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Effect of Different Pre-Milking Udder Wash Treatments Using Marigold Leaf Extract on the Bacteriological Quality of Raw Milk in Crossbred Cows

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Abstract
The present study evaluated the efficacy of different concentrations of Marigold leaf extract used as a pre-milking udder wash on the bacteriological quality of raw milk in crossbred cows. Twelve healthy lactating crossbred cows were randomly allotted to four udder wash treatments, namely clean water wash as control, Marigold leaf extract at 20 g per litre of water, 40 g per litre of water and 60 g per litre of water. Milk samples were collected aseptically after milking and analyzed for total bacterial load and different physiological groups of bacteria using standard microbiological techniques. The results revealed a significant reduction in bacterial counts in Marigold-treated groups compared to the control, with the highest reduction observed at the 60 g per litre concentration. Coliform bacteria were absent in all treatments, indicating satisfactory hygienic conditions. The study demonstrated that Marigold leaf extract can be effectively used as a natural, economical and eco-friendly alternative to chemical udder disinfectants for improving the bacteriological quality of raw milk under farm conditions.

Keywords: Marigold leaf extract; udder hygiene; raw milk quality; bacterial load; clean milk production; crossbred

Introduction
Milk is one of the most complete and nutritious foods for humans; however, its high nutritive value also makes it an ideal medium for the growth of microorganisms. The bacteriological quality of raw milk is therefore considered a critical determinant of its shelf life, processing suitability and consumer safety. Microbial contamination of milk generally occurs at the farm level and is influenced by factors such as animal health, housing hygiene, milking practices and post-milking handling. Among various farm-level interventions, pre-milking udder hygiene plays a pivotal role in controlling the entry of microorganisms into milk. The teat surface often harbors dirt, manure, bedding material and a wide variety of microorganisms, which can easily contaminate milk during milking if proper udder sanitation is not followed. Poor udder hygiene has been reported as one of the major contributors to increased Standard Plate Count and spoilage organisms in raw milk (Murphy and Boor, 2000) [5]. Chemical udder sanitizers such as iodine-based solutions, chlorhexidine and quaternary ammonium compounds are commonly used for pre-milking udder washing. Although effective, their regular use is often limited in smallholder dairy systems due to higher cost, limited availability, concerns regarding chemical residues in milk and possible development of microbial resistance. These limitations have led to increasing interest in exploring plant-based, safe and cost-effective alternatives for udder hygiene management.
Marigold (*Calendula officinalis*) is a well-known medicinal plant extensively used in traditional medicine for its antimicrobial, anti-inflammatory, antioxidant and wound-healing properties. The antimicrobial activity of Marigold is attributed to its rich content of flavonoids, saponins, carotenoids and phenolic compounds, which are known to inhibit the growth of a wide range of bacteria. Considering its medicinal value, local availability and low cost, Marigold leaf extract appears to be a promising natural alternative for use as a pre-milking udder wash. Despite the well-documented medicinal properties of *Calendula*

officinalis, scientific information on its application as an udder hygiene agent and its influence on the bacteriological quality of raw milk is limited. Hence, the present study was undertaken to evaluate the effect of different concentrations of Marigold leaf extract used as a pre-milking udder wash in raw milk of crossbred cows with the following objectives:

1. To determine the extent of total bacterial load, expressed as Standard Plate Count (SPC), in raw milk as influenced by different pre-hand milking udder wash treatments using Marigold (*Calendula officinalis*) leaf extract.
2. To evaluate the viable counts of different physiological groups of bacteria in raw milk as affected by various pre-hand milking udder wash treatments using Marigold (*Calendula officinalis*) leaf extract.

Materials and Methods

The present experiment was conducted at the Dairy Farm of Sam Higginbottom University of Agriculture, Technology and Sciences (SHUATS), Prayagraj, during the pre-monsoon season for a period of 30 consecutive days. Twelve clinically healthy crossbred cows in mid-lactation were selected and screened for both clinical and subclinical mastitis using the California Mastitis Test (CMT); only mastitis-free animals were included in the study. Milking was carried out twice daily (morning and evening) under uniform feeding, housing, and management conditions. Four pre-milking udder wash treatments were evaluated: T_0 (clean water wash, control), T_1 (Marigold leaf extract prepared using 20 g fresh leaves per litre of water), T_2 (Marigold leaf extract prepared using 40 g fresh leaves per litre of water), and T_3 (Marigold leaf extract prepared using 60 g fresh leaves per litre of water). For extract preparation, freshly collected marigold leaves were thoroughly washed, boiled in one litre of water for 10 minutes, cooled to room temperature, and filtered before use. Prior to milking, the udder and teats of each animal were washed with the respective treatment solution and allowed a contact time of approximately one minute, followed by drying with a clean and separate cloth for each cow to prevent cross-contamination. Milk samples were aseptically collected immediately after milking in sterile containers and transported to the laboratory under refrigerated conditions. Each treatment was replicated ten times through repeated milk sampling. Microbiological quality of milk was assessed using standard procedures described by Chalmers (1953) [3] for Standard Plate Count (SPC), Lactic Acid Bacteria Count (LABC), Proteolytic Bacteria Count (PBC), Lipolytic Bacteria Count (LBC), and Coliform Count. The data obtained were analyzed using one-way analysis of variance (ANOVA), and treatment means were compared using the Critical Difference (CD) test at the 5% level of significance as described by Snedecor and Cochra. Results are expressed as Mean±Standard Error (SE).

