International Journal of Advanced Biochemistry Research

ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2025): 5.29
IJABR 2025; 9(10): 111-115
www.biochemjournal.com
Received: 26-07-2025
Accepted: 30-08-2025

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Effect of essential oil-based teat spray on sub-clinical mastitis and milk yield in dairy cows

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DOI: https://www.doi.org/10.33545/26174693.2025.v9.i10b.5899

Abstract
The present study was conducted at Gorakshan Sabha, Dhantoli, Nagpur, for 90 days to study the "Effect of essential oil-based teat spray on sub-clinical mastitis and milk yield in dairy cows". 71 dairy cows were screened using the Modified California Mastitis Test (MCMT), of which 24 tested positive and were randomly allocated into three treatment groups (T_1, T_2, and T_3) of eight cows each. T_1 received 2% of essential oil-based teat dip (post-milking), T_2 received 2% of essential oil-based teat spray (post-milking), and T_3 received 1% of essential oil-based teat spray (post-milking). The essential oils used included eucalyptus, lavender, peppermint, and tea tree. Milk samples were collected on days 0, 15, 30, 45, 60, 75, and 90 to evaluate Somatic Cell Count (SCC) and milk yield. Results showed a significant reduction in SCC and a gradual increase in milk yield across all groups, with the most notable improvement in T_2. These findings highlight the potential of essential oil-based teat sprays as an effective, natural alternative for managing subclinical mastitis.

Keywords: Subclinical mastitis, essential oils, teat spray, somatic cell count, dairy cows, milk yield

1. Introduction
India's dairy sector is the backbone of its agriculture, significantly influencing rural livelihoods and the economy. It holds cultural, religious, and culinary importance, with India producing 239.30 million tonnes of milk annually (BAHS, 2024) [5] and boasting over 300 million bovines. Dairy production traces back 8,000 years to the domestication of zebu cattle, with milk being a staple since the Vedic period. Post-independence, the sector saw remarkable growth, aided by dairy cooperatives and Operation Flood (BAHS, 2023) [4]. India now contributes 25% of global milk production (PIB, 2024) [18]. Its total livestock population stands at 536.76 million, marking a 4.8% increase since 2012, with 95.78% in rural areas. The bovine population, which includes cattle, buffalo, Mithun, and yak, reached 303.76 million in 2019. The global demand for high-quality milk has spurred efforts to manage mastitis, a major issue for India's dairy sector, causing significant economic losses. Mastitis reduces daily income per cow by ₹306-335 (Wani *et al*., 2022) [37] and costs ₹1,390 per lactation, with losses stemming from decreased milk value and veterinary expenses. Treatment averages ₹509, encompassing medicine and service charges (Sinha *et al*., 2014) [32].

Preventive strategies focus on improved milking hygiene and post-milking teat care. However, reliance on antibiotics raises concerns about antimicrobial resistance (AMR), a global health threat as it hampers the effectiveness of treatments for bacterial infections (Palma *et al*., 2020) [22]. Indiscriminate antibiotic use in livestock contributes to AMR, altering gut microbiota and posing risks to public health. Udder health is integral to sustainable milk production (Neculai-Valeanu *et al*., 2022) [19]. Mastitis, caused by bacteria, yeast, or algae, leads to reduced milk yield and quality and poses significant financial challenges for farmers. Understanding mastitis is complex due to its varied causative agents (Rathaur *et al*., 2020) [26].

Teat dipping, introduced in 1916 by Moak using pine oil solutions, remains a vital practice for preventing udder infections (Johns, 1966) [15]. Proper udder and teat management, prioritizing animal welfare, is essential for productivity and farm sustainability. Teats regulate milk flow and act as barriers against infections, influencing milk quality (Smolenski,

2018) [33]. Diseases affecting teats cause discomfort and increase mastitis susceptibility, reducing milk production and composition quality, raising treatment costs, and leading to early culling. Mastitis pathogens are categorized as contagious (e.g., Staphylococcus aureus, Streptococcus agalactiae) or environmental (e.g., Escherichia coli, Streptococcus uberis, Streptococcus dysgalactiae) (Zigo *et al*., 2021) [39]. Contaminated milk, antibiotic residues, and chronically infected cows exacerbate financial losses. Post-milking teat disinfection minimizes infection risk, involving germicidal solutions like iodine, chlorhexidine, and sodium hypochlorite (Hemling *et al*., 2002; Nickerson, 2001) [14, 20]. Limitations of chemical agents include costs, availability, and residue concerns in milk products. Essential oils offer a natural alternative to chemical agents due to their antimicrobial, anti-inflammatory, antioxidant, and skin-conditioning properties (Adorjan and Buchbauer, 2010) [1]. They are safe for teat application, with no bacterial resistance reported (Rasool *et al*., 2021) [25]. Eucalyptus camaldulensis oil inhibits Gram-positive (S. aureus) and Gram-negative bacteria (E. coli, Streptococcus spp.) (Sabo and Knezevic, 2019) [27]. Peppermint oil is effective against fungi (candida) and bacteria (Mahboubi and Kazempour, 2014; Balakrishnan, 2015) [16, 3], while lavender and tea tree oils target various pathogens (Cavanagh and Wilkinson, 2002; Yadav *et al*., 2017) [9, 38]. Essential oils present a promising solution for managing subclinical mastitis without antibiotic residues, ensuring sustainable dairy practices.

