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Inhibitory effects of Hoof-Zink, Copper Sulfate and Zinc Sulfate on the growth of *Treponema medium*, *T. denticola*, and *T. vincentii*

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OBJECTIVE

The objective of this study was to determine if different concentrations of Hoof-Zink, copper sulfate and zinc sulfate solutions have antibacterial activity against the spirochete bacteria *Treponema medium, T. denticola,* and *T. vincentii*. These three bacteria are genetically and phenotypically very similar to the *Treponema* strains that cause hairy warts (*digital and interdigital dermatitis*) on dairy cattle.

MATERIALS AND METHODS

Bacterial strains

The bacterial strains used for this study were obtained from the American Type Culture Collection (ATCC).

Treponema medium	ATCC No. 700293
Treponema denticola	ATCC No. 33779
Treponema vincentii	ATTC No. 35580

The bacterial strains were propagated and frozen stocks were made for the study. Frozen stocks were resuspended in oral treponeme enrichment (OTE) broth and grown overnight in an anaerobic incubator. Wet mounts were prepared after incubation to determine bacterial viability.

Bacterial inhibition assay

The Hoof-Zink was received as a liquid and diluted with distilled water to achieve concentrations of 2.5, 5, 10 and 15% on a weight per weight basis. The copper sulfate ($CuSO_4$; feed grade crystals) and zinc sulfate ($ZnSO_4$; spray-dried powder) were resuspended with distilled water to achieve concentrations of 2.5, 5, 10 and 15% on a weight per weight basis.

	Concentrations
Hoof-Zink	2.5%, 5%, 10%, 15%
$CuSO_4$	2.5%, 5%, 10%, 15%
$ZnSO_4$	2.5%, 5%, 10%, 15%

After resuspension, each solution was filter sterilized to ensure sterility (0.45 µm Supor filter, Pall Gellman) and pH was determined using a pH meter with an ion exchange electrode. Each solution was plated on a 96-well plate and bacterial strains were added to three replicate wells for each solution at a concentration of 10^4 cfu/mL. Positive and negative controls were also included on the plates. Three replicate plates of the bacterial inhibition assay were performed for each mineral concentration and all procedures were completed in an anaerobic hood.

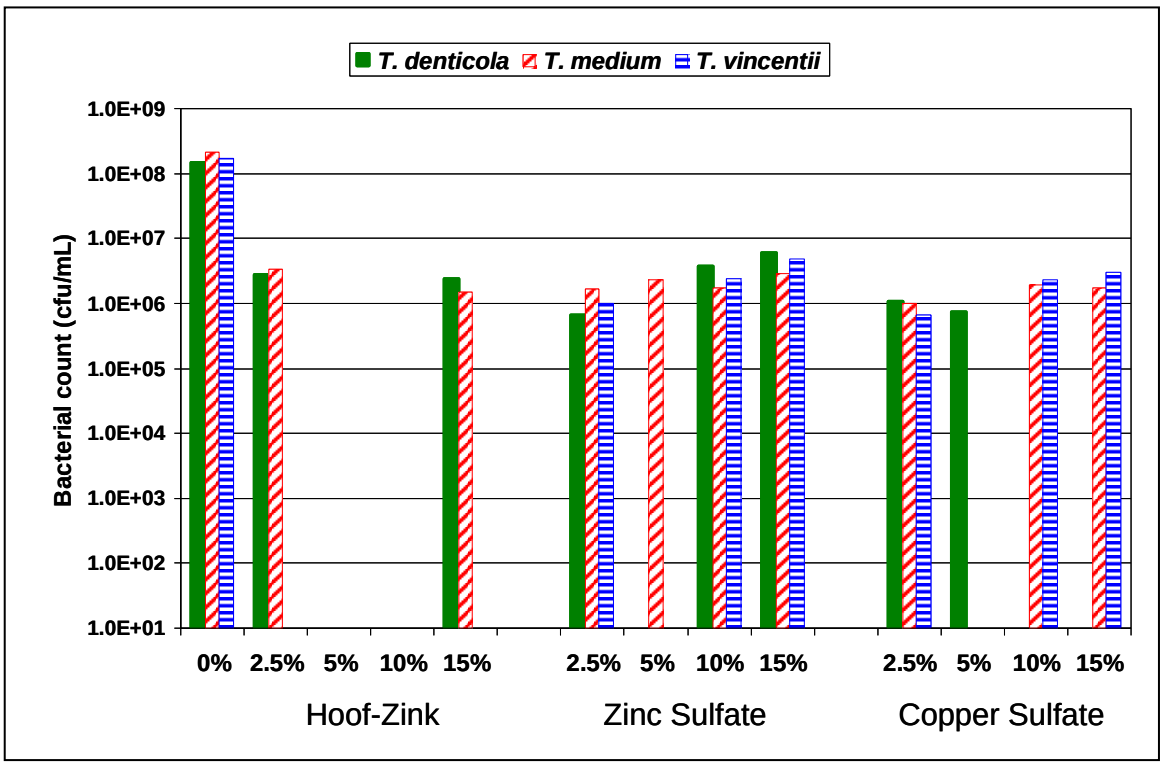
The solutions were added to the plate in 100 µL volumes, with the exception of the positive and negative control for each bacterium. For each assay replicate, the plates were incubated for 24 hours at 37°C in the anaerobic hood. After incubation the three replicate rows of solution/bacteria were pooled in a microcentrifuge tube. Positive and negative control wells were also pooled for each bacterium. A wet mount of each sample was examined under dark-field microscopy for bacterial viability. If any viable bacteria were observed, the sample was counted using a Petroff-Hauser cell counter.