Results

Standard Plate Count (SPC)

The Standard Plate Count (SPC) values of raw milk under different pre-milking udder wash treatments are presented in Table 1. Statistical analysis showed that SPC differed significantly ($p<0.05$) among the treatments. The control group (T_0) recorded the highest SPC (36.5±0.42 $\times10^3$/ml). A progressive reduction in SPC was observed in all Marigold-treated groups, with the lowest value recorded in T_3 (31.7±0.42 $\times10^3$/ml).

Lactic Acid Bacteria Count (LABC)

The lactic acid bacteria count (LABC) of raw milk differed significantly ($p<0.05$) among the treatment groups. The highest LABC was observed in the control group (28.4±0.44 $\times10^2$/ml), whereas the lowest count was recorded in T_3 (26.3±0.44 $\times10^2$/ml). Treatments T_1 and T_2 showed intermediate values.

Proteolytic Bacteria Count (PBC)

Proteolytic bacteria count showed significant ($p<0.05$) variation among treatments. The control group recorded the highest PBC (27.5±0.58 $\times10^2$/ml), while the lowest value was observed in T_3 (21.4±0.58 $\times10^2$/ml). A gradual decline in proteolytic bacteria count was evident with increasing concentration of Marigold leaf extract.

Lipolytic Bacteria Count (LBC)

Lipolytic bacteria count also differed significantly ($p<0.05$) among treatments. The highest LBC was recorded in the control group (30.2±0.33 $\times10^2$/ml), whereas the lowest count was observed in T_3 (26.6±0.33 $\times10^2$/ml). All Marigold-treated groups showed lower lipolytic bacterial counts compared to the control.

Coliform Count

Coliform bacteria were not detected in any of the milk samples collected from all treatment groups.

Table 1: Effect of Marigold udder wash on bacterial counts of raw milk

Treatment	SPC ($\times10^3$/ml)	LABC ($\times10^2$/ml)	PBC ($\times10^2$/ml)	LBC ($\times10^2$/ml)
T_0	36.50±0.42^a	28.40±0.44^a	27.50±0.58^a	30.20±0.33^a
T_1	33.00±0.42^b	26.80±0.44^b	23.60±0.58^b	28.30±0.33^b
T_2	33.00±0.42^b	27.10±0.44^b	23.20±0.58^b	28.90±0.33^b
T_3	31.70±0.42^c	26.30±0.44^c	21.40±0.58^c	26.60±0.33^c

Means bearing different superscripts within a column differ significantly ($p<0.05$).

Graphical representation of SPC reduction across treatment groups is shown below.

