 2016), who reported that chemical composition of propolis strictly depends on the plant sources and is a result of geographical location, climate conditions, and environmental factors.

The highest time recorded in low land (Guangua) woreda allows more time for the milk to stay fresh for different purposes especially for rural areas which have not enough infrastructure to keep the milk quality. The result agrees with FAO, (2005; Tewodros Bimerow *et al*.,(2016), who reported that, raw cow's milk preservation with lactoperoxidase system allows adequate time for the milk to be transported from the collection point to a processing center without refrigeration. The result also agreed that geographic location from which propolis is collected affected its antibacterial potential that contaminated raw milk (Przybyłek and Karpiński., 2019).

The main effect of propolis concentration was statistically highly significant (*p <.0001*) on clot on boiling and alcohol test of raw cow's milk as shown in table 4.5 below. The results indicated below Table (4.6) showed that gradually increasing coagulation time (hours) on alcohol and clot on boiling tests which supplemented high doses of concentrations. The highest coagulation times (hours) on clot on boiling and alcohol test of milks treated with extracted honey bee propolis than untreated raw cow's milk might be due to anti-bactericidal effects of extracted honey bee propolis. The result of the present study was consistent with El-Deeb (2017), who reported that supplementing of 2% ethanol extracted honey bee propolis (20% extract) to raw milk resulted in freshness of the milk up to 12 and 48 hours at 30 and5±1 º C, respectively. This finding is also in line with Elsherif,et *al*., (2012), who reported that clot on boiling test for raw milk indicated that samples expired at third day may be due to the increased acidity with the addition of honey.

In raw cow's milk treated with T5 ethanol extracted honey bee propolis in a clot on boiling and alcohol test, the coagulation time was 12 hours. This indicates that more 10 hours kept the milk fresh in additional to control (T1) 2 hours of raw cow's milk. This allows adequate time for the milk producer to be transported from the collection point to a processing center without

refrigeration. This more additional time on raw cow's milk treated with extracted honey bee propolis than control raw cow's milk (T1) coagulation time might be due to the nature of ethanol extraction to extract bio active chemicals used in antimicrobial role in raw cow's milk. The justification agreed with Mello, (2010), who reported that ethanol extract honey bee propolis was the richest extract in bioactive components which inhibit microbial properties in supplemented food.

In the interaction effect, which is shown in below Table (4.7), the coagulation times (hours) taken on clot on boiling and alcohol test was increased with increasing the extracted honey bee propolis concentrations in three study Woredas. Moreover, significantly the highest coagulation time was recoreded in Guangua woredas treated with T5 treatment level. However, the current values of coagulation hours in clot on boiling and alcohol test was lower than the finding that conducted in Andassa Livestock Research Center On-Farm Evaluation of raw cow's milk by lactoperoxidase System, the raw cow's milk shelf life of increase up to 24 hours under ambient conditions can permit transportation of milk from far-off zones to the /market places without fear of spoilage (Tewodros Bimerow *et al*., 2016).

A significant coagulation time /hour variation in a clot on boiling and alcohol test of raw cow's milk might be due to the variation in extracted honey bee propolis concentration which supplemented in raw cow's milk and different in agro-ecologies from which propolis harvested. These additional coagulation time hours allow for the raw cow's milk to stay fresh for different purposes, especially for rural areas which have not enough infrastructure to keep the milk quality. The result agrees with FAO, (2005); Tewodros Bimerow *et al*.,(2016), who reported that raw cow's milk preservation with lactoperoxidase system allows adequate time for the milk to be transported from the collection point to a processing center without refrigeration. The result also agrees with El-Deeb (2017), who reported that supplementing of 2% water-extracted honey bee propolis (20% extract) to raw milk resulted in freshness of the milk up to 12 and 48 hours at 30 and5±1 º C, respectively. The current result also agreed with, geographic location from which propolis is collected affects its antibacterial potential (Przybyłek and Karpiński, 2019).

4.3.2. Effects of honey bee propolis on Milk methylene blue reduction test

The methylene blue reduction test was very highly significantly *(P < 0.001)* influenced by the main effects of agroecologies of propolis harvested and extracts propolis concentrations. However, the interaction effect had significantly influenced (*P < 0.05*) on the methylene blue reduction test of raw cow's milk as indicated in the below Table (4.6).

Methylene blue reduction tests of control (T1) raw cow's milk; were positive results at 2 hours. This short time might be the milk contamination through internal and external factors and the result revealed that the control raw cow's milk (T1) is considered as poor quality milk. The result meets with (Mayra.P.*et al*., 2020) who reported that if reduction occurs between 30 minutes to 2 hours, it is considered as poor quality milk. The result was higher than the finding reported by Estifanos Hawaz *et al.*, (2015), who reported that shorter decolorization times (1:52, 1:46 h) of raw cow's milk were recorded for Haramaya and Kersa districts of eastern Ethiopia respectively. While the result was lower than, longer decolorization times (2:44, 2:35h) were recorded in Babile and Kulubi districts of eastern Ethiopia respectively (Estifanos Hawaz *et al*, 2015).

The mean value of methylene blue reduction test color decolorization time result showed that increment times taken in hours were proportional to the addition of honey bee propolis extracted. This might be anti-microbial properties of supplementing extracted honey bee propolis to raw cow's milk improve the quality of raw milk by reducing the microbial load existed in raw cow's milk. The result showed that similar result with Bongard *et al*., (1995) and Marker *et al*., (1997) reported that blue color disappearance in a short time indicates higher microbial load and blue color disappearance in a longer time indicates low microbial load in the milk sample. In this study, T1 (control) milk shows a very short decolorization time of the dye. This might be due to poor milk handling practices during milking, poor animal health services, and the use of poor potable water. However, More color disappearance times were taken in sample raw cow's milk in low land than mid and high land this might be due to variation of bio active chemicals existed in extracted honey bee propolis harvested from difference geographical source against microbial properties. The result agrees with the antimicrobial impact of propolis depends on its source, chemical composition; extract concentration and extraction methods (Stepanovic *et al*., 2003).

