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To determine the extent of total bacteria as Standard plate bacterial count (SPC) and Lactic acid bacterial count (LABC) in raw milk as influenced by different post-hand milking udder wash treatments using moringa leaf extract in cross-bred cows

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Abstract
The purpose of the present investigation was to determine the "To determine the extent of total bacteria as Standard plate bacterial count (SPC) and Lactic acid bacterial count (LABC) in raw milk as influenced by different post-hand milking udder wash treatments using moringa leaf extract in cross-bred cows." Experimental included randomly selected twelve apparently healthy cross-breed cows housed in tail to tail barn under similar management condition. Clean milk was obtained by using the dry full-hand technique for milking, which met all health and safety requirements. Representative samples of milk were collected in different post-hand milking udder wash treatments as T_0 (udder of cows washed after milking with clean water and wiped off with clean towel as control). T_1 (udder of cows washed after milking with moringa leaf extract, prepared by boiling 50 gram fresh green moringa leaves in 1-liter Distilled water for 10 minutes), T_2 (udder of cows washed after milking with moringa leaf extract, prepared by boiling 75 gram green moringa leaves in 1-liter Distilled water for 10 minutes), T_3 (udder of cows washed after hand milking with moringa leaf extract, prepared by boiling 100 gram green moringa leaves in 1-liter Distilled water for 10 minutes). Lactic acid bacterial count (LABC) & standard plate count (SPC) in milk were calculated from analyses of reference milk representative samples. Statistical analysis of different bacterial parameters for different post-milking udder wash treatments of moringa leaf extract revealed significant effect on SPC and LABC of milk. The experimental results showed that the post-milking udder wash treatments using moringa leaf extract were more effective to the control as T_0 in terms of the bacteria count in raw milk judged on the basis of SPC and physiological groups of bacteria.

Keywords: Post-milking, udder wash, moringa leaf extract, bacteriological and raw milk

Introduction
Indian dairy farming is increasing very faster. Many government and private dairy farm is opening day by day in the India. Dairy farming is becoming a good source of income for Indian village and urban farmers. Not only milk is used by people but dairy farm wastage also used as manure for fertility of soil and highest crop production Quddus (2012) [11].
Milk production in India relies heavily on cattle and buffalo. About half (54%) of the milk sold comes from cooperatives and/or unorganized sources like milkmen and contractors Shrivastava (2008) [15]. Uttar Pradesh (16%), Rajasthan (13%), Madhya Pradesh (9%), Andhra Pradesh (8%) and Gujarat (7%) are the five main milk-producing states in India, together accounting for more than half of the country's total production. FASSI New Delhi predicts that in 2022, India would consume 85 MMT of fluid milk, a 2.5% increase over the 83 MMT the USDA officially reported for MY 2021 Kumar *et al.* (2021) [7].
Post says that demographic shifts and improvements in retail and restaurant distribution have contributed to this growth. More and more Indians are drinking milk as a means of improving their protein intake. The country of India is now both the world's greatest producer and consumer of milk.

The USDA estimates 680,000 MT for India's output in 2021; Post predicts 700,000 MT for 2022 Bathla and Hussain (2022) [2].

Milk has been recognized as a complete food by nutritionists all over the world. It has all the ingredients and nutrients necessary for growth and maintenance of a healthy human body. Clean and hygienic milk production is a main aspect of Indian dairy framers. The term is common in dairy industry that "Where milk is healthy for human health also it is harmful for human health", because bacteria in milk affect the human health, therefore our dairy cattle milk should be free from bacteria Górska-Warsewicz *et al.* (2019) [5].

We can produce free bacterial milk with the help of using clean and fresh feed, fodder and water provide to our animals as well as before the milking use of different kind of washing of body and udder of dairy animals Lukuyu *et al.* (2012) [8].

At the time of milking dairy animal's udder plays an important for bacterial quality in milk production. If udder will safely clean, washed and wiped properly then bacterial quality in milk will be count low Bekuma and Galmessa (2018) [3].