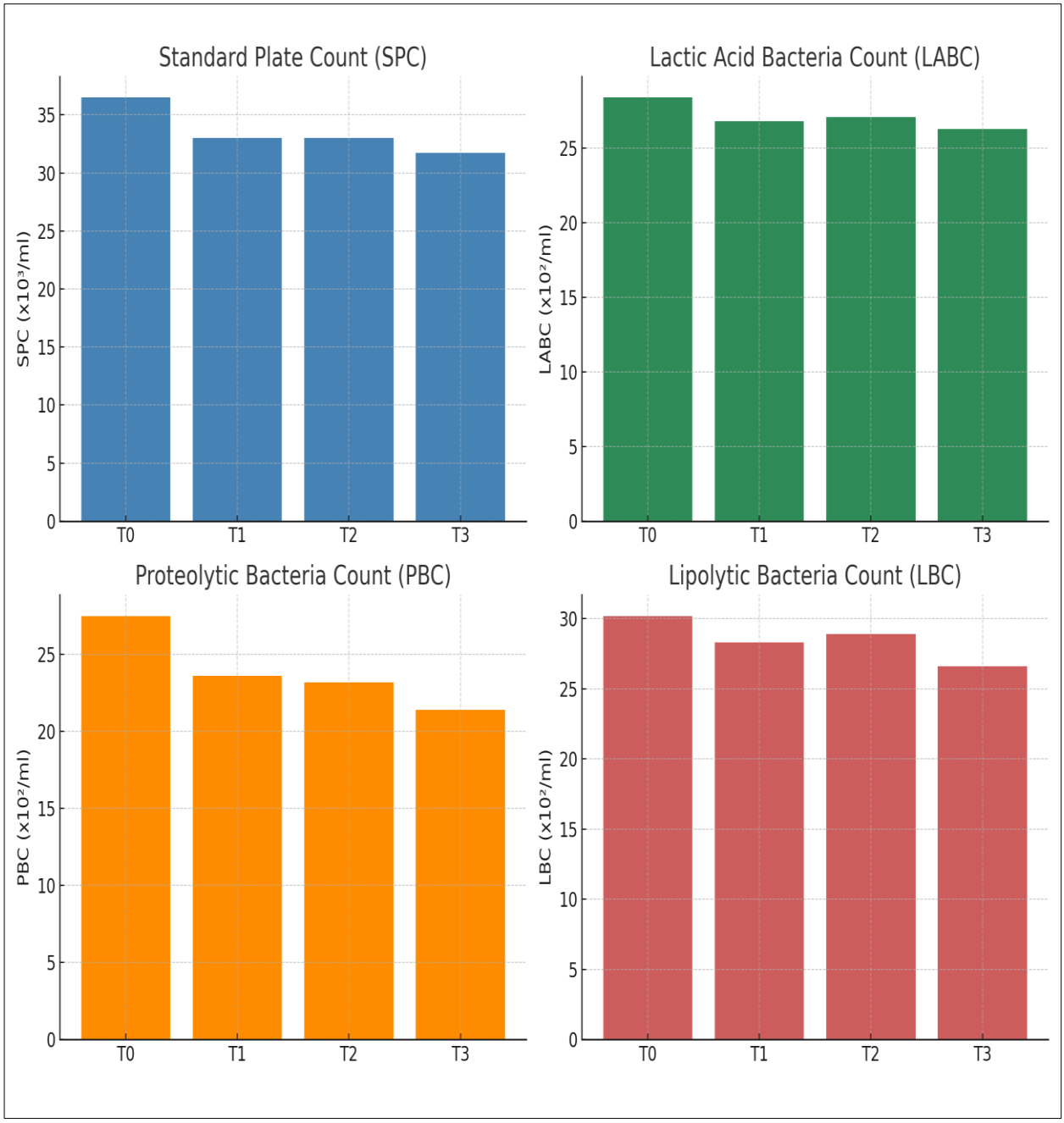

Fig 1: Effect of Marigold-Based Udder Wash Treatments on Microbial Quality of Milk

Discussion

The present study demonstrated that the application of Marigold leaf extract as a pre-milking udder wash significantly improved the bacteriological quality of raw milk in crossbred cows. The significant reduction in Standard Plate Count observed in Marigold-treated groups compared to the control indicates the effectiveness of this herbal udder hygiene practice in reducing microbial contamination at the point of milking.

The progressive decline in bacterial counts with increasing concentration of Marigold leaf extract suggests a dose-dependent antimicrobial effect. Higher concentrations of the extract likely provided greater amounts of bioactive phytochemicals such as flavonoids, saponins and phenolic compounds, which are known to inhibit bacterial growth by disrupting cell membranes and enzymatic activity. Similar reductions in Standard Plate Count following the use of herbal udder wash solutions have been reported earlier by Pandey and Prasad (2001) [6] and Aneja (1992) [1].

The reduction in lactic acid bacteria, proteolytic bacteria and lipolytic bacteria is of particular significance, as these physiological groups are responsible for spoilage of milk through degradation of lactose, proteins and fats, leading to off-flavours and reduced shelf life. Improved udder hygiene has been reported to lower these bacterial populations and enhance the keeping quality of raw milk (Rajorhia, 2001; Sharma and Sharma, 2001) [7, 8].

The lowest proteolytic and lipolytic bacterial counts observed in the highest concentration treatment indicate that Marigold leaf extract was effective in suppressing spoilage-causing microorganisms. The antimicrobial activity of *Calendula officinalis* against spoilage and pathogenic bacteria has also been documented in earlier studies (Burtscher et al., 2023) [2].

The absence of coliform bacteria across all treatments reflects satisfactory farm hygiene and effective udder sanitation practices. Overall, the findings of the present study support the potential use of Marigold leaf extract as a

natural, economical and eco-friendly alternative to chemical udder disinfectants for improving milk hygiene under farm conditions.

Conclusion

The present study demonstrated that the application of Marigold (*Calendula officinalis*) leaf extract as a pre-milking udder wash significantly improved the bacteriological quality of raw milk in crossbred cows. Among the different concentrations evaluated, the 60 g per litre treatment was found to be the most effective in reducing total bacterial load as well as various physiological groups of bacteria. The findings indicate that Marigold leaf extract can be recommended as a safe, economical and eco-friendly alternative to chemical disinfectants for improving udder hygiene and promoting clean milk production under farm conditions.

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