In the interaction effect, the highest methylene blue color disappearance time/hour was registered at GT5 (11.50 hour), DT5 (10.50 hour) and BT5 (9 hour) and the lowest time was recorded at GT1 (2 hours), DT1 (2 hour) and BT1 (2 hour) on evaluated methylene blue reduction test of raw cow's milk. The results from this study showed that the addition of extracted honey bee propolis improve methylene blue color disappearance hours of raw cow's milk directly proportional to the dose of the supplemented extracted honey bee propolis. Similarly, milk treated with Guangua (low land) woreda harvested honey bee propolis had the

highest color disappearance time than Dangla(mid land) woreda and Banja(high land) woreda harvested honey bee propolis.

This might be due to low microbial load that existed in law land propolis treated milk than mid and high land and also chemical composition difference of the extracted honey bee propolis which harvested from Guangua, Dangla and Banja woredas. These results is in close agreement with the findings of Stepanovic *et al*.,(2003), who reported that the antimicrobial impact of propolis depends on its source, chemical composition; extract concentration and extraction methods. The result also comparable with Bongard *et al*., (1995) and Marker *et al*., (1997) reported that blue color disappearance in a short time indicates higher microbial load and blue color disappearance in a longer time indicates low microbial load in the milk sample.

Table 4.6. Main effects of agro-ecologies of propolis harvested and propolis concentration on shelf life of raw cow's milk (hours/hrs)

	Parameters		
Agro ecologies	C.O.B TEST	Alcohol test	MBRT
Banja	6^{c}	7^{c}	6.50^{c}
Dangla	7^{b}	7.30^{b}	7.40^{b}
Guangua	8^{a}	8^{a}	8^{a}
P-values	***	***	***
Concentration			
T1	2^{e}	2^{e}	2^{e}
T2	5^{d}	6.30^{d}	5.30^{d}
T3	7.20^{c}	8.30^{c}	7.50^{c}
T4	9.30^{b}	9.10^{b}	9^{b}
T5	10.50^{a}	11^{a}	10.30^{a}
P-values	***	***	***

Where; * = Significant ($P < 0.05$); ** = highly significant ($P < 0.01$); *** = very highly significance ($P < 0.001$); T1= control sample raw milk, T2= sample raw cow's milk supplemented 5 milileter propolis; T3=sample raw cow's milk supplemented 10 milileter propolis; T4=sample raw cow's milk supplemented 15 milileter propolis; T5=sample raw cow's milk supplemented 20 milileter propolis; and; means followed by the different letter (s) within a column are significantly different.

Table 4.7. Interaction effects of agro ecologies of propolis harvested and propolis concentration on shelf life of raw cow's milk (hours/hrs).

Ag*con	Parameters		
	Clot on boiling test	Alcohol test	Methylene blue taste
BT1	2^{g}	2^{j}	2^{i}
BT2	4^{f}	5.50^{i}	4^{h}
BT3	6.50^{d}	7.50^{fgh}	7.50^{ef}
BT4	8^{c}	8^{efg}	7.50^{ef}
BT5	9.50^{b}	9.50^{bcd}	9^{cd}
DT1	2^{g}	2^{j}	2^{i}
DT2	5^{ef}	6.50^{hi}	5.50^{g}
DT3	7^{cd}	8.50^{def}	7.50^{ef}
DT4	9.50^{b}	9.50^{bcd}	8.50^{de}
DT5	10^{b}	10.50^{b}	10.50ab
GT1	2^{g}	2^{j}	2i
GT2	6^{de}	7^{gh}	6.50^{fg}
GT3	8^{c}	9^{cde}	8.50^{de}
GT4	10.50b	10^{bc}	10^{bc}
GT5	12^{a}	12^{a}	11.50^{a}
P-values	*0.05*	*0.05*	*0.05*

T1= control sample raw milk, T2= sample raw cow's milk supplemented 5 milileter propolis; T3=sample raw cow's milk supplemented 10 milileter propolis; T4=sample raw cow's milk supplemented 15 milileter propolis; T5=sample raw cow's milk supplemented 20 milileter propolis; and; means followed by the different letter (s) within a column are significantly different.

4.4. Effects of Honey Bee Propolis on Microbial Quality of Raw Milk

The main effects of agro-ecologies of propolis harvested and propolis concentration have shown very highly significant (($p < 0.001$) effects as shown in Table (4.7), in all microbial quality analyses of sample raw cow's milk in the current study. Except the main effect of agro-ecologies of propolis harvested on total coliform counts at the initial time was non-significant ($p > 0.05$).

4.4.1. Total bacterial count

The main effect of propolis concentration and agro-ecologies of propolis harvested on total bacterial count (log 10CFU/ml) in sample raw cow's milk at initial, 24 hours, and 72 hours of storage period was shown in Table (4.7) below. The analysis of variance showed that agro ecologies of propolis harvested and propolis concentration had a highly significant effect ($P < 0.001$) on total bacterial counts of sample raw cow's milk. However, the interaction effect of propolis by agro-ecologies and concentration had no significant ($P > 0.05$) influence on total bacterial counts of sample raw cow's milk. But, total bacterial count at 72 hours had a highly significant ($p < 0.001$) interaction effects.