Moringa oleifera belongs to a small medicinal tree native to South America and Brazil. Moringa is currently found in tropical and subtropical areas all over the planet. For the reason that it includes various pharmacologically active compounds such flavonoids, guayavolic acid, guavanoic acid, guajadinal, guajaverin, and other active principles, Moringa has been utilised as a traditional medicine Das (2015) [4].

Moringa oleifera leaves have anti-inflammatory, antioxidant, antibacterial, antifungal, anticancer, antimicrobial, antispasmodic, anti-stomach-ache, anti-cancer, anti-hyperglycaemia, hepatoprotection, and endothelial progenitor cell characteristics. Moringa leaves have antibacterial properties in a variety of ways Ramamurthy *et al.* (2021) [12].

Moringa oleifera is related to phytotherapy plant that has been used in folk medicine to cure and manage a variety of ailments Gupta *et al.* (2018) [6]. Since independence, India's entire effort has been focused on building an allopathic-based veterinary infrastructure that is wholly funded by the government. Traditional systems and knowledge have been abandoned to the point where many of us, including veterinary medical specialists, are uninformed of ancient literature and are even sceptical of it. On recent years, the emphasis in livestock maintenance and development has switched to modern science. On a dairy farm, one of the most important goals is to produce clean and healthy milk. Fresh milk can include pathogenic and saprophytic bacteria, but clean milk is free of these contaminants and is manufactured in conditions that allow for just micrograms of microbial growth. Singh *et al.* (2021) [16].

Table 1: The BIS has laid down following standards for grading row milk in India:

SL. No.	Grade	SPL/ml	Methylene Blue Reduction Test (hrs.)	Coliform Test
1.	Very Good	Not over 200,000	5 and above	Absent in 0.001/ml
2.	Good	2,00,000	3 and 4	Absent in 0.001/ml
3.	Fair	1million-5 million	1 and 2	Absent in 0.001/ml
4.	Poor	Over 5 million	½	Present in 00.001/ml

Materials and Methods

From the herd consisting of cross-bred cows at SHUATS dairy farm, Prayagraj, The California Mastitis Test revealed that twelve of the cows were healthy and clear of mastitis. (Schalm and Noorlender, 1957) [13] and random selection of animals with additional obvious udder infections or injuries. All selected cows were housed in tail to tail milking-ready barn and dry full hand method of milking was followed. Samples of milk were collected for different four post-hand milking treatments using moringa leaf extract. There were a total of ten replicates for every intervention, including the untreated group. First two streams of milk from all quarters were discarded as a measure of recommended routine practice. Milk samples collected raw milk samples were analyzed for their total bacterial count per milliliter by Standard plate bacterial count (SPC) and viable count of four physiological groups of bacteria enzyme, (LABC).

Collection of samples

Representative It was decided to take milk samples for different post-hand milking udder wash treatments using moringa leaf separately in sterile conical flasks of 250 ml. capacity, and filled with sterile cotton plugs to prevent contamination. Specimens of milk that was unprocessed were taken, and sent to the lab to be tested for bacteria.

Experiment Treatments (T) using *Moringa oleifera* leaf extract used for udder washing after milking

T_0: Udders of cows washed after post-hand milking using clean water and wiped off with clean towel as control.

T_1: Udders of cows washed after post-hand milking with Moringa leaf extract (prepared by boiling 50 grams green Moringa leaf in 1-liter Distilled water for 10 minutes, cooled then filtered) and wiped off with clean towel.

T_2: Udders of cows washed after post-hand milking with Moringa leaf extract (prepared by boiling 75 grams green Moringa leaf in 1-liter Distilled water for 10 minutes, cooled than filtered) and wiped off with clean towel.

T_3: Udders of cows washed after post-hand milking with Moringa leaf extract (prepared by boiling 100 grams green Moringa leaf in 1-liter Distilled water for 10 minutes, cooled than filtered) and wiped off with clean towel.

Parameters of study

The following methods were used to measure the characteristics of the microorganisms (Chammers, 1953).

- Standard plate count per millimeter (SPC) intended for overall bacteria
- Lactic acid bacterial count (LABC)

Sterilization of glassware

Conical Flasks

Before being put to use, each and every conical flask was meticulously scrubbed, dried, glugged with cotton of the absorbent type, and then sterilized in an autoclave at a temperature of 120 degrees Celsius for sixty minutes.

