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Teat-end Bacterial Load in Buffaloes with Subclinical Mastitis and its Amelioration using Indigenously Prepared Teat Dip

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ABSTRACT: Mastitis is a worldwide problem of dairy industry that has been the cause of concern from decades with no concrete preventive tool yet. Teat dips have been recommended but still not much in practice by our rural folk. This study determines the efficacy of some essential oils as an alternate to commercially available teat dips in reducing the teat end bacterial count and to optimize the time for post milking teat dips to reduce pathogen loads on teat skin. The percentage reduction of mastitis pathogens on teat skin with 3 teat dips: plain water, commercial antiseptic solution and mixture of cinnamon oil and tea tree oil at 30 second and 15 minute after post dipping was evaluated. The teat- end bacterial load ($\log_{10}$ TBC value) after washing of udder with water as pre-milking teat disinfectant was 7.814 $\log_{10}$ cfu/ml. Use of dettol as teat disinfectant reduces teat- end bacterial load ($\log_{10}$ TBC) to 6.59 $\log_{10}$ cfu/ml after 30 sec. of application of dettol disinfectant, but after 15 minute it again increased tolog10 TBC value 7.309 $\log_{10}$ cfu/ml. Teat dipping with the mixture of Cinnamon and Tea- tree oil reduced the teat- end bacterial load to log10 TBC value 6.759 $\log_{10}$ cfu/ml within 30 second and 6.944 $\log_{10}$ cfu/ml at 15 min. interval. It can be concluded that different mastitis pathogens may possess different sensitivities to teat dips, and essential oils of Cinnamon and tea tree oil can be effectively used as post milking teat dip. Furthermore, a 15 minute post-milking dip contact time for oil and 30 s for dettol dips may be optimal in reducing pathogen load.

Keywords: Mastitis, Teat disinfectant, Buffaloes, Cinnamon oil, Tea tree oil.

INTRODUCTION

Bovine mastitis, an inflammation of mammary gland, is one of the most devastating disease caused by variety of pathogens, such as *Staphylococcus aureus* (*S. aureus*), *Escherichia coli*, and *Streptococcus agalactiae*, *S. dysgalactiae*, *S. uberis* (Thompson *et al.*, 2014) and results in decreased milk quality and economic losses (Nickersonand Oliver 2014). The disease can be prevented by reducing the bacterial load on the teat surface which reduces the chances of mastitis. This can be effectively achieved by use of teat disinfection. Teat disinfection Pre- and post-milking is important to reduce the number of bacterial load on surface (Dufour *et al.*, 2011), reduce the possibility of mastitis and also the risk of bacterial contamination of milk (Suriyasathaporn and Chupia 2011; Zucali *et al.*, 2011). Alternative plant or herbal solutions that have been studied and are useful for teat dipping consist of *Morinda citrifolia* extract (Purwantiningsih *et al.*, 2017), star fruit leaf extract, or *Averrhoa bilimbi* Linn. (Julianto *et al.*, 2017), essential oils from *M. alternifolia* (Dore *et al.*, 2019) and leaf extracts of babadotan or *Ageratum conyzoides* (Mahpudin *et al.*, 2017). Study on the leaves of the cherry leaf (*Muntingia calabura* L.) as a teat dipping has been done by Kurniawan *et al.* (2013).

Treatment of bovine mastitis with alternative essential oils and plant-derived antimicrobials has been assessed *in vitro* on major bacterial and fungal mastitis pathogens (Dal Pozzo *et al.*, 2011 and Ksouri *et al.*, 2017). Essential oils are safe and, unlike antibiotics, no resistance has been reported after a prolonged exposure to bacteria (Dal Pozzo *et al.*, 2011). Furthermore, synergism between plant metabolites and antibiotics has been described by (Hemaiswarya *et al.*, 2008), which suggests the use of essential oils as adjuvant. Cinnamon (*Cinnamomum zeylanicum*) is one of the most influential antimicrobial medicinal plant belonging to Lauraceae family (Prabuseenivasan *et al.*, 2006). Cinnamon essential oil (CEO) has antimicrobial, antioxidant and anti-carcinogenic activities (Yuce *et al.*, 2012, Zouheyr *et al.*, 2014; Raeisi *et al.*, 2015).

