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PLANT PATHOLOGY AND NEMATOLOGY

Activity of Commercial Detergents Against Conidia and Chlamydospores of *Fusarium oxysporum* f. sp. *vasinfectum*

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ABSTRACT

Current containment recommendations for limiting the spread of race 4 of *Fusarium oxysporum* f. sp. *vasinfectum* in California lack non-corrosive yet effective alternatives to bleach for sanitizing equipment used in farming operations. To find an equivalent to Farmcleanse, an Australian product recommended for disinfecting equipment contaminated with the Fusarium wilt pathogen, 26 cleansers and degreasers commercially available in the United States were tested for their ability to kill spores of race 4 of *F. oxysporum* f. sp. *vasinfectum*. All treatments were tested at 1:10 and 1:100 (v/v) aqueous dilutions against conidia and chlamydospores in suspension. All treatments were also tested against chlamydospores in soil at a 1:10 dilution. Treatments demonstrating strong anti-fungal activity against spore suspensions at a 1:100 dilution were tested against conidia and chlamydospores at increasing aqueous dilutions (up to 1:100,000). Six products (Clorox, Simple Green d Pro 3, Trewax Nature's Orange, Formula 409 Antibacterial All Purpose Cleaner, Formula 409 Orange Cleaner Degreaser, and Lysol Antibacterial Kitchen Cleaner Citrus Scent) were effective against conidia and chlamydospores at a 1:100 dilution. Two products (Simple Green d Pro 3, Trewax Nature's Orange) gave results similar to bleach at dilutions up to 1:1000 on both conidia and chlamydospores in suspension. Quaternary ammonium compounds were present in four of the six most effective products. None of the cleansers performed as well as bleach against chlamydospores in soil. These results reveal useful alternatives to bleach, but similarity in brand names of distinctly different products should be noted. The results also point to the importance of completely removing soil from equipment prior to spraying with anti-fungal cleansers.

Limiting the movement of vehicles and equipment contaminated with pathogen-infested soil to non-infested sites is a prudent safeguard against the spread of soilborne diseases. Simple sanitation measures, such as removing soil and applying disinfectants to equipment, have been recommended for limiting the spread of soilborne diseases, such as clubroot (*Plasmodiophora brassicae* Woronin) of canola (*Brassica napus* L.) in Alberta (Hartman, 2007), sudden oak death (*Phytophthora ramorum* Werres, De Cock & Man in't Veld) in California (Anonymous, 2006), and Fusarium wilt (*Fusarium oxysporum* Schltd.:Fr. f. sp. *vasinfectum* (Atk.) W.C. Snyd. & H. N. Hans.) of cotton (*Gossypium* spp.) in Australia (Kay, 2003; Moore and O'Neill, 2000). In the last example, a commercially available detergent, Farmcleanse (Castrol Australia, Guildford, NSW), was the most effective at killing spores of *F. oxysporum* f. sp. *vasinfectum* in soil among locally available products.

In the San Joaquin Valley of California, Fusarium wilt caused by race 4 has become the dominant disease concern for cotton production (Kim et al., 2005). Although sanitation is recommended to limit the spread of this disease, containment of the pathogen is particularly challenging because of the diversity and intensity of agriculture practiced in the region. In most areas of the Valley, cotton is rotated with a variety of grain, forage, and vegetable crops, several of which are harvested with specialized machinery that travels among farms. Disinfectants must be effective against macro- and micro-conidia, the asexual spore forms produced in quantity within the plant, as well as the long-lived and thick-walled chlamydospores, which populate the soil

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environment. In the laboratory, chlamydospores of *F. oxysporum* were capable of surviving 17 years in a soil tube without losing pathogenicity (McKeen and Wensley, 1961).

Most farm equipment is made of metal that is vulnerable to corrosion, so growers may be reluctant to use bleach solutions (sodium hypochlorite) for equipment sanitation. Farmcleanse, the detergent recommended for sanitation in Australia, is not available in the United States, and limited information is available regarding other effective, non-corrosive, and inexpensive alternatives to bleach. Our objective was to test a range of detergents and cleansers, readily available in the United States, for their efficacy against spores in aqueous suspension and chlamydospores of *F. oxysporum* f. sp. *vasinfectum* in soil.