Pipettes

The bacteriological pipettes with capacities of 1 ml and 10 ml were each submerged in a solution of chromic acid for an entire night before being washed in running water and allowed to dry. They were disinfected by being heated in a hot air oven at a temperature of 120 degrees Celsius for one hour while being wrapped in paper first.

Test tubes
The object under examination was washed in detergent and then in running water. Following this step, 9 ml blanks of Ringer's solution were made and placed in test tubes so that they could be used later on in the process of sample dilution. They were autoclaved for twenty minutes at a temperature of 120 degrees Celsius and a pressure of 1.2 pounds per square centimeter after having sterile absorbent cotton plugs inserted.

Petri plates
Following a thorough cleaning with detergent and warm water, these were flipped over to dry on a clean table. Paper was used to wrap each of the four packages containing the dry plates. These were subjected to an autoclaving process in an oven with hot air at a temperature of 120 degrees Fahrenheit for one hour.

Preparation of media for microbial examination of samples
Ringer's Solution
It was needed for dilution milk samples desired before plating per Prasad and Neeraj 2004.
Sodium chloride (NaCl) 9 g
Potassium chloride (KCI) 0.42 g
Calcium chloride ($CaCl_2$) 0.24 g
Sodium bicarbonate ($NaHC0_2$) 0.20 g
Distilled water 1000ml.
*0.48 in the event of salt water, ($CaC12.6H_20$)

(SPC) Standard plate count total bacteria
For total bacteria obtained nutrient agar medium **(**Chalmers, 1953) Composition.
Composition:
Agar-Agar 15 g(20 g)
Peptone 5 g
Sodium chloride 5 g
Beef extract 3 g
Distilled water 1000 ml.
pH 7.2
At a temperature of sixty degrees Celsius, 1000 milliliters of distilled water containing peptone, sodium chloride, and beef extract were given a pH adjustment with bromothymol blue to get it up to 7.2. The agar powder was dissolved in 900 ml of distilled water by heating it for 15 minutes in an autoclave at a pressure of 1.25 kg/cm2 for 20 minutes. After that, it was combined with peptone that had been filtered, sodium chloride, and beef extract, and then it was divided among conical flasks.

Lactic acid bacterial count (LABC)
For the determination of LABC, the lactose agar medium was utilized.

Composition
Agar-Agar 15 g (20 g)
Peptone 5g
Sodium chloride 5 g
Beef extract 3 g
Lactose 20 g
Distilled water 1000 ml.
Andred's indicator 10 ml (Acid fuchsine 0.05% aq. Solution i.e. 50 mg acid fuchsine in 100 ml water)
Nutrient agar was prepared, melted butter Fat and Nile blue sulphate indicator is added and in 250 ml capacity flasks. In order to sterilize the medium, it was steamed for 30 minutes on three separate days. Fat globules are emulsified by forcefully shaking the liquid just before use. Underneath the colonies, lipolytic bacteria degraded pink fat globules, resulting in a blue appearance. Nile blue sulphate turns the unhydrolyzed fat globules a pinkish color.

Standard plate bacterial count (SPC/ml)
The following procedure was used for SPC in milk:
- Milk samples collected were shaken gently 25 times in back and motion on a leveled table, in a time of about 7seconds
- Dilution of agitated samples of milk was prepared with the help of sterilized 9 ml blank of finger's solution such as 1:10. 1:100, 1:1000. Care was taken to shake the diluted sample as stated above.
- Sterilized pipettes were used to measure quantity of 1 ml suitable milk dilution and transferred to priority marked sterilized Petri plates in duplicates.
- As the dilution was transferred into the Petri plates, the month of agar flasks were flame safety and approximately 15 ml of the nutrient agar medium was poured into each dish to cover about 3 mm deep.
- Agar medium was mixed with the dilution by gently rotating and tilting the dishes. After agar medium became solid, the plates were inverted and incubated for two days at 37 °C.