2. Materials and Methods

2.1 Location of the Experiment: The present study was conducted at the Gorakshan Sabha Gaushala, Dhantoli, in Nagpur district, Maharashtra.

2.2 Screening and Selection of Animals

For the study, 71 cows were screened for mastitis by CMT after screening the samples from the animals, they were assessed for subclinical mastitis by determining the Somatic Cell Count (SCC). The somatic cells were determined by manually counting as described by (Rana *et al*., 2023) [24], and the animals having SCC between 2×10^5 to 5.0×10^5 cells/ml were selected for experimentation. A total of 24 lactating cows in the mid-lactation period were selected, and they were distributed randomly in three experimental groups.

The study utilized the Modified California Mastitis Test (MCMT) following the procedures outlined by Schalm and Noorlander (1957) [28] and Bhatnagar and Malhotra (1969) [6]. The test was performed in the milking shed before milking on day 0 of the experiment. A plastic paddle with four shallow cups (marked LF, LH, RF, and RH) was used to screen for subclinical mastitis.

Fore milk samples (2-3 ml) from each udder quarter were collected in the respective cups, followed by an equal amount (2-3 ml) of MCMT reagent. The paddle was gently rotated to mix the contents, and visual observations were made. Gel formation indicated a positive result, signaling subclinical mastitis in the corresponding quarter, while a watery solution indicated a negative result. Out of 71 cows tested, 25 were MCMT-positive, and 24 cows were selected for experimentation.

2.3 Experimental Design

T_1: 2.0 ml EO-based teat dip post-milking
T_2: 2.0 ml EO-based teat spray post-milking
T_3: 1.0 ml EO-based teat spray post-milking

2.4 Preparation of essential oil-based teat dip Solution

An aqueous solution of essential oils was prepared by mixing 2-3 ml of liquid soap, 2-4 drops of Tween 20 as a stabilizer, and 2.0 ml Essential oils (0.5ml of Eucalyptus oil, 0.5 ml of Lavender oil, 0.5ml of Peppermint oil and 0.5ml of Tea tree oil), and distilled water to make it 100 ml volume.

2.5 Preparation of essential oil-based teat spray Solution

An aqueous solution of essential oils spray was prepared by mixing,

1. 2-3 ml of liquid soap
2. 2-4 drops of Tween 20
3. (a) Essential oil 0.5 ml each of: Eucalyptus oil, Lavender oil, Peppermint oil, and Tea tree oil for T_2 Group
   (b) Essential oil 0.25 ml each of: Eucalyptus oil, Lavender oil, Peppermint oil, and Tea tree oil for T_3 Group
4. Distilled water to make it 100 ml volume.

2.6 Somatic Cell Count

The SCC was determined by manually counting as described by Rana *et al*. (2023) [24]. To prepare the sample, 20 ml of milk and 10 ml of Phosphate buffer solution (PBS) were taken in a Centrifuge tube, which was then subjected to a Centrifuge machine at 4000 rpm for 15 minutes at 25°C. After centrifugation, the supernatant was removed by Pasteur pipette, and in the remaining pellet, 10 ml PBS was added again to remove fats and centrifuge at 8000 rpm for 5 minutes. After the process of centrifugation, the supernatant was removed and 2 ml PBS was added again in a fatless pallet and left undisturbed for 5 minutes. 50 µl of methylene blue dye was added to 50 µl of fatless mixture in Eppendorf serum tubes and left undisturbed for 10 to 15 minutes. With the help of a micropipette inject 50 µl of sample in a Neubauer chamber and observe the sample under a microscope.

3. Results

3.1 Somatic Cell Count (SCC)

The results depicted in Table 1 showed a non-significant ($p<0.05$) value of SCC on days 0^{th} was observed for the Groups T_1, T_2, and T_3. The values for Groups T_1, T_2, and T_3 were 4.0±0.15, 4.125±0.16, and 4.35±0.13, respectively, which reveals that all the experimental animals exhibit sub-clinical mastitis. With the progress of the treatment, there was a decrease in the somatic cell count every fortnight i.e. on the 15^{th}, 30^{th}, 45^{th}, 60^{th}, $75^{th,}$ and 90^{th} day in all the groups.