The main effects of Agro ecologies of propolis harvested, TBC results of the raw cow's milk treated with ethanol extracted honey bee propolis at initial, 24 hours and 72 hours of evaluated time harvested from Banja were 5.83 log10/cfu/ml, 5.73 log10/cfu/ml, 5.51 log10/cfu/ml; Dangla 5.65 log10/cfu/ml, 5.55 log10/cfu/ml, 5.03 log10/cfu/ml and Guangua were 5.58 log10/cfu/ml, 5.48 log10/cfu/ml and 4.83 log10/cfu/ml of milk respectively. The result revealed that the lowest total bacterial count was registered in Guangua(low land) woredas and the highest was recorded in Banja(high land) and Dangla(midland) woreds in decreasing order. Similarly in all study districts, the highest total bacteria was recorded at initial time and showed gradually decrease in total bacterial counts at 24 and 72 evaluated hours. These might be due to difference in propolis chemical composition to inhibit the growth of microbes and also propolis needs time to activate bio active chemicals used as a preservative role in food.

The result agreed with (Hadi Koohsari *et al*., 2020), who reported that on the microbial load of raw milk, antimicrobial activity of the propolis extracts is more observed after 24, 48, and 72 hours, and no changes are observed at the initial times. The result in line with (Hendi et *al*., 2011), the potential antibacterial activity of extracted propolis was affected by variations in the geographical origin of the propolis harvested. Also agree with (Bankova V., 2005 and Pozippe *et al*., 2011) who reported that, antimicrobial activity is present in all propolis of different regions, but different geographical origins, areas vegetation, and botany affect the rate of this antimicrobial activity.

The main effects of honey bee propolis concentration on TBC results of raw cow's milk showed in Table (4.7) below, at initial evaluated hours TBC results were 7.42 log10/cfu/ml, 6.30 log10/cfu/ml, 5.67 log10/cfu/ml, 5.49 log10/cfu/ml, and 3.54 log10/cfu/ml; in 24 evaluated hours results were 7.42 log10/cfu/ml, 6.30 log10/cfu/ml, 5.67 log10/cfu/ml, 5.49 log10/cfu/ml,

and 3.54 log10/cfu/ml and at 72 hours were 7.46 log10/cfu/ml, 5.15 log10/cfu/ml, 4.9 log10/cfu/ml, 4.7 log10/cfu/ml and 3.3 log10/cfu/ml in TI, T2, T3, T4 and T5 treatment levels of extracted honey bee propolis concentration respectively. The result was higher than Walaa Mohamed *et al*(2023), who found that at the initial time of TBC values in control, 1% , 2% and 3% EEP were 4.99, 4.91, 4.85 and 4.79 log10 CFU/ ml, respectively. While at 24 hours, TBC results were 6.00, 4.30, 4.08, and 3.92 log10 CFU/ ml, and also at 72 hours of examined time 6.05, 3.99, 3.88 and 3.62 log10 CFU/ ml of milk in control, 1%, 2% and 3% EEP propolis treatment levels respectively.

TBC results revealed that the control (T1) had higher TBC than others and gradually decreased the TBC in raw cow's milk, which was treated with a high dose of extracted honey bee concentration as indicated Table (4.7) below. Similarly, the control (T1) group TBC was gradually increased from initial up to 72 hours of storage period. While the raw cow's milk which supplemented different concentrations of EEP total bacterial count (log CFU/ml) values showed a gradual decrease from the initial to 72 hours of storage period.

This might be due to the amount of extracted honey bee concentrations which, supplemented in raw cow's milk and propolis need time to inhibit the growth of microbes in milk. The result is in line with Hadi Koohsari *et al*. (2020), who reported that there is a significant difference between the concentration of 3% and 5% ethanol extracts with other concentrations. Thus, a significant antimicrobial activity could be observed at these concentrations. The result is also similar to (Hadi Koohsari *et al*., 2020), who reported that there is a significant difference between the amount of microbial load in the control treatment and the raw milk containing different concentrations of the ethanol extract and also, the antimicrobial activity of the propolis extracts is more observed after 24, 48, and 72 hours, and no changes are observed at the initial times suggesting that propolis needs time to be effective.

In control raw cow's milk (T1), the result of the total bacterial count was a comparable figure with the study conducted by Asamnew Tassew and Eyassu Seifu (2011), at Bahir Dar Zuria with the overall mean of 7.58log10cfu/ml, Beyene Faekadu (1996) in Southern, Ethiopia that he got average total bacterial count of 7.7log cfu/ml, Tola (2002) in Eastern, Wollega that he got average total bacterial count of 7.4log10, Worku Tollossa *et al*. (2012), who reported that total bacterial count from 7.36 -7.88 log10 cfu/ml of raw cows' milk in Borana, Ethiopia and Mosu Solomon *et al*. (2013) at selected dairy farms in Debre Zeit town that he got average aerobic bacterial count of 7.07log cfu/ml. Moreover, this study was in line with a study by (Shunda. *et*

al., 2013), the overall mean total bacterial count of cow's milk in Mekelle was 7.39log10 cfu/ml at different points. However, the bacterial count obtained from the current result was higher than the work done by Ashenafi Mogessie and Fekadu Beyene (1994) reported as 6.32log10 cfu/ ml, Ombui et *al.* (1995) reported as 5log10 cfu/ml.