Plates with 30–300 colonies were chosen post-incubation for counting with the use of a Quebec colony counter. After averaging the bacterial count from two plates, we multiplied that number by the dilution ratio to get the total number of bacteria in one milliliter of milk.

Incubation period
The incubation times for various physiological groups of bacteria were as follows:

Bacterial group	Temperature (^{0}C)	Incubation Period
Standard plate count (SPC)	37	48 hrs.
Lactic acid bacterial count (LABC)	35	48 hrs.

Statistical analysis of data
The data collected on bacterial parameters were collected recorded, tabulated and analyzed statistically using Analysis of Variance Techniques (ANOVA) as per Snedecor and Cochran (2004) [14].
Indicators such as the Lactic Acid Bacterial Count (LABC) and the Standard Plate Count (SPC) can help determine whether or not a patient has an infection. Photolytic bacterial count (PBC), Lipolytic bacterial count (LBC) and coliform per ml of raw milk were collected and presented in Table from 4.1.1 to 4.5.10 Data were statistically analyzed to determine the level of significance in bacterial counts due to different post-hand milking udder wash treatments using moringa leaf extract on various bacterial parameters in raw milk. The results of this experiment have been presented and discussed in this chapter.

Standard Plate Count (SPC × $10^{3)}$ per ml in milk for total viable count
- The data regarding SPC (10^3) per ml in different post-hand milking treatments using Moringa leaf extract on bacteriological quality of raw milk are presented in (Table 2) and analysis of variance (ANOVA) of the same

is given in (Table 3). Following observations were made:

- The SPC (10^3) per ml. of fresh milk in general ranged from 29.00 to 37.00.
- Irrespective of different post-hand milking treatments using Moringa leaf extract as udder wash mean SPC (10^3) per ml of raw milk ranged from 30.70 to 35.10.
- SPC (10^3) per ml in raw/fresh milk of cows pertaining to T_0, T_1, T_2 and T_3 post -hand milking treatments using Moringa leaf extract ranged from 33.00 to 37.00, 31.00 to 33.00, 29.00 to 32.00 and 30.00 to 32.00 respectively.
- Mean SPC (10^3) per ml in fresh milk of cows in T_0, T_1, T_2 and T_3 was 35.10,31.50,30.70, and 31.20 respectively.
- The differences in SPC (10^3) per ml of fresh milk outstanding to dissimilar post-milking treatments using Moringa leaf extract were found significant (Table 4.1.2). However, differences in the values of SPC in raw/fresh milk between T_0 and T_1 and also between T_3 and T_2 were found at per.

From the perusal of data on SPC (10^3) of raw milk as influenced by different post-milking treatments using Moringa leaf extract as udder wash contained in (Table 4.1.1) and (Figurer.1) it was that lowest SPC/ml (10^3) was recorded in milk of T_2 (37.70) followed by T_3 (31.20), T_1 (31.50) and T_0 (35.10). The disparity between these numbers was found statistically significant indicating there by significant effect of post-milking udder wash treatment of moringa leaf extract on SPC/ml of raw milk. Result revealed that SPC per ml in raw milk of T_2 was significantly lower than milk samples of other post-milking udder wash treatments. Therefore, T_2 with lowest registered SPC was the most superior post-milking udder wash treatment over remaining treatments of moringa leaf extract to obtain milk of low bacterial count. The observations with regard to SPC ml in raw milk are in agreement with Pandey and Prasad (2001) [9]. But not in line with Banka *et al.* (2007) [1] reporting higher SPC/ml milk.

Table 2: Average Standard plate count (SPC×10^3) per ml in raw/fresh milk of different Post- milking udder wash treatments using *Moringa oleifera* leaf extract (T_0, T_1, T_2, and T_3) in cross-bred cows.

Replications	SPC/ml (10^3) in raw milk			
	T_0	T_1	T_2	T_3
1	37	32	32	31
2	36	31	29	32
3	33	31	30	30
4	35	33	31	32
5	36	31	30	31
6	35	31	31	31
7	35	32	31	32
8	35	31	31	31
9	36	31	31	31
10	33	32	31	31
Mean	35.10	31.50	30.70	31.20

Table 3: Analysis of variance (ANOVA) for the data on SPC/ml in raw/fresh milk contained in table 2.