MATERIALS AND METHODS

Twenty-six commercial products were examined based on availability, cost, and potential similarity in active ingredients to Farmcleanse (i.e., degreasers) (Table 1). Ingredients listed on product labels or Material Safety Data Sheets were examined in the PAN Pesticide Database for indications of known anti-microbial activity (Kegley et al., 2008). These products were evaluated in addition to 6.5% sodium hypochlorite (Clorox Company, Oakland, CA). Positive and negative controls (spores mixed with water with no detergent and water without spores, respectively) were included in each trial. Positive controls were used to ensure that adequate numbers of viable spores were present. All treatments were tested at a 1:10 aqueous dilution (v/v) against conidia, chlamydospores, and soil, and at a 1:100 dilution (v/v) against conidia and chlamydospores. A subset of treatments that were efficacious at the 1:100 dilution was tested further (dilutions to 1:100,000) against conidia and chlamydospores. All experiments were conducted three times (trials).

Assays against conidia and chlamydospores in aqueous suspension. Conidia and chlamydospores of CA-14, an isolate of *F. oxysporum* f. sp. *vasinfectum* race 4 from California (Kim et al., 2005), were used. Different batches of spores (conidia and chlamydospores) were produced for each trial. Conidia were produced by inoculating 150 ml of sterile ¼-strength potato dextrose broth (PDB; Difco, BD, Franklin Lakes, NJ) with 1 ml of a conidial suspension. The inoculant suspension was made by adding 2 ml of sterile water to 4-d-old cultures grown on ¼-potato dextrose agar (PDA) in 60-mm Petri plates. Flasks of inoculated PDB were continuously agitated (90 rpm) on an orbital shaker at room temperature for 4-7 d before spores were harvested by filtration through 8 layers of cheesecloth.

Chlamydospores were produced using a modification of the soil broth method described by Goyal et al. (1973). One-hundred and twenty-five g of dried Super Soil (Scotts Miracle-Gro, Marysville, OH) in 500 ml of reverse osmosis water was agitated at 90 rpm for 1 hour on an orbital shaker, and filtered through 8 layers of cheesecloth. To every 50 ml of filtered soil broth, 0.025 g of glucose was added before autoclaving at 121°C for 20 min. The soil broth was autoclaved a second time the following day. Sediment remaining in the sterilized broth was allowed to settle completely before the liquid fraction was decanted into a sterile flask. The clear soil broth (~150 ml) was inoculated with 1 ml of a conidial suspension from a 3- to 7-d-old culture on ¼-strength PDA in a 60-mm Petri plate. Flasks of inoculated soil broth were agitated at room temperature on an orbital shaker at 90 rpm. Approximately 5 d after inoculation, chlamydospores formed in clumps of mycelia in the soil broth. Micro- and macroconidia also developed in the broth, occasionally in high numbers. To reduce the number of conidia, the mycelium-chlamydospore aggregates were rinsed with sterile water over sterile cheesecloth. Chlamydospores were then harvested by transferring the rinsed mycelium-chlamydospore aggregates into 30 ml of sterile water. A blender with sterile stainless steel mini-containers (Waring Laboratory, Torrington, CT) was used to disperse the aggregates into clusters of 1 to 3 chlamydospores. Each 30-ml sample of chlamydospores in water was subjected to five, 30-s bursts of the blender. The blending container was cooled in ice for 30 s between every two bursts of blending to minimize heating of the spore suspension.

The respective concentrations of conidia and chlamydospores were determined using an improved Neubauer hemacytometer (Hausser Scientific, Horsham, PA). Only suspensions in which chlamydospores composed ≥38% of all fungal propagules (conidia, chlamydospores, and mycelial fragments) were used in the chlamydospore and soil assays. Serial dilutions were made from suspensions to achieve spore concentrations of 10,000 CFU in 9 ml of sterile water in round-bottom 15-ml tubes.