All treatment groups T_1, T_2, and T_3, showed statistically significant ($p<0.05$) reductions in somatic cell count from day 0^{th} to day 90^{th} within the rows.

Group T_1 had shown non-significant ($p<0.05$) difference within the treatment group till day 15^{th}. However, a significant difference within group T_1 was observed between day 30^{th} and day 45^{th}. Also, a significant difference ($p<0.05$) was observed between day 45^{th} and day 60^{th}. However, no significant difference ($p<0.05$) was observed between day

60th and day 75th for group T_1. Further, a significantly lower SCC ($p<0.05$) was also observed for group T_1 between day 75th and day 90th.

For group T_2, a non-significant ($p<0.05$) difference within the treatment group was observed between day 0th and day 15th. However, a significant difference ($p<0.05$) within group T_2 was observed between day 15th and day 30th. Also, a significant difference ($p<0.05$) was observed between day 30th and day 45th. Similarly, a significant difference ($p<0.05$) was also observed for group T_2 between day 75th and day 90th. However, no significant ($p<0.05$) difference in SCC in group T_2 was observed between day 45th and day 60th; further, no significant ($p<0.05$) difference in SCC in group T_2 was observed between day 75th and day 90th. For group T_3 a non-significant ($p<0.05$) difference within the treatment group was observed between day 0 and day 15. However, significantly lower SCC values ($p<0.05$) within group T_2 were observed between day 15 and day 30. Also, significantly lower values for SCC ($p<0.05$) were observed between day 30 and day 45. Further, with the advancement of the experiment, there is a reduction in the SCC on days 60, 75, and 90. However, no significant ($p<0.05$) difference in SCC in group T_3 was observed between day 45, day 60, day 75, and day 90.

3.2 Milk Yield

The data in Table 2 revealed that there was no significant ($p<0.05$) difference in milk yield for groups T_1 (10.56±0.23), T_2 (10.71±0.40), and T_3 (10.9±0.33) at the initiation of the experiment. All three groups have shown an increase in milk yield over the experimentation period.

A non-significant difference was observed within the group for Group T_1, Group T_2, and Group T_3 throughout the experimentation period. However, all three experimental Groups have shown an increase in the milk yield during the experimentation period.

4. Discussion

4.1 Somatic Cell Count (SCC)

Significant difference ($p<0.05$) in Group T_1 (2.77±0.12) and T_2 (2.9±0.16) and Group T_1 (2.77±0.12) and T_3 (3.45±0.09) was observed from day 45th. A significant difference ($p<0.05$) in Group T_1 and T_2 and Group T_1 and T_3 continues up to day 90th with values as 1.97±0.04, 1.95±0.07, and 1.97±0.04, 2.25±0.07 in Group T_1 and T_2 and Group T_1 and T_3 respectively, whereas, the non-significant difference was observed in values of Group T_1 (1.97±0.04) and T_2 (1.95±0.07). The present findings were in line with the findings of Deshmukh (2023) [10] who reported a decrease in SCC in groups treated with Essential Oil (EO)-based teat dip and Iodine-based teat dip. The readings of SCC in Groups T_1 and T_2 show that the animals have recovered from subclinical mastitis on the 90th day of experimentation. The results of in line with the findings of Rasool *et al*., (2021) [25] who reported a decrease in SCC of the treatment group in dairy cows on the application of EO based post milking teat dips (Eucalyptus oil and lavender oil with dimethyl sulphoxide). Rathaur *et al*., (2020) [26] reported a significant decrease in SCC on the 10th day of treatment with the application of herbal paste (turmeric, castor oil, lemon, and aloe vera) after milking in cows with subclinical mastitis. Similarly, Waghmare *et al*., (2013) [36], and Vala *et al*., (2013) [35] also reported a significant reduction in the SCC in cows on the application of Mastidip (*Berberis lyceum*, *Curcuma longa*, *Eucalyptus globulus*) post-milking teat dip. Biradar *et al*., (2022) [7] also reported a significant decrease in somatic cell count on the 60th day with the application of Aloe vera-based teat dip in HF crossbred cows positive for subclinical mastitis.

The somatic cells in milk include Macrophages, Neutrophils, Epithelial cells, and Mononuclear cells whereas Neutrophils and PMN Leukocytes act as the second line of defense in the mammary gland and they act as both morphological and chemical barriers for pathogens. Macrophages are found abundant (66-88%) in the mammary gland, they identify the pathogens and signal the leukocytes and immune cells resulting in the accumulation of leucocytes and immune cells in mammary glands and thereby increasing the count of somatic cells (Alhussien & Dang (2018) [2], Orlandini & Bijgaart (2011) [21], and Harmon, 1994) [13].