In the interaction effect of agro-ecologies of propolis harvested and propolis concentration total bacterial count was highly significant ($p<0.001$) at 72 hours of examined time. As shown in the interaction Table (4.8) below, the highest total bacterial counts were recorded in BT1 (7.46), DT1 (7.46), and GT1 (7.46) log1ocfu/ml of milk. However, the lowest TBC were registered in BT5 (3.43), DT5 (3.19) and GT5 (3.27) log1ocfu/ml of milk. As a general, the result revealed that significantly lower TBCs were recoreded in Guangu Woreda(lowland) and Dangla woreda(midland). While, the highest values were recoreded in Banja (high land) Woreda with in the same treatment level supplemented to raw cow's milk. These results might be associated with the chemical composition of propolis which inhibited the growth of microbes varies in low, mid, and high land areas based on botanical sources in which propolis harvested and due to the treatment level of propolis which, is supplemented to raw cow's milk. The results are in agreement with Hadi Koohsari *et al*. (2020) who reported that on the microbial load of raw milk, antimicrobial activity of the propolis extracts is more observed after 24, 48, and 72 hours, and no changes are observed at the initial times and there is a significant difference between the concentration of 3% and 5% ethanol extracts with other concentrations. Thus, a significant antimicrobial activity could be observed at these concentrations. This also agrees with (Bankova ., 2005, Pozippe *et al*., 2011), who reported that, antimicrobial activity is present in all propolis of different regions, but different geographical origins, areas vegetation, and botany affect the rate of this antimicrobial activity.

Figure 4.3. Total bacterial count

4.4.2. Total coliform count

The main effect of agro-ecologies of propolis harvested on total coliform counts of raw cow's milk showed that non-significant (*P >0.05*) effect at initial evaluation time. However, at 24 and 72 hours of evaluated time, the analysis of variance showed that very highly significant (*P < 0.001*) effect on total coliform counts of raw cow's milk as indicated in Table (4.7) below.

These results revealed that low total coliform bacteria was recorded in raw cow's milk which, was treated with honey bee propolis harvested from Guangua(lowland) woredas. However, the highest total coliform bacteria were registered in raw cow's milk treated with propolis which was harvested from Banja(highland) and Dangla(midland) woredas. This result variation in total coliform bacteria of raw cow's milk might be due to the chemical composition difference of propolis which inhibits microbes besides the agro-ecology difference from which propolis is harvested. These findings were similar to those of (Hendi et *al*., 2011) who reported that the potential antibacterial activity of extracted propolis was affected by variations in the geographical origin of propolis harvested. Also agree with (Bankova ., 2005, Pozippe *et al*., 2011) who reported that antimicrobial activity is present in all propolis of different regions, but different geographical origins, areas vegetation, and botany affect the rate of this antimicrobial activity. The current finding is comparable with Walaa Mohamed *et al* (2023), who reported that total coliform bacteria at control treatment were(3.04, 3.04, and 4.04); 1%EEP(3.04, 3.04 and 2.56); 2%EEP(3.04, 2.66 and 2.04) and at 3%EEP(3.04, 2.46 and 2.32) log10/cfu/ml of raw cow's milk at initial, 24 and 72 evaluation hours respectively.

Main effects of honey bee propolis concentration, the analysis of variance showed that had a very highly significant (*P < 0.001*) effect at initial and 24 hours and a significant (*P < 0.05*) effect at 72 hours on total coliform bacterial of raw cow's milk as showed in Table(4.8) below. Total coliform bacteria at initial evaluated hours were T1 (5.99), T2 (5.05), T3 (3.88), T4 (3.34), and T5 (3.2) log10/cfu/ml of raw cow's milk. At 24 hours, evaluation results were, T1 (6.05), T2 (4.86), T3 (3.95), T4 (3.57), and T5 (2.9) log10/cfu/ml of raw cow's milk. Finally, the results evaluated at 72 hours total coliform bacteria results were T1 (6.3), T2 (4.55), T3 (3.87), T4 (3.15), and T5 (2.83) log10/cfu/ml of raw cow's milk.

The results revealed that TCFCs of the untreated (control) sample raw cow's milk showed an increased event, whereas the treated samples (T2, T3, T4, and T5) showed a retarded decrease of total coliform bacteria up to 72 hours of storage time. This might be due to propolis needs time to killed microbes in milk and different amount of concentrations of extracted honey bee

propolis which is supplemented in raw cow's milk. This correlates with the findings Sadia Afrin Khan, (2020), who reported that the TCFC of the untreated samples showed a gradual increase, whereas the treated samples showed a retarded increase up to 20 h of storage.

The interaction effect of agro-ecologies of propolis harvested and propolis concentration on coliform bacteria was a significant ($p<0.05$) effect at 72 hours of evaluation time. However, at initial and 24 hours, the interaction effect of agro-ecologies of propolis harvested and propolis concentration was non-significant ($p>0.05$) on coliform bacteria of raw cow's milk. As indicated in the interaction Table (4.8) below, the lowest total coliform bacteria recorded in all agro-ecologies of propolis harvested interacted with a high amount of extracted honey bee propolis. Especially the lowest total coliform bacteria was registered in GT5 (2.8 log10/cfu/ml) of raw cow's milk. The result might be due to propolis harvested agro-ecologies difference. The justification is in line with Hadi Koohsar *et al*. (2020), who reported that compounds responsible for the biological activity of propolis are different and variable in samples of different origins.

Figure 4.4. Total coliform count of raw cow's milk

4.4.3. Total yeast and mold count

The analysis of variance revealed that the main effect of agro-ecologies of propolis harvested and propolis concentration had a very highly significant ($P < 0.001$) effect on the yeast and mold counts of raw cow's milk evaluated up to 72 hours of storage period. However, yeast and mold counts of evaluated raw cow's milk were not influenced by the interaction effect of agro-ecologies and propolis concentration ($p>0.05$).