Tea tree oil (TTO), an essential oil extracted from the leaves of *Melaleuca alternifolia* (*M. alternifolia*), also possesses antibacterial activity and anti-inflammatory properties (Low *et al.*, 2013). These studies determine the efficacy of essential oils as an alternate to commercially available teat dips in reducing the teat end bacterial count and to optimize the time for post milking teat dips to reduce pathogen loads on teat skin.

MATERIALS AND METHODS

Teat-end swabs collection and analysis of bacterial count. The study was conducted on 30 buffaloes divided in 3 groups of 10 animals each. In I Group Washing of udder with water, udder was dipped in essential oils (Cinnamom and Tea tree oil) in IInd group whereas III Group were washed with dettol. Teat-end swabs from buffaloes were collected individually from both hind quarters by rotating a moistened cotton swab, covering an area of 2 cm² outside the teat orifice. The first swab was taken immediately after udder washing with water (Group I)whereas in II and III Group swab were collected from quarter after applying with teat dip solution(mixture of Cinnamon and Tea tree oil) or (dettol) and removing the surplus teat dip solution. Teats were dipped in a cup of dipping solution and allowed to remain in contact for 30sec.to teat skin. First swab was collected after 30sec. applying teat-dip second after 15min. and subsequently dried using a dry and sterile towel to remove the surplus teat dip solution.

The teat-end swabs were placed in sterile test tube containing 0.1% peptone water and stored in ice container until analyzed. All teat-end swabs were analyzed for bacterial counts, *i.e.* total bacterial count (TBC), staphylococcal count (STA), streptococcal count (STR) and coli form count (COL) by Standard plate count by Pour plate method as per AOAC (Association of Official Analytical Chemists, 1990).

Statistical analysis: Statistical analysis of data was done by using SPSS 20 software.

RESULTS AND DISCUSSION

The teat end total bacterial count (log_{10}TBC) after washing of udder with water was 7.814 log_{10} cfu/ml. After 30 sec. (W_1) the teat end bacterial count (log_{10}TBC) decreased to of 6.939log_{10} cfu/ml but increased to 7.422 log_{10} cfu/ml after 15 min (W_2). In oil dipped group the teat end bacterial count (log_{10}TBC) decreased to 6.759log_{10} cfu/ml at 30 sec (O_1) but increased to 6.944 log_{10} cfu/ml after 15 min (O_2). In the standard antiseptic treatment group, the bacterial load at 30 second (D_1) & 15 min(D_2) was 6.591 log_{10} cfu/ml and 7.309 log_{10} cfu/ml respectively.

In control group, the log_{10} *E. coli* count was 7.386log_{10} cfu/ml. After 30 sec. (W_1) the log_{10} *E. coli* count was 6.326log_{10} cfu/ml but increased to 7.535 log_{10} cfu/ml after 15 min (W_2). In oil dipped group the count decreased to 6.328 log_{10} cfu/ml at 30 sec (O_1) but increased to log_{10} 6.591 log_{10} cfu/ml after 15 min(O_2).The standard antiseptic treatment group *E. coli* count at 30 sec (D_1) and 15 min(D_2) was 6.3404 log_{10} cfu/ml and 7.240log_{10} cfu/ml respectively.

In control group the Log_{10} *S. aureus* count was 7.209 log_{10} cfu/ml. After 30 sec.(W_1) the log_{10} *S. aureus* count decreased to 6.39 log_{10} cfu/ml but increased to log_{10} *S. aureus* 7.38 log_{10} cfu/ml after 15 min (W_2). In oil dipped group the count decreased to *S. aureus* 5.9 log_{10} cfu/ml at 30 sec (O_1) but increased to log_{10} *S. aureus* 6.0 log_{10} cfu/ml after 15 min(O_2). The standard antiseptic treatment group *S. aureus* count at 30 sec. (D_1) &15min (D_2) was log_{10} *S. aureus* 5.87 log_{10} cfu/ml and 5.79log_{10} cfu/ml respectively.