Sources of variance	d.f.	S.S.	M.S.S.	F.cal.	F.tab. (5%)	Result	C.D. at (5%)
Treatments	3	121.275	40.425	60.722	0.000	S	0.749
Replications	9	11.125	1.236	1.857	0.103		
Error	27	17.975	0.666	--	--		
Total	39	--	--	--	--		

Post-milking treatments using *Moringa oleifera* leaf extract as udder wash

Treatments	T_0	T_1	T_3	T_2
Mean SPC (10^3) per ml in raw milk	35.10	31.50	31.20	30.70

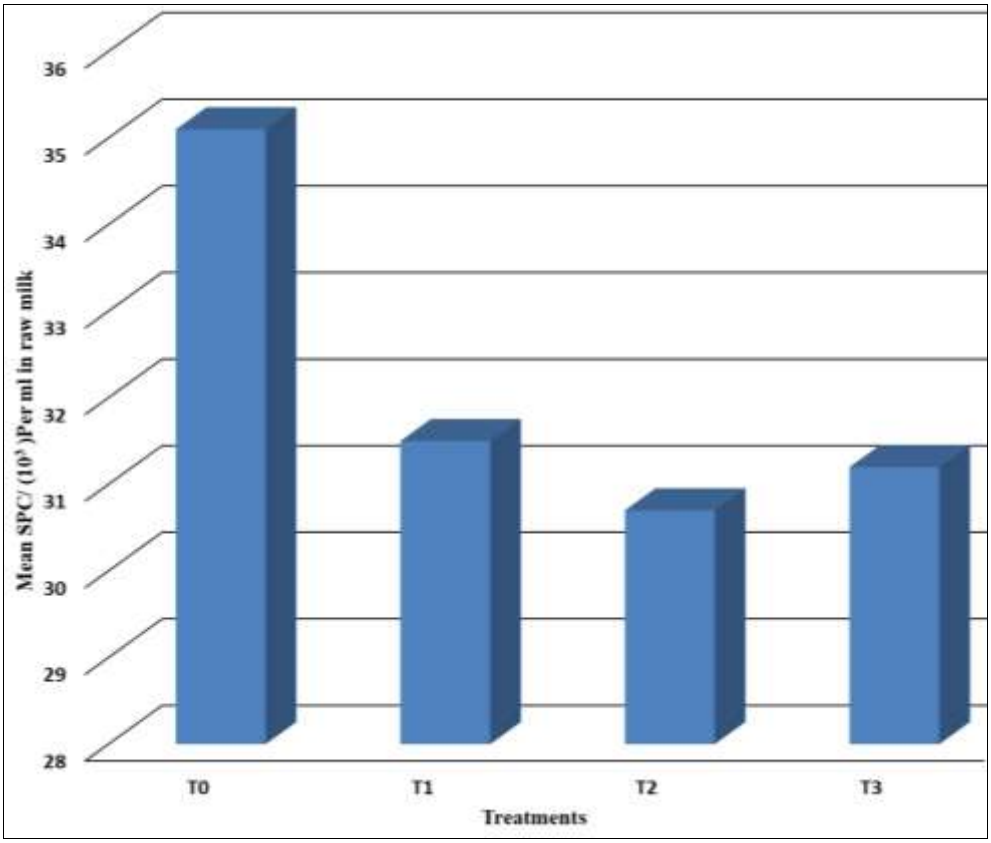
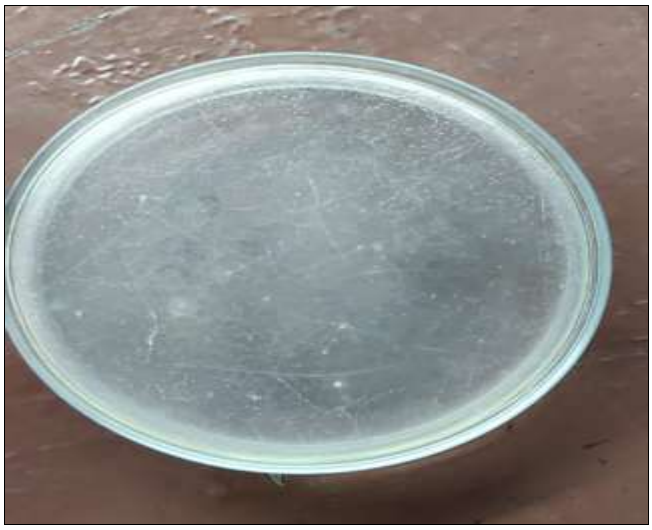


