

In-vitro Trial of Sierra Natural Science SNS AG DC+ Bactericide and Fungicide

March 28, 2023. Acre, Inc. Greenhouse & Laboratory

Objective: Trails are designed to determine the activity of DC+ bactericide and fungicide against the plant pathogenic bacterium *Xanthomonas compestris* and the pathogenic fungi *Phytophthora cactorum* (Oomycota) and *Alternaria alternata* (Ascomycota). *Phytophthora* and *Alternaria* were contaminated and re-tested. Due to contamination of *Alternaria*, Ascomycota (*Stemphylium*) was used in subsequent tests.

Background: These 3 plant pathogens are representative of 3 major classes; Basidiomycota include the rust and smuts and other pathogenic fungi. Generally, pathogens that control Ascomycota fungi also control Basidiomycota. The preliminary trial demonstrated the activity of pure DC+ and at low rates. This trial was refined to determine the activity of DC+ using the cellulose disk technique. The trial provides dilution rates for application to crops. All tests were made using 3 replications.

Materials & Methods: DC+ was impregnated in 1 cm cellulose disks at dilution rates 1:10, 1:20 and 1:40 compared to untreated disks (UTC). *Xanthomonas* cells were evenly distributed over the surface of potato dextrose peptone agar (PDP). *Phytophthora* and *Alternaria* spores and mycelium were evenly distributed on potato dextrose agar. Impregnated disks were placed on the center of the agar plates. Three plates were used for each treatment and the UTC. After 5 days the longest diameter inhibition zones were measured including the 1 cm diameter of the disk.

(This trial was flawed. *Phytophthora* was contaminated with a bacterium. An antibiotic medium and selective medium have been used to purify the fungus. Three cellulose disks, 1 cm diameter, appeared to be contaminated with a fungus (*Xanthomonas* test) but did not interfere with the results. Disks have been sterilized by autoclaving. Laminar flow chamber: A new filter has been installed and the chamber cleaned. A new M&M will be sent. See Table 4 – *Phytophthora* & *Stemphylium*)

TRIAL RESULT SUMMARY

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Table 1. Inhibition Zones in Centimeters

Pathogen	DC+ Rate	Inhibition (cm)	Average (cm)	Notes
<i>Xanthomonas</i>	UTC	0	0	Inhibition was greatest at 1:10 dilution Cellulose disks were one cm in diameter.
	1:10	2.8, 2.5, 1.8	2.37	
	1:20	2.5, 1.6, 1.6	1.90	
	1:40	1.8, 1.2, 2	1.40	

TRIAL RESULTS SUMMARY Cont.

Pathogen	DC+ Rate	Inhibition (cm)	Average (cm)	Notes
<i>Alternaria -Initial Test</i>	UTC	0	0	The trial was flawed due to contamination. Re-tested. See Ascomycota Stemphylium test results below.
	1:10	0	0	
	1:20	0	0	
	1:40	0	0	
<i>Stemphylium -Re-test</i> The initial test was contaminated. Re-tested. See Table 4 Stemphylium	UTC	0	0	Stemphylium (Ascomycota) was used in subsequent tests.
	1:10	0	0	
	1:20	0.953	0	
	1:40	3.708	0	
<i>Phytophthora-Initial Test</i> All replications contained bacteria	UTC	0	0	There were only patches of Phytophthora. The contaminant bacteria were measured. Control bacteria was positive.
	1:10	1.7, 1.5, 2.2	1.67	
	1:20	1.5, 2, 1.5	1.73	
	1:40	1.1, 1.5, 1.2	1.27	The trial was flawed due to contamination. Re-tested. See below
<i>Phytophthora -Re-test</i> The initial test was contaminated. Re-tested. See Table 3 Phytophthora	UTC	0	0	Inhibition at all levels.
	1:10	0	0	
	1:20	0	0	
	1:40		0	