The main effect of agro-ecologies of propolis harvested on total yeast and mold counts of raw cow's milk as indicated Table (4.8) below the result obtained from Banja woredas were 2.94, 2.87, 2.85; Dangla 2.80, 2.78, 2.67; and Guangua were 2.70, 2.65 and 2.5 log10cfu/ml of raw cow's milk at initial, 24 and 72 hours of evaluated time in each study Woredas respectively. The

result revealed that the lowest total yeast and mold was recorded in Gangua(low land) woredas. However, the highest was registered in Dangla and Banja woredas in increasing order. Similarly, the result of total yeast and mold count reduces gradually from initial to 24 and 72 hours of evaluated time in the same trend at all study districts. This might be due to propolis needs time to killed microbes and the difference of propolis harvested areas. The justification is similar to Hadi Koohsari *et al*.(2020) who reported that on the microbial load of raw milk, antimicrobial activity of the propolis extracts is more observed after 24, 48, and 72 hours, and compounds responsible for the biological activity of propolis are different and variable in samples of different origins.

The main effect of extracted honey bee propolis concentration was highly significant ($p<0.001$) effect on total yeast and mold counts of raw cow's milk as indicated in Table (4.8) below. The initial counts of total yeast and mold were 4.04, 3.17, 2.88, 2.64, and 2.36 log10 CFU/ml in T1 (control) T2, T3, T4, and T5 treatment groups of raw cow's milk respectively. At the end of the evaluated time(72 hours), total yeast and mold counts were 3.5, 2.84, 2.62, 2.3, and 2.1 log10 CFU/ml in T1 (control) T2, T3, T4, and T5 treatment groups of raw cow's milk respectively. The result showed that the number of yeast and mold in the control (T1) milk group increased substantially, suggesting that propolis might be used as an antifungal agent to preserve milk during storage. The reduction in yeast and mold counts was more obtained in raw cow's milk supplemented with high propolis concentration when at 72 hours of evaluated time, the lowest total yeast and mold counts were recorded than 24 and initial hours evaluated raw cow's milk in all treatment groups. The result agreed with Walaa Mohamed *et al* (2023), who reported that the initial count of total yeast and mold count were 3.84, 3.45, 3.65, and 3.68 ± 1.76 log10 CFU/ml in control, 1% EEP, 2% EEP, and 3% EEP groups, respectively and increased to 4.34, 3.10, 2.85 and 2.67 log10 CFU/ml at the 72 hours of evaluated time.

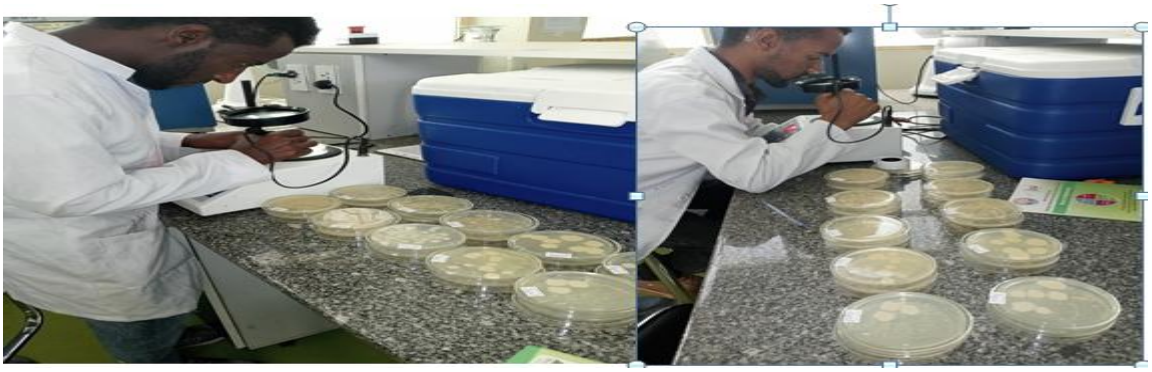

Figure 4.5. Total yeast and mold count of raw cow’s milk.

Table 4.2. Main effects of agro-ecologies of propolis harvested and propolis concentration on microbial quality of raw cow's milk at 0, 24, and 72 hours

Agro ecologies	TBC_{0hr}	TBC_{24hr}	TBC_{72hr}	$TCFC_{0hr}$	$TCFC_{24hr}$	$TCFC_{72hr}$	$TYMC_{0hr}$	$TYMC_{24hr}$	$TYMC_{72hr}$
Banja	5.83^{a}	5.73^{a}	5.51^{a}	4.34^{a}	4.51^{a}	4.16^{a}	2.94^{a}	2.87^{a}	2.85^{a}
Dangla	5.65^{b}	5.55^{b}	5.03^{b}	4.34^{a}	4.27^{b}	4.23^{a}	2.80^{b}	2.78^{a}	2.67^{ab}
Guangua	5.58^{b}	5.48^{b}	4.83^{c}	4.20^{a}	4.1^{b}	3.88^{b}	2.70^{c}	2.67^{b}	2.5^{b}
P-values	***	***	***	*ns*	***	***	***	***	***
LSD(0.05)	0.136	0.12	0.0198	0.228	0.1984	0.2596	0.0815	0.0862	0.2131
Concentration									
T1	7.42^{a}	7.46^{a}	7.48^{a}	5.99^{a}	6.05^{a}	6.3^{a}	3.17^{a}	3.26^{a}	3.5^{a}
T2	6.30^{b}	6.28^{b}	5.15^{b}	5.05^{b}	4.86^{b}	4.55^{b}	3.04^{b}	2.95^{b}	2.84^{b}
T3	5.67^{c}	5.56^{c}	4.9^{c}	3.88^{c}	3.95^{d}	3.87^{c}	2.88^{c}	2.78^{c}	2.62^{c}
T4	5.49^{d}	5.35^{c}	4.7^{d}	3.34^{d}	3.57^{d}	3.15^{d}	2.64^{d}	2.57^{d}	2.3^{c}
T5	3.54^{e}	3.44^{d}	3.3^{e}	3.2^{d}	2.9^{e}	2.83^{d}	2.36^{e}	2.3^{e}	2.1^{c}
P-values	***	***	***	***	***	**	***	***	**
LSD(0.05)	0.1755	0.1755	0.0256	0.2944	0.2562	0.3352	0.1053	0.1113	0.2751
CV (%)	2.4	2.49	0.4	5.5	4.8	6.6	3.01	3.2	8.2