Fig 1: Graphical analysis of post-milking udder wash treatments of using *Moringa oleifera* leaf extract on standard plate count (SPC×10^3) per ml in raw milk



Standard Plate Count

Lactic acid bacterial count (LABCx10^2) per ml of raw milk.

The data regarding LABC (10^2) per ml different post-hand milking treatments using Moringa leaf extract on bacteriological quality of raw milk are presented in (Table 4) and (ANOVA) of the same is given in (Table 5). Following observations were made:

- The LABC (10^2) per ml of raw/fresh milk in general ranged from 19.00 to 29.00.
- Independently of the post-hand-milking therapy that is used Moringa leaf extract as udder wash, mean LABC (10^2) per ml of raw/fresh milk ranged from 20.40 to 25.60.
- LABC (10^2) per ml in raw/fresh milk of cows pertaining to T_0, T_1, T_2, and T_3 ranged from 22.00 to 29.00, 21.00 to 27.00, 20.00 to 24.00 and 19.00 to 23.00, respectively.
- Mean LABC (10^2) per ml in raw milk in T_0, T_1, T_2 and T_3 post-hand milking treatments via Moringa leaf extract was 25.60, 22.90, 21.50 and 20.40, respectively.
- The differences in LABC (10^2) per ml of raw milk due to different post-hand milking treatments via Moringa leaf extract were found significant (Table 4.2.4).

The perusal of data on LABC/ ml (10^2) of raw milk as influenced by different post-hand milking treatments using Moringa leaf extract milking contained in (Table 4.2.3) and (Figure.2) showed that lowest LABC/ml (10^2) was recorded in milk at T_2 (21.50) followed by T_3 (20.40), T_1 (22.90) and T_0 (25.60). The differences in values of LABC between T_1 and T_3 were found at per (Table 4.2.4) indicating there by a significant effect of post-milking udder wash treatments using Moringa leaves extract on LABC/ml of milk. Results ravelled significantly less count of LABC/ml in milk of T_3, T_1, and T_0 as compared to T_2 remaining post-milking udder wash treatments via moringa leaf extract. However, T_3, T_1, and T_0 registered significantly higher LABC than milk of T_2 indicating thereby inferiority of T_3, T_1 and T_0 over T_2 of the post-milking udder wash treatments of LABC in milk. The average LABC/ml in raw milk obtained in the present study is in the agreement through the information of Singh and Prasad (1987) [17], Pandey and Prasad (2001) [10]. Pandey and Prasad (1991) [9].