Table 1. Commercial disinfectants, household cleansers, and auto degreasers tested for efficacy against spores of *F. oxysporum* f. sp. *vasinfectum*.

Product	Manufacturer	Antimicrobial Component(s)*
Clorox	Clorox Company, Oakland, CA	sodium hypochlorite
Clean-Rite Purple Power	Aiken Chemical Co., Inc., Greenboro, SC	2-butoxyethanol ethylene glycol butyl ether
Clean-Rite Purple Power Citrus All Purpose Detergent	Aiken Chemical Co., Inc., Greenboro, SC	natural citrus oils 2-butoxyethanol
Clean-X Pressure Wash Degreaser	Unelko, Scottsdale, AZ	sodium metasilicate ethylene glycol n-butyl ether sodium hydroxide
Dawn Ultra Antibacterial Orange	Proctor & Gamble Co., Cinncinati, OH	Triclosan
Fantastik OxyPower Multi-Purpose Cleaner	S.C. Johnson & Son, Inc., Racine, WI	hydrogen peroxide
Farmcleanse	Castrol, Guildford, NSW, Australia	alkali metal salts of alkylbenzene sulfonic acid coconut diethanolamide
Formula 409 Orange Cleaner Degreaser	Clorox Company, Oakland, CA	quaternary ammonium
Formula 409 Antibacterial All Purpose Cleaner	Clorox Company, Oakland, CA	quaternary ammonium n-propoxypropanol monoethanolamine
Greased Lightning High Performance Auto & Shop	A & M Cleaning Products, Inc., Clemson, SC	sodium hydroxide 2-butoxyethanol
Greased Lightning All-Purpose Cleaner Degreaser	HomeCare Labs, Lawrenceville, GA	sodium hydroxide
Lysol Disinfectant Antibacterial Kitchen Cleaner Citrus Scent	Reckitt Benckiser, Inc., Wayne, NJ	quaternary ammonium
OilEater	Kafko International Ltd., Skokie, IL	sodium metasilicate 2-butoxyethanol
Palmolive Oxy Plus	Colgate Palmolive, New York, NY	n/a
Pine Sol	Clorox Company, Oakland, CA	pine oil
Power Clean 101	Classic Chemical Corp., Batesville, IN	sodium metasilicate ethylene glycol butyl ether
Purple Passion All Purpose Cleaner Degreaser	South Win Ltd., Greensboro, NC	n/a
RCHD-Special Concentrated All Purpose Citrus Cleaner	Classic Chemical Corp., Batesville, IN	citrus terpenes
Simple Green	Sunshine Makers, Inc., Huntington Beach, CA	2-butoxyethanol
Simple Green Max	Sunshine Makers, Inc., Huntington Beach, CA	2-butoxyethanol
Simple Green d Pro 3	Sunshine Makers, Inc., Huntington Beach, CA	quaternary ammonium
Super “C”	National Colloid Research & Development, Santa Ana, CA	n/a
Superclean Multi-Purpose Cleaner Degreaser	Super Clean Brands, St. Paul, MN	2-butoxyethanol sodium hydroxide sodium metasilicate
Trewax Nature’s Orange	Beaumont Products, Inc., Kennesaw, GA	n/a
Zep 505 All Purpose Degreaser	Enforcer Products, Cartersville, GA	n/a
Zep All Purpose	Enforcer Products, Cartersville, GA	n/a
Zep Citrus	Enforcer Products, Cartersville, GA	d-limonene 2-aminoethanol 2-butoxyethanol
Zep Industrial Purple Cleaner and Degreaser	Enforcer Products, Cartersville, GA	sodium hydroxide 2-butoxyethanol

* Products that did not list ingredients or with ingredients not identified as anti-microbial in the PAN Pesticide Database are labeled “n/a”.

