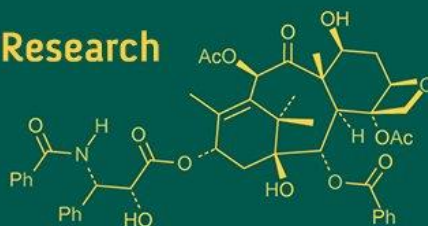


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Dr. Sakshi Vijay Tirpude
Department of Livestock
Production and Management,
Post Graduate Institute of
Veterinary and Animal
Sciences, Akola, Maharashtra,
India

Dilip M Badukale
Department of Livestock
Production and Management,
Post Graduate Institute of
Veterinary and Animal
Sciences, Akola, Maharashtra,
India

Girish J Panchbhai
Department of Livestock
Production and Management,
Post Graduate Institute of
Veterinary and Animal
Sciences, Akola, Maharashtra,
India

Prashant Mohanrao Sathe
Department of Livestock
Production and Management,
Post Graduate Institute of
Veterinary and Animal
Sciences, Akola, Maharashtra,
India

Corresponding Author:
Dr. Sakshi Vijay Tirpude
Department of Livestock
Production and Management,
Post Graduate Institute of
Veterinary and Animal
Sciences, Akola, Maharashtra,
India

Total viable count and ABST of milk in cows with subclinical mastitis

Sakshi Vijay Tirpude, Dilip M Badukale, Girish J Panchbhai and Prashant Mohanrao Sathe

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Abstract

Subclinical mastitis adversely affects milk quality and dairy productivity, while post-milking teat disinfection is an important measure for reducing bacterial contamination. The present study evaluated the effect of an essential oil-based teat dip on the total viable count of milk from crossbred cows affected with subclinical mastitis. Thirty-six lactating crossbred cows were selected and randomly allotted to three groups of 12 animals each. The control group received distilled water, while the second and third groups received 1% iodine and an essential oil-based teat dip, respectively. The essential oil formulation contained eucalyptus, lavender, peppermint and tea tree oils. Total viable count was estimated by serial dilution and pour plate technique at 10^{-3} , 10^{-4} and 10^{-5} dilutions. At the 10^{-3} dilution, post-treatment counts were 36.00 ± 0.32 , 25.83 ± 0.20 and 24.08 ± 0.22 in the distilled water, iodine and essential oil groups, respectively. At the 10^{-4} dilution, corresponding values were 34.16 ± 0.27 , 24.16 ± 0.20 and 22.16 ± 0.20 , whereas at the 10^{-5} dilution, the values were 32.33 ± 0.25 , 21.91 ± 0.31 and 20.75 ± 0.25 , respectively. The lowest post-treatment counts were consistently observed in the essential oil-treated group. The essential oil blend also exhibited antibacterial activity against *Streptococcus* spp. and *Escherichia coli*, with maximum inhibition zones of 15.50 ± 0.42 mm and 14.50 ± 0.42 mm, respectively, in the undiluted preparation. The findings indicated that the essential oil-based teat dip was associated with lower post-treatment viable bacterial counts and demonstrated *in vitro* antibacterial activity against the tested organisms.

Keywords: Antibacterial activity, essential oils, subclinical mastitis, teat dip, total viable count

Introduction

Subclinical mastitis is an important disease affecting dairy animals and is associated with reduced milk quality and economic losses. Unlike clinical mastitis, the disease may occur without visible abnormalities in milk or the udder, making early detection and preventive management particularly important.

Post-milking teat disinfection is an important component of mastitis prevention. Conventional chemical disinfectants such as iodine are commonly used to reduce microbial contamination and minimise the risk of new intramammary infections. However, increasing interest in natural products has encouraged the investigation of plant-derived compounds as alternative antimicrobial agents.

Essential oils contain biologically active compounds that may exhibit antimicrobial activity against a variety of microorganisms. Eucalyptus, lavender, peppermint and tea tree oils have individually been investigated for their antimicrobial properties. Their incorporation into a post-milking teat dip may therefore provide a plant-based approach for reducing bacterial contamination associated with the teat surface and milk.

Total viable count is commonly used as an indicator of the viable bacterial population present in milk. Therefore, the present study was undertaken to evaluate the effect of an essential oil-based post-milking teat dip on the total viable count of milk from crossbred cows affected with subclinical mastitis. The antibacterial activity of the essential oil blend against *Streptococcus* spp. and *Escherichia coli* was also evaluated.

