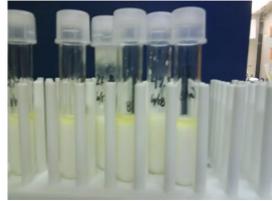


Antibacterial Activity of an Herbal Product *in vitro* on Mastitis Pathogens



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Introduction and Objective

There is anecdotal evidence of the efficacy of plant essential oils for treatment of mastitis in dairy cattle¹ and there is need for alternatives to antibiotics in the growing U.S. organic dairy industry. The potential mechanism of action of essential oils in mastitis therapy has not been well studied. An essential oil-based botanical intramammary infusion, Phyto-Mast (Penn Dutch Cow Care, Narvon, PA), has been used with some effectiveness as a dry treatment in mature cows². One component of this botanical infusion, thyme essential oil, has documented evidence of antibacterial activity^{3,4}. The objective of the current study was to evaluate the antibacterial activity of Phyto-Mast and thyme essential oil against mastitis-causing pathogens.

Materials and Methods

Phyto-Mast testing and analysis

American Type Culture Collection strains of *E. coli*, *Staph. aureus*, and *Strep. agalactiae* were grown on plates overnight at 37°C and tested using a modified CLSI standard for broth dilution testing⁵ with nutrient broth and 2.45% Tween 80 to emulsify the Phyto-Mast or canola oil, which served as a control.

Phyto-Mast and canola oil were tested at 9% vol/vol and run in triplicate for each species. Multivariate ANOVA with repeated measures was used to analyze the Phyto-Mast data, comparing growth of the mastitis organisms in the Phyto-Mast to the growth of organisms in canola oil.

Thyme essential oil testing and analysis

Isolates of *Staph. aureus*, *Strep. uberis*, and *Staph. chromogenes* were obtained from clinical mastitis samples from dairy herds in North Carolina for testing thyme essential oil (TEO). Whole ultra high-temperature pasteurized milk was used as the growth medium for TEO. Thyme essential oil was tested using a modified CLSI standard for broth dilution testing⁵: TEO was vortexed into growth media and bacteria were added to the prepared TEO solutions. The vials were incubated overnight at 37°C. Following incubation, serial dilutions (10⁻³ – 10⁻⁸) were performed and bacterial growth was recorded following another 24 hour incubation.

- TEO was tested by itself at 1%, 2%, and 3% vol/vol.
- Phyto-Mast and canola oil were tested at 9% vol/vol.

- Controls for TEO included milk only, milk + bacteria, and milk + penicillin-streptomycin (at 1% and 5% dilutions).
- Replicates were randomized by date, bacteria, and treatment to minimize experimental bias.

Phyto-Mast Results⁶

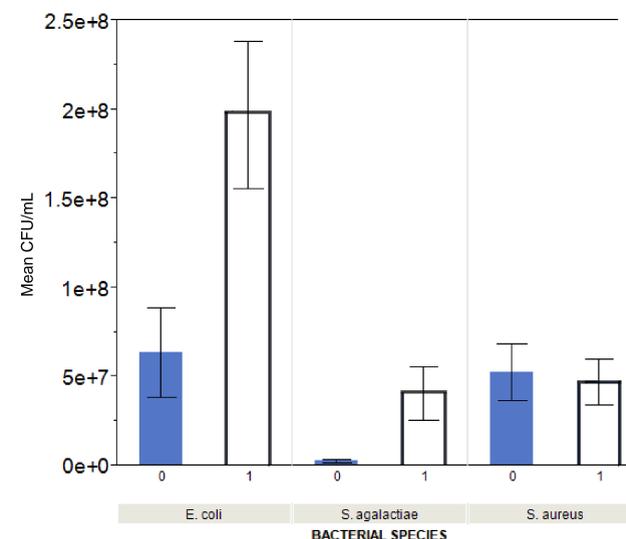
Canola oil and Phyto-Mast not inoculated with bacteria did not exhibit any growth.

Table 1: Results of MANOVA comparing growth in Phyto-Mast to growth in canola oil

Bacterial Species	P-value
<i>E. coli</i>	0.0065*
<i>S. agalactiae</i>	0.0315*
<i>S. aureus</i>	0.3152

* P-values indicate a significant difference between Phyto-Mast® and canola oil control where Phyto-Mast® had less bacterial growth than canola oil.

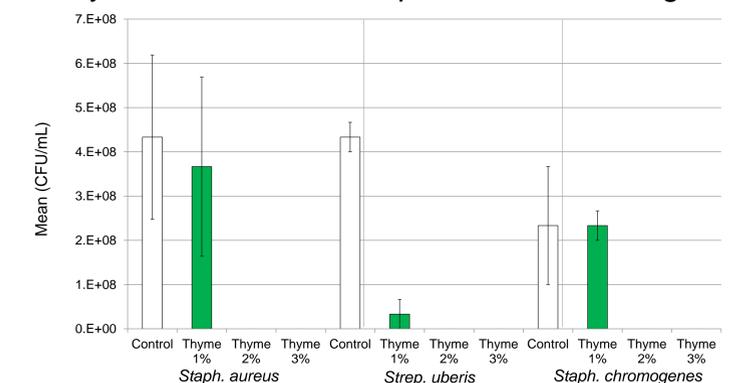
Figure 1: Growth of bacteria in either Phyto-Mast (0) in canola oil vehicle or in canola oil only (1)



Thyme Essential Oil Results⁷

The milk only (no bacteria) control had no microbial growth for all replications and the penicillin-dihydrostreptomycin control completely eliminated all bacterial growth.

Figure 2: Growth of bacteria exposed to various concentrations of thyme essential oil compared with control growth



Thyme essential oil completely eliminated all bacterial growth at 2% and 3% concentrations in all replicates of all bacteria tested.

Conclusions

- Phyto-Mast® has a bactericidal effect on *E. coli* and *Strep. agalactiae* *in vitro*.
- *Thymus vulgaris* essential oil was consistently antibacterial at or above 2% concentration.
- Further testing of Phyto-Mast® and TEO is recommended to determine their physiological effects in dairy cattle.

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