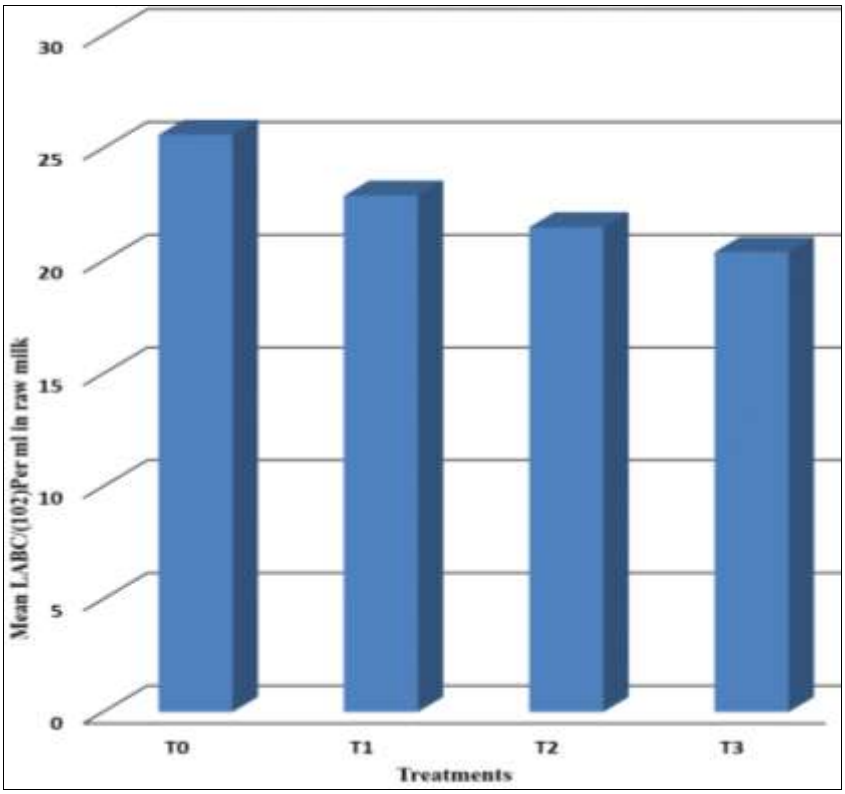


Fig 2: Graphical analysis of post-milking udder wash treatments of using *Moringa oleifera* leaf extract on Lactic acid bacterial count (LABC×10^2) per ml in raw milk

Fig 3: Lactic Acid Bacterial Count

Table 4: Average lactic acid bacterial count (LABC×10^2) per ml in raw/fresh milk of different Post-milking udder wash treatments (T_0, T_1, T_2 and T_3) using *Moringa oleifera* leaf extract in cross bred cows.

Replications	LABC/ml (10^3) in raw/fresh milk			
	T_0	**T_1**	**T_2**	**T_3**
1	22	21	21	19
2	29	22	21	21
3	26	27	24	23
4	24	21	21	21
5	26	21	20	19
6	26	25	22	22
7	25	23	21	19
8	26	21	21	19
9	26	23	21	20
10	26	25	23	21
Mean	25.60	22.90	21.50	20.40

Table 5: (ANOVA) for the data on LABC/ml in raw milk contained in Table 4.

Sources of variance	d.f.	S.S.	M.S.S.	F.cal.	F.tab. (5%)	Result	C.D. at (5%)
Treatment	3	151.400	50.467	34.849	0.001	**S**	1.104
Replications	9	61.100	6.789	4.688	0.000		
Error	27	39.100	1.448	--	--		
Total	39	--	--	--	--		

Treatments	T_0	T_1	T_3	T_2
Mean LABC (10^2) per ml in raw milk	25.60	22.90	21.50	20.40

Summary and Conclusion

The temperature category T2 had the lowest mean SPC (103) per ml of milk (30.70) followed by T_3 (31.20), T_1 (31.50) and T_0 (35.10) and the differences in these values were found significant. However, differences in the values of SPC in milk between T_2 and T_3 were found at par. Lowest mean LABC (10^2) /ml was recorded in milk of T_3 (20.40) followed by T_2 (21.50) T_1(22.90) and T_0 (26.50) and the differences in these values were found significant.

The findings obtained from the experiment revealed significant effect of different post-hand milking treatments using Moringa leaf extract on Standard plate count (SPC) and Lactic acid bacterial count (LABC) in raw milk. Overall rating of quality of raw/fresh milk as determined by various bacterial parameters was originate most excellent in T_3 (100 g. moringa leaf extract) followed by T_2 (75 g. moringa leaf extract) T_1 (50 g. leaf extract) and T_0 indicating there by superiority of T_3 over rest of the post-milking udder wash treatments using moringa leaf extract.

The moringa leaf extract can be used as an alternative of post-milking udder disinfectants for reducing the teat-end bacterial loads. This may lead to lower chemical uses, which may promote more hygienic, safe milk for consumers, and decrease cost of mastitis and control, especially in the developing countries where moringa is native and easy to obtain.

Therefore, use of Moringa leaf extract as post -hand milking udder wash (prepared by boiling 100 gm Moringa leaves in 1-liter water for 10 minutes then filtered) may be recommended to the dairy farmers to produce milk of low bacterial count.

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