Materials and Methods

Experimental animals and study period

The experiment was conducted at Shri Gaurakshan Sanstha, Akola, Maharashtra, for 90 days from 13 November 2024 to 17 February 2025.

Thirty-six lactating crossbred cows affected with subclinical mastitis were selected for the experiment. The selected cows were randomly divided into three groups containing 12 animals each.

Experimental treatments

The treatment groups were as follows

T₀: Distilled water post-milking teat dip

T₁: 1% iodine post-milking teat dip

T₂: Essential oil-based post-milking teat dip

The essential oil-based teat dip was prepared using 0.5 ml each of eucalyptus, lavender, peppermint and tea tree oils. The oils were mixed with 2 ml liquid soap and two drops of glycerine, and the volume was made up to 100 ml with distilled water.

Collection of milk samples

Milk samples were collected aseptically from the experimental animals. The initial streams of milk were discarded, and approximately 50 ml of milk was collected in sterile containers. Samples were labelled and transported under suitable conditions for microbiological examination.

Estimation of total viable count

Total viable count was estimated by serial ten-fold dilution and pour plate technique. Milk samples were serially diluted, and aliquots from the selected dilutions were inoculated into sterile Petri plates. Molten agar was added to the plates, mixed thoroughly and allowed to solidify. The plates were incubated at 35-37 °C for 24 h. Following incubation, visible colonies were counted.

Total viable count observations were recorded at 10⁻³, 10⁻⁴ and 10⁻⁵ dilutions for pre-treatment and post-treatment milk samples.

Antibacterial activity of the essential oil blend

The antibacterial activity of the essential oil blend was evaluated against *Streptococcus* spp. and *Escherichia coli*. The blend was tested in undiluted form and at 1:2, 1:4, 1:8, 1:16 and 1:32 dilutions. Antibacterial activity was assessed by measuring the zone of inhibition.

Statistical analysis

The experimental data were analysed using IBM SPSS Statistics version 22. The data were subjected to appropriate statistical analysis, and mean values with their standard errors were calculated. Analysis of variance and Duncan's multiple range test were used for comparison among treatment means. Pre-treatment and post-treatment observations were also compared using the t-test.

Results

Total viable count

The total viable counts observed before and after treatment at different dilutions are presented in Table 1.

At the 10⁻³ dilution, the pre-treatment total viable counts were 28.91 ± 0.25, 29.66 ± 0.28 and 29.33 ± 0.35 in T₀, T₁ and T₂, respectively. The post-treatment counts were 36.00 ± 0.32, 25.83 ± 0.20 and 24.08 ± 0.22, respectively. The lowest post-treatment count was recorded in T₂.

At the 10⁻⁴ dilution, the pre-treatment total viable counts were 27.33 ± 0.25, 27.25 ± 0.17 and 27.83 ± 0.38 in T₀, T₁ and T₂, respectively. The corresponding post-treatment

counts were 34.16 ± 0.27, 24.16 ± 0.20 and 22.16 ± 0.20. The lowest post-treatment count was again recorded in T₂.

At the 10⁻⁵ dilution, the pre-treatment counts were 25.91 ± 0.25, 25.83 ± 0.20 and 25.16 ± 0.24 in T₀, T₁ and T₂, respectively. The post-treatment counts were 32.33 ± 0.25, 21.91 ± 0.31 and 20.75 ± 0.25, respectively. The essential oil-treated group recorded the lowest post-treatment count.

The post-treatment values differed significantly among the treatment groups at all three dilutions. However, the paired comparison between pre-treatment and post-treatment observations within the individual groups was reported as non-significant.

Antibacterial activity of the essential oil blend

The antibacterial activity of the essential oil blend against *Streptococcus* spp. and *Escherichia coli* is presented in Table 2.

The undiluted essential oil blend produced the maximum zone of inhibition against *Streptococcus* spp. and *Escherichia coli*, measuring 15.50 ± 0.42 mm and 14.50 ± 0.42 mm, respectively. The inhibition zones decreased progressively with increasing dilution of the essential oil blend.

Against *Streptococcus* spp., inhibition zones of 12.50 ± 0.42, 10.50 ± 0.42 and 8.83 ± 0.30 mm were observed at 1:2, 1:4 and 1:8 dilutions, respectively. No zone of inhibition was observed at 1:16 and 1:32 dilutions.

Against *Escherichia coli*, inhibition zones of 10.50 ± 0.42, 8.50 ± 0.42 and 7.00 ± 0.25 mm were observed at 1:2, 1:4 and 1:8 dilutions, respectively. No zone of inhibition was observed at 1:16 and 1:32 dilutions.