Where; * = Significant ($P < 0.05$); ** = highly significant ($P < 0.01$); *** = very highly significant ($P < 0.001$); T1= control sample raw milk, T2= sample raw cow's milk supplemented 5 milileter propolis; T3=sample raw cow's milk supplemented 10 milileter propolis; T4=sample raw cow's milk supplemented 15 milileter propolis; T5=sample raw cow's milk supplemented 20 milileter propolis; and; means followed by the different letter (s) within a column are significantly different.

Table 4.3. Interaction effects of agro-ecologies of propolis harvested and propolis concentration on microbial quality of raw cow's milk

Agro*con	TBC_0	TBC_{24}	TBC_{72hr}	$TCFC_0$	$TCFC_{24hr}$	$TCFC_{72}$	$TYMC_0$	$TYMC_{24}$	$TYMC_{72}$
BT1	7.38^{a}	7.50^{a}	7.46^{a}	6.04^{a}	6.13^{a}	6.18^{a}	3.23^{a}	3.29^{a}	3.97^{a}
BT2	6.65^{b}	6.15^{b}	6.02^{b}	4.98^{b}	4.80^{b}	4.58^{b}	2.97^{bc}	2.84^{de}	2.69^{cd}
BT3	5.82^{de}	5.35^{cde}	5.47^{c}	4.01^{c}	3.91^{cd}	4.21^{bc}	2.73^{de}	2.58^{fgh}	2.45^{def}
BT4	5.66^{ef}	5.10^{e}	5.17^{d}	3.43^{def}	3.39^{ef}	3.17^{def}	2.53^{fg}	2.47^{gh}	2.14^{ef}
BT5	3.64^{g}	3.49^{f}	3.43^{j}	3.23^{ef}	3.11^{fg}	3.07^{ef}	2.15^{h}	2.15^{i}	2.0^{f}
DT1	7.44^{a}	7.50^{a}	7.46^{a}	6.00^{a}	6.02^{a}	6.18^{a}	3.23^{a}	3.28^{a}	3.97^{a}
DT2	6.17^{c}	5.90^{bcd}	4.63^{g}	5.14^{b}	4.9^{b}	4.56^{b}	3.02^{bc}	2.95^{cd}	2.84^{bcd}
DT3	5.70^{ef}	5.60^{bcde}	4.48^{h}	3.89^{cd}	4.29^{c}	4.21^{bc}	2.86^{cd}	2.77^{def}	2.65^{cde}
DT4	5.45^{f}	5.34^{ced}	4.39^{i}	3.36^{def}	4.27^{c}	3.51^{de}	2.60^{ef}	2.55^{fg}	2.34^{def}
DT5	3.49^{g}	3.49^{f}	3.19^{l}	3.27^{ef}	3.07^{fg}	2.84^{f}	2.38^{g}	2.18^{h}	2.15^{ef}
GT1	7.44^{a}	7.50^{a}	7.46^{a}	6.04^{a}	6.01^{a}	6.18^{a}	3.23^{a}	3.22^{ab}	3.97^{a}
GT2	6.08^{cd}	6.03^{bc}	4.88^{e}	5.03^{b}	4.87^{b}	4.50^{b}	3.11^{ab}	3.06^{bc}	3.0^{bc}
GT3	5.49^{f}	5.44^{cde}	4.78^{f}	3.73^{cde}	3.69^{de}	3.60^{de}	3.05^{abc}	2.97^{cd}	2.77^{cd}
GT4	5.38f	5.25^{de}	4.74f	3.23^{ef}	3.04^{fg}	2.77^{f}	2.77^{de}	2.65^{efg}	2.38^{def}
GT5	3.50^{g}	3.36^{f}	3.27^{k}	3.07^{f}	2.8^{g}	2.56^{f}	2.53^{fg}	2.38^{h}	2.13^{ef}
P-values	*0.1983*	*0.6494*	*<.0001*	*0.9928*	*0.0174*	*0.4979*	*0.4674*	*0.1852*	*0.6385*

Where; T1= control sample raw milk, T2= sample raw cow's milk supplemented 5 milileter propolis; T3=sample raw cow's milk supplemented 10 milileter propolis; T4=sample raw cow's milk supplemented 15 milileter propolis; T5=sample raw cow's milk supplemented 20 milileter propolis; and; means followed by the different letter (s) within a column are significantly different.

CHAPTER 5. CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

This study aimed to evaluate the effectiveness of ethanol-extracted honey bee propolis concentration harvested from three different study areas in Awi zone namely Banja,Dangla and Guangua woredas. Chemical composition(protein, fat, solid nonfat, ash, pH, and titrable acidity), clot on boiling, alcohol test, methylene blue reduction test, organoleptic test, and microbial quality (total bacterial, total yeast, and mold and total coliform) test of raw cow's milk were evaluated. The addition of high concentrations of EEP as well as, milk treated with propolis concentration harvested from Guangua woredas than Dangla and Banja woreda result showed that a significant ($p<0.05$) improved its protein, fat, solid nonfat, and ash contents of raw cow's milk. The main effects of agro-ecologies of propolis harvested and propolis concentration had a highly significant ($P < 0.001$) effect on the main and significant ($P < 0.05$) interaction effect on clot on boiling, alcohol, and methylene blue reduction tests of raw cow's milk. In the microbial quality test, gradual reduction of the total bacterial, coliform, yeast, and mold counts of raw cow's milk was recorded in milk treated with high propolis concentration compared to the control raw cow's milk, and the significantly lower TBC, TCFC, and TYMC were recorded in Guangua woreda harvested propolis concentration. Then, it can be concluded that use of extracted honey bee propolis concentration to raw cow's milk can significantly improves the chemical composition, shelf life, and microbial quality of raw cow's milk. Finally, use of 5ml and 10ml propolis concentrations extends the shelf life of raw cow's milk for 8-9 hours under ambient temperatures without affecting the organoleptic/sensory characteristics of raw cow's milk.