On the 90th day, all the groups showed a decrease in SCC, however, the lowest values were observed in Group T_2 (1.95±0.04) followed by Group T_1 (1.97±0.04) and Group T_3 (2.25±0.07), indicating better efficacy of 2% EO-based Teat spray over 2% EO-based teat dip and 1% EO-based Teat spray.

4.2 Milk yield

The increase in milk yield can be attributed to the fact that essential oils have antimicrobial, antibacterial anti-inflammatory activities (Tomanic *et al*. 2022; Hammer *et al*., 2006; Carson *et al*., 2006) [34, 12, 8], which have led to the alleviation of sub-clinical mastitis conditions. It was also to be noted that the experiment started in the mid-lactation period for a total of 90 days and hence it tended towards late lactation, thereby not increasing the milk yield. The result revealed that the differences among different groups for milk yield were non-significant on the 90th day of the experiment.

A decrease in milk yield due to bovine subclinical mastitis was reported by (Gonçalves *et al*., 2018) [11]. The results of the present study are in line with Rasool *et al*. (2021) [25], who found that using essential oils (lavender and eucalyptus) as a post-milking teat dip in dairy cows with subclinical mastitis led to a significant increase in milk yield. According to Waghmare *et al*. (2013) [36] and Vala *et al*. (2013) [35], subclinical mastitis in cows' milk yield increased after receiving Mastidip herbal teat dip for 30 or 60 days. According to Patil *et al*. (2014) [23], the subclinical mastitis cows' milk yield increased gradually on the 14th day after using Mastidip herbal teat dip, which contains herbal components of *Eucalyptus globus*, *Curcuma longa*, and *Berberis lyceum*.

In the present study, Essential oil blends containing eucalyptus, peppermint, tea tree, and lavender were used to treat dairy cows with subclinical mastitis. Pathogens that cause subclinical mastitis are characterised by tissue alterations that slowly affect the mammary system, leading to reduced milk production (Sharif and Muhammad, 2008; Sharma *et al*., 2011) [30, 31]. The essential oils have anti-microbial, anti-viral, anti-mutagenic, anti-cancer, antioxidant, anti-inflammatory, immunomodulatory, and tick-repellent activities (Meenu *et al*., 2023; Tomanic *et al*., 2022; Serafino *et al*., 2008) [17, 34, 29], which may enhance udder health and improve the milk yield, and their properties of essential oils might have been reflected in the present study.

Table 1: Mean ± SE values of dairy cows' somatic cell count (×10^5 cells/ml) under different treatment groups.

Groups	0th Day	15th Day	30th Day	45th Day	60th Day	75th Day	90th Day	CD
T_1	4.00^{e}±0.15	3.62^{de}±0.18	3.40^{d}±0.18	2.77^{Bc}±0.12	2.10^{Cb}±0.1	2.05^{Bb}±0.05	1.97^{Ba}±0.04	0.291
T_2	4.12^{e}±0.16	4.12^{cd}±0.16	3.65^{c}±0.15	2.90^{Bb}±0.16	2.47^{Bb}±0.1	2.22^{Ba}±0.08	1.95^{Ba}±0.07	0.312
T_3	4.35^{c}±0.13	4.17^{c}±0.12	3.85^{b}±0.10	3.45^{Aa}±0.09	3.02^{Aa}±0.05	2.55^{Aa}±0.07	2.25^{Aa}±0.07	0.205
CD				0.381	0.323	0.212	0.192	

The mean bearing different superscripts (A, B, C) within the column differ significantly (p<0.05)
The mean bearing different superscripts (a, b, c, d, e) within the row differ significantly (p<0.05)

Table 2: Mean ± SE values of dairy cows' milk yield (kg) under different treatment groups.

Groups	0th Day	15th Day	30th Day	45th Day	60th Day	75th Day	90th Day
T_1	10.56±0.23	10.56±0.23	10.58±0.23	10.58±0.21	10.63±0.18	10.70±0.26	11.01±0.25
T_2	10.71±0.40	10.71±0.40	10.87±0.34	10.98±0.34	11.05±0.31	11.13±0.33	11.17±0.34
T_3	10.90±0.33	10.91±0.33	10.85±0.32	10.91±0.30	10.96±0.32	10.97±0.30	11.00±0.30

6. Conclusion

The teat spray, composed of Eucalyptus, Lavender, Tea tree, and Peppermint oils, significantly (P<0.05) reduced somatic cell count (SCC), alleviating subclinical mastitis. Demonstrating its effectiveness in enhancing udder health. While milk yield showed no significant difference (P<0.05) between groups treated with 2% EO-based teat spray (T_2) and 2% EO-based teat dip (T_1). The 2% EO-based teat spray emerged as the most cost-effective and labor-efficient option, requiring fewer refills, easier handling, and broader coverage of the udder.

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