Discussion

The present study demonstrated lower post-treatment total viable counts in the iodine-treated and essential oil-treated groups compared with the distilled water control. Among the treatment groups, the essential oil-based teat dip consistently recorded the lowest post-treatment count at 10⁻³, 10⁻⁴ and 10⁻⁵ dilutions.

The increase in total viable count in the distilled water control group indicated that distilled water alone did not provide an antimicrobial effect. In contrast, the lower post-treatment counts in the iodine and essential oil groups suggested an association between post-milking antimicrobial teat treatment and reduced viable bacterial counts.

The lowest counts recorded in the essential oil-treated group may be associated with the antimicrobial properties of the constituent essential oils. The formulation contained eucalyptus, lavender, peppermint and tea tree oils, which together demonstrated measurable antibacterial activity against both *Streptococcus* spp. and *Escherichia coli* in the present investigation.

The *in vitro* findings supported the microbiological observations. The undiluted essential oil blend produced the largest zones of inhibition against both tested bacterial organisms. The progressive reduction in the zone of inhibition with increasing dilution demonstrated a concentration-dependent antibacterial response.

Previous studies cited in the thesis have also evaluated plant-derived and essential oil-based teat preparations for their potential role in reducing microbial contamination and improving udder health. The present results were consistent with the concept that plant-derived antimicrobial

preparations may have potential application as post-milking teat disinfectants.

An important consideration in interpreting the present findings is the statistical outcome of the pre-treatment and post-treatment comparison. Although lower numerical values were consistently recorded in the essential oil-treated

group after treatment, the paired t-test comparison within the groups was reported as non-significant. Therefore, the results indicate a favourable treatment-associated pattern rather than establishing a statistically significant pre-treatment to post-treatment reduction within the essential oil group.

Table 1: Total viable count of milk samples before and after treatment at different dilutions

Dilution	Observation	T ₀	T ₁	T ₂	Overall mean
10 ⁻³	Pre-treatment	28.91 ± 0.25	29.66 ± 0.28	29.33 ± 0.35	29.30 ± 0.17
	Post-treatment	36.00 ± 0.32 ^c	25.83 ± 0.20 ^b	24.08 ± 0.22 ^a	28.63 ± 0.89
10 ⁻⁴	Pre-treatment	27.33 ± 0.25	27.25 ± 0.17	27.83 ± 0.38	27.47 ± 0.16
	Post-treatment	34.16 ± 0.27 ^c	24.16 ± 0.20 ^b	22.16 ± 0.20 ^a	26.83 ± 0.89
10 ⁻⁵	Pre-treatment	25.91 ± 0.25	25.83 ± 0.20	25.16 ± 0.24	25.63 ± 0.14
	Post-treatment	32.33 ± 0.25 ^c	21.91 ± 0.31 ^b	20.75 ± 0.25 ^a	25.00 ± 0.89

Values are expressed as mean ± standard error. Means bearing different superscripts within the same row differed significantly at $p < 0.01$. The paired pre-treatment and post-treatment comparisons within the individual groups were non-significant.

Table 2: Antibacterial activity of the essential oil blend against *Streptococcus* spp. and *Escherichia coli*

Dilution	<i>Streptococcus</i> spp.	<i>Escherichia coli</i>
Undiluted	15.50 ± 0.42 ^d	14.50 ± 0.42 ^d
1:2	12.50 ± 0.42 ^c	10.50 ± 0.42 ^c
1:4	10.50 ± 0.42 ^b	8.50 ± 0.42 ^b
1:8	8.83 ± 0.30 ^a	7.00 ± 0.25 ^a
1:16	No zone	No zone
1:32	No zone	No zone

Values are expressed as mean ± standard error. Different superscripts within the same column indicate significant differences at $p < 0.01$.

Conclusion

The essential oil-based teat dip containing eucalyptus, lavender, peppermint and tea tree oils recorded the lowest post-treatment total viable counts among the experimental groups at 10⁻³, 10⁻⁴ and 10⁻⁵ dilutions. The essential oil blend also demonstrated concentration-dependent antibacterial activity against *Streptococcus* spp. and *Escherichia coli*.

The findings indicated the potential of the tested essential oil formulation as a natural post-milking teat disinfectant. However, because the paired pre-treatment and post-treatment comparisons within the groups were reported as non-significant, further studies involving larger sample sizes and repeated observations are required to confirm its microbiological efficacy.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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