In control group the Log_{10} *Streptococcal* count was 5.477 log_{10} cfu/ml. After 30sec. (W_1) the log_{10} *Streptococcal* count was 6.42 log_{10} cfu/ml but increased to log_{10} *Streptococcal* count 7.260 log_{10} cfu/ml after 15 min (W_2). In oil dipped group the count decreased to log_{10} *Streptococcal* 6.42log_{10} cfu/ml at 30 sec (O_1) and increased to log_{10} *Streptococcal* 6.514 log_{10} cfu/ml after 15min(O_2). The standard antiseptic treatment group *Streptococcal* count at 30 sec. (D_1) &15min (D_2) was log_{10} *Streptococcal* 7.049 log_{10} cfu/ml and 6.839 log_{10} cfu/ml respectively (Table 1).

Data reveals that at 30sec the decrease was maximum in D_1 followed by O_1 & W_1, suggesting the maximum efficacy of dettol as teat dip for transient period. At 15minute interval oil dipped group exhibited least total bacterial count log_{10} TBC of 6.759 that was significantly lower than all other treated groups at 15 min post dip, justifying the prolonged effect of essential oils as teat dips the potential that can be exploited to effectively prevent mastitis.

The main compounds of cinnamon bark and leaf essential oils (*E*-cinnamaldehyde and eugenol, respectively) are responsible of diverse biological activities such as peripheral vasodilator, antitumor, antifungal, sedative, germicide, antioxidant and anti-mutagenic properties (Raoand Gan, 2014; Jayaprakasha and Rao, 2011).

Studies by different researchers suggest the use of essential oils as teat dips in reducing the load of pathogenic bacteria causing subclinical mastitis

(Pisestyani *et al*. 2017; Piotr *et al.*, 2018; Pozzo *et al.*, 2012). Dore *et al.* (2019) reported that the use of essential oil from *M. alternifolia (*Tea tree). The antibacterial activity and anti-inflammatory properties of tea tree oil has also been reported by Low *et al.*, 2013. Dore *et al.* (2019) reported that Terpinen-4-ol (T-4-ol), component of the *Melaleuca alternifolia* essential oil, shows antibacterial properties without inducing resistance. Tea tree oil has also been utilized for formulating a phyto-derivative solution for intra-mammary mastitis treatment. T-4-ol used as active principle of post-dipping alternative to chemical products routinely applied for prevention of subclinical mastitis, contributing to both milk quality and to animal welfare.

Table 1: Teat end total bacterial count (log_{10} cfu/ml).

Sr. No.	Sample code	NA	MAM	MSA	BAM
		Log $_{10}$ value	Log $_{10}$ value	Log $_{10}$ value	Log $_{10}$ value
1.	Control	7.814^{a}	7.386^{a}	7.209^{a}	5.477^{a}
2.	Post(water)30 sec (W1)	6.939^{b}	6.326^{b}	6.39^{b}	6.42^{b}
3.	Post(water)15 min(W2)	7.422^{a}	7.535^{a}	7.38^{a}	7.260^{c}
4.	O1 (30 sec)	6.759^{b}	6.328^{b}	5.9^{b}	6.42^{b}
5.	O2 (15 min)	6.944^{b}	6.591^{b}	6.00^{b}	6.514^{b}
6.	D1 (30 sec)	6.591^{b}	6.3404^{b}	5.87^{b}	7.049^{b}
7.	D2 (15 min)	7.309^{b}	7.240^{b}	5.79^{b}	6.839^{b}

Different superscript in a column after significantly ($p<0.05$)

CONCLUSION

Teat dip was effective in reducing the bacterial load at teat end inhibiting the invasion and inflammation and thus can be of immense use in the prevention of sub-clinical mastitis in dairy animals as well as against the control of pathogens which poses serious health hazards in human beings. Hence, farmer should be encouraged to adopt teat dip to reduces losses due sub-clinical mastitis and avoid health issues in human beings due to consumption of contaminated milk.

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Conflict of Interests. None.

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