5.2. Recommendations

Based on the findings of the current study the following issues are recommended:-

- ❖ Further investigation should be done on

- Chemical components of propolis which has antimicrobial activities to preserve raw cow's milk.
- Propolis pollen analysis to identify plants which have chemical components of high antimicrobial activities
- Propolis' anti-microbial role at different preservative temperatures to identify recommended temperatures to preserve dairy products by extracted honey bee propolis.

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7. APPENDICES TABLE AND FIGURES

7.1 Appendice Tables

SAS output results

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	15	48.67614656	3.24507644	161.51	<.0001
Error	14	0.28129658	0.02009261		
Corrected Total	29	48.95744315			

R-Square	Coeff Var	Root MSE	TBC0 Mean
0.994254	2.491003	0.141748	5.690415

Source	DF	Type III SS	Mean Square	F Value	Pr > F
A_Eco	0	0.00000000	.	.	.
CON	0	0.00000000	.	.	.
Rep	1	0.01767048	0.01767048	0.88	0.3642
A_Eco_CON	8	0.26450439	0.03306305	1.65	0.1983

Total bacterial count at initial time

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	15	48.67614656	3.24507644	161.51	<.0001
Error	14	0.28129658	0.02009261		
Corrected Total	29	48.95744315			

R-Square	Coeff Var	Root MSE	TBC24 Mean
0.994254	2.491003	0.141748	5.690415

Source	DF	Type III SS	Mean Square	F Value	Pr > F
A_Eco	0	0.00000000	.	.	.
CON	0	0.00000000	.	.	.
Rep	1	0.01767048	0.01767048	0.88	0.3642
A_Eco_CON	8	0.26450439	0.03306305	1.65	0.1983

Total bacterial count at 24hrs

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	15	57.90265112	3.86017674	9028.64	<.0001
Error	14	0.00598567	0.00042755		
Corrected Total	29	57.90863679			

R-Square	Coeff Var	Root MSE	TBC72 Mean
0.999897	0.403481	0.020677	5.124715

Source	DF	Type III SS	Mean Square	F Value	Pr > F
A_Eco	0	0.00000000	.	.	.
CON	0	0.00000000	.	.	.
Rep	1	0.00502898	0.00502898	11.76	0.0041
A_Eco_CON	8	1.44748947	0.18093618	423.20	<.0001

Total bacterial count at 72hrs

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	15	35.18985274	2.34599018	41.51	<.0001
Error	14	0.79128975	0.05652070		
Corrected Total	29	35.98114249			

R-Square	Coeff Var	Root MSE	TCFC0 Mean
0.978008	5.536592	0.237741	4.293992

Source	DF	Type III SS	Mean Square	F Value	Pr > F
A_Eco	0	0.00000000	.	.	.
CON	0	0.00000000	.	.	.
Rep	1	0.55284088	0.55284088	9.78	0.0074
A_Eco_CON	8	0.07361144	0.00920143	0.16	0.9928

Total coliform count at initial time

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	15	36.71669506	2.44777967	57.20	<.0001
Error	14	0.59910823	0.04279344		
Corrected Total	29	37.31580328			

R-Square	Coeff Var	Root MSE	TCFC24 Mean
0.983945	4.823933	0.206866	4.288322

Source	DF	Type III SS	Mean Square	F Value	Pr > F
A_Eco	0	0.00000000	.	.	.
CON	0	0.00000000	.	.	.
Rep	1	0.19835386	0.19835386	4.64	0.0492
A_Eco_CON	8	1.23676144	0.15459518	3.61	0.0174

Total coliform count at 24hrs

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	15	41.47642243	2.76509483	37.74	<.0001
Error	14	1.02567882	0.07326277		
Corrected Total	29	42.50210125			

R-Square	Coeff Var	Root MSE	TCFC72 Mean
0.975868	6.613395	0.270671	4.092769

Source	DF	Type III SS	Mean Square	F Value	Pr > F
A_Eco	0	0.00000000	.	.	.
CON	0	0.00000000	.	.	.
Rep	1	0.72289280	0.72289280	9.87	0.0072
A_Eco_CON	8	0.56665665	0.07083208	0.97	0.4979

Total coliform count at 72hrs

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	15	2.89300207	0.19286680	26.68	<.0001
Error	14	0.10119062	0.00722790		
Corrected Total	29	2.99419269			

R-Square	Coeff Var	Root MSE	TYMC0 Mean
0.966204	3.016060	0.085017	2.818812

Source	DF	Type III SS	Mean Square	F Value	Pr > F
A_Eco	0	0.00000000	.	.	.
CON	0	0.00000000	.	.	.
Rep	1	0.02394598	0.02394598	3.31	0.0902
A_Eco_CON	8	0.05872033	0.00734004	1.02	0.4674

Total yeast and mould count at initial time

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	15	3.51052275	0.23403485	28.95	<.0001
Error	14	0.11319377	0.00808527		
Corrected Total	29	3.62371652			