RESULTS AND DISCUSION

In this trial, the pH of the Hoof-Zink and $ZnSO_4$ was similar at each of the concentrations (Table 1). Even though the pH of the concentrated Hoof-Zink is approximately pH 2.5, when diluted with distiller water, the pH of the Hoof-Zink solutions remained higher than copper sulfate solutions at each concentration.

Table 1. pH of Hoof-Zink, copper sulfate and zinc sulfate solutions

Items	2.5%	5%	10%	15%
Hoof-Zink	5.60	5.53	5.29	4.36
$CuSO_4$	4.71	4.58	4.54	3.94
$ZnSO_4$	5.67	5.61	5.47	4.40



The positive control (0%) was calculated to be 1.44×10^8, 3.0×10^8, and 1.65×10^8 cfu/mL for *T. denticola*, *T. medium* and *T. vincentii*, respectively. Hoof-Zink at 5 and 10% completely inhibited the growth of all three *Treponema* strains. Hoof-Zink at 2.5 and 15% inhibited the growth of both *T. denticola* and *T. medium* by almost 2 logs and completely inhibited the growth of *T. vincentii*.

In contrast to Hoof-Zink, zinc sulfate and copper sulfate was not able to completely inhibit the growth of the three bacteria consistently at any of the four concentrations. Zinc sulfate at 5% was able to completely inhibit the growth of both *T. denticola* and *T. vincentii*, but only reduced the growth of *T. medium* by approximately 2 logs. Zinc sulfate at 2.5, 10 and 15% only reduced the growth to the three *Treponema* strains by approximately 2.5, 2.0 and 1.5 logs compared to the positive control, respectively.

Copper sulfate at 2.5% reduced the growth of the three *Treponema* strains by just over 2 logs. When copper sulfate was increased to 5%, *T. medium* and *T. vincentii* was completely inhibited, but the growth of *T. denticola* was not completely inhibited until the concentration of copper sulfate was at 10 and 15%. However, at these higher copper sulfate levels (10 and 15%), the growth of *T. medium* and *T. vincentii* was measured at 10^6 cfu/mL.

SUMMARY

In this experiment, efforts were made to ensure all of Hoof-Zink, copper sulfate and zinc sulfate were dissolved into the solutions before the test. This is not always the case in the field when using copper sulfate and zinc sulfate in footbaths, due to their solubility. Hoof-Zink at 5 and 10% completely prevented the growth of all three *Treponema* strains. In contrast, both the copper sulfate and zinc sulfate at 5 and 10% did not consistently prevent the growth of the three *Treponema* strains. The level of copper sulfate needed to completely prevent the growth of the three *Treponema* strains was variable.

Results of this trial also demonstrate that the inhibition of these *Treponema* strains was not dose dependent, but in most cases these spirochete bacteria are able to remain viable in the presence of both low and high concentrations of the minerals solutions. As the levels of the products are increased and the pH was subsequently reduced to around 4, these bacteria were able to remain viable. This research suggests that just decreasing pH to around 4.0 either just the trace mineral or with an acidifier may not consistently inhibit the growth of these spirochete bacteria.