R-Square	Coeff Var	Root MSE	TYMC24 Mean
0.968763	3.237683	0.089918	2.777237

Source	DF	Type III SS	Mean Square	F Value	Pr > F
A_Eco	0	0.00000000	.	.	.
CON	0	0.00000000	.	.	.
Rep	1	0.01387011	0.01387011	1.72	0.2114
A_Eco_CON	8	0.10965012	0.01370626	1.70	0.1852

Total yeast and mould count at 24hrs

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	15	8.41652896	0.56110193	11.37	<.0001
Error	14	0.69074304	0.04933879		
Corrected Total	29	9.10727200			

R-Square	Coeff Var	Root MSE	TYMC72 Mean
0.924155	8.270705	0.222123	2.685664

Source	DF	Type III SS	Mean Square	F Value	Pr > F
A_Eco	0	0.00000000	.	.	.
CON	0	0.00000000	.	.	.
Rep	1	0.00485873	0.00485873	0.10	0.7583
A_Eco_CON	8	0.30193289	0.03774161	0.76	0.6385

Total yeast and mould count at 72hrs

7.2. Appendix Figure

Figure1. Honey bee propolis collection and extraction

A B

Figure 2. Titrable acidity of milk (A) and organoleptic test of milk (B).

Figure 3. Total bacterial count

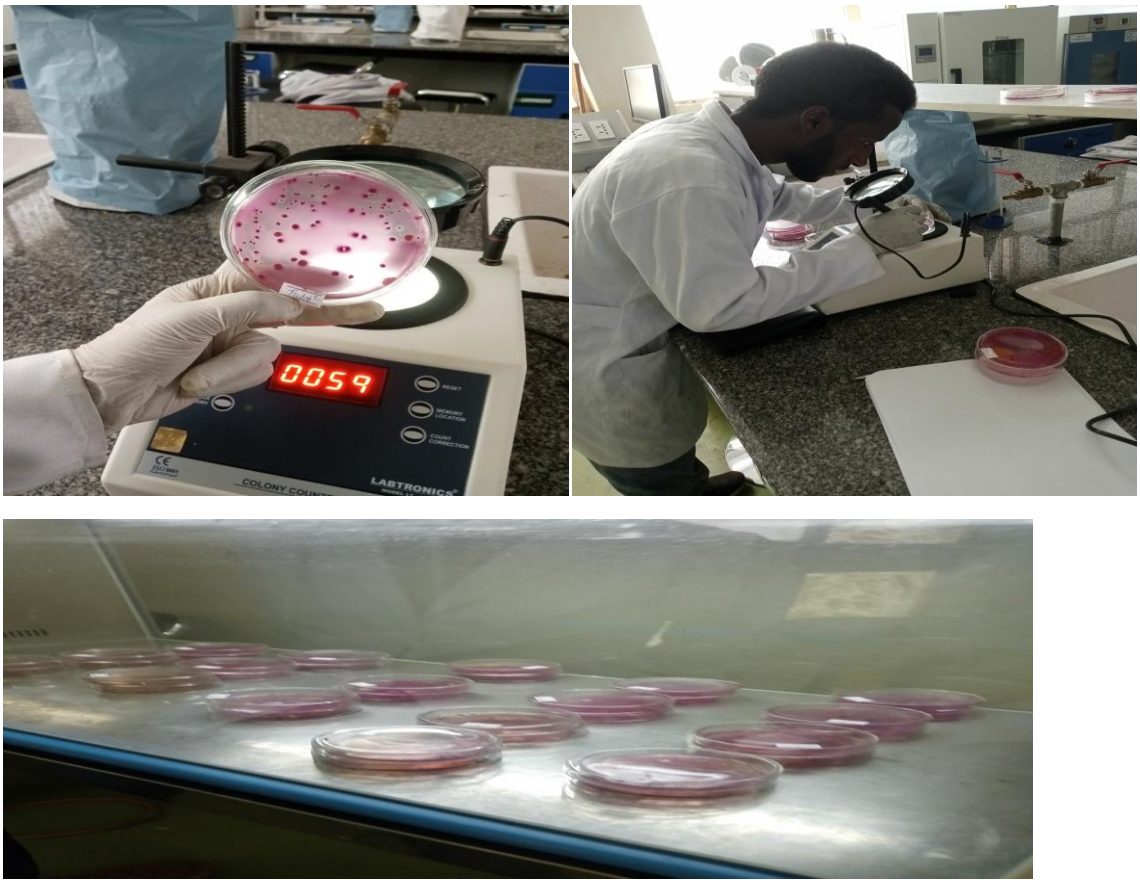

Figure 4. Total coliform count of raw cow's milk

Figure 5. Total yeast and mold count of raw cow's milk

7.3. Author Biography

The author “Abebe Melese Tirfie” was born in April 1995 G.C from his father Ato Melese Tirfie and his mother W/ro Adanech Birhan in Tej bahir kebele hulet eju enesie woreda(Motta) of east Gojjam zone. He attends and completed his elementary school from 2002- 2009 at Awjja elementary school. He attends his secondary school at Keranyo secondary school and his preparatory school at bahire giorgis secondary and preparatory school from September 2010- 2011 G.C and 2012-2013 G.C respectively.

In October 2015 G.C he joined Debre Tabor University and in June 2017 G.C he was graduated with a bachelor's degree in animal sciences by scored the highest point(**CGPA=3.98**) from the department and the second from agriculture and environmental science college. After graduation, he was worked for six month in East Gojjam zone sedie woreda at aposition of apiculture and sericulture woreda expert. After six mounth work experience may 2018 G.c, Abebe joined to Injibara University as a cheife technical assistant. Then, In December 2022 G.c he joined Bahir Dar University for the M.Sc program in the college of agriculture and environmental sciences, department of animal production and technology and follows M.Sc program in animal production and he scored **3.98 CGPA.**