Final 1:10 dilutions of cleansers and degreasers were made by addition of 1 ml of the commercial formulation of each cleanser to a tube of spore suspension. Especially viscous detergents (Ajax, Dawn, Farmcleanse, RCHD, Super "C", and Power Clean 101) were dispensed using reverse mode pipetting with a 5 ml pipet, following manufacturer instructions (Rainin Instrument, LLC, Oakland, CA). Reverse mode pipetting minimized error from fluid retention on the pipet tip. An excess volume of detergent was aspirated into the pipet tip by releasing the pipet plunger from the second stop. One ml of the detergent was dispensed into the spore suspension by pressing the pipet plunger to the first stop. The pipet tip with excess detergent was discarded. For treatments with dilutions greater than 1:10, commercial formulations were pre-diluted to a concentration 10× the desired final concentration in sterile reverse osmosis water immediately before addition of 1 ml of the pre-dilution to spore suspensions. Each 15-ml tube was vortexed for 5 s immediately after addition of the treatment. Spore suspensions were exposed to treatment for 5 min and aliquots of each treated suspension were immediately spread onto six 100-mm plates of ¼-strength PDA using a sterile glass rod. Aliquots of 50 µl (= 50 propagules/plate in positive controls) were plated for the conidia assays, and 75-µl aliquots (= 75 propagules/plate in positive controls) were plated for the chlamydospore assays. All plates were incubated at room temperature with a 12-h photoperiod maintained with fluorescent lights. Counts of CFUs per plate were made after three and five days.

Assays against chlamydospores in soil. Dry clay (Lokern and Buttonwillow clay; 27% sand, 33% silt, 40% clay) was ground to a uniform texture (75-100 mesh) using a Quaker City grinding mill (model 4-E, Straub Co., Phoenixville, PA). The soil was autoclaved for 1 h on each of two consecutive days before it was inoculated. Each cleanser treatment was tested in 1 g of sterile soil in a round-bottom 15-ml tube. Chlamydospore suspensions were prepared as previously described. A volume of spore suspension containing approximately 4000 propagules (conidia, chlamydospores, and mycelial fragments), a level of inoculum representative of a heavily infested field (Zhou and Everts, 2003), was pipetted into each tube. The inoculated soil was allowed to air-dry for 4-7 days, after which it was stirred with sterile wooden applicators. Preliminary trials indicated 500 µl of water would completely wet 1 g of soil. Therefore, 500 µl of each cleanser treatment diluted 1:10 in sterile water were added to each tube. The contents in each tube was mixed briefly with a vortexer and allowed to incubate for five minutes. Nine ml of sterile water was then added to each tube and the contents were thoroughly vortexed. Six 187.5 µl (= 75 propagules/plate in positive controls) aliquots of the soil slurry from each tube were spread on plates of ¼-strength PDA. Plates were incubated and the CFU were counted as for the conidia and chlamydospore assays.

Experimental design and data analyses. Data from all experiments were analyzed by mixed-model ANOVA using PROC GLIMMIX of SAS (SAS, ver. 9.2, SAS Institute, Cary, NC). In all analyses, the response variable was the number of CFU per plate. Fixed effects included detergent treatment, detergent dilution, and their interaction. Trial (repetition of the experiment) was included as a random effect. Because six plates were assayed for each combination of treatment, dilution, and trial, counts from individual plates represented subsamples. The random effect of trial*dilution*detergent was used as the error term for testing the fixed effects. Denominator degrees of freedom were corrected using the Kenward-Rogers option. Because residual plots indicated heterogeneous variances, the data were analyzed after a square root-transformation of the number of CFU. Results are presented as means of untransformed data.

Because only a subset of detergent treatments was evaluated at greater dilutions (<1:100), separate analyses were conducted for each range of dilutions (1:10 to 1:100; 1:1000 to 1:100,000). Data corresponding to spore types (conidia or chlamydospores in suspension, chlamydospores in soil) were analyzed separately. Therefore, a total of five analyses were conducted.

In each analysis, when the detergent*dilution interaction was significant simple effects were examined using the SLICE option of the LSMEANS statement. When these analyses indicated significant differences in CFU counts among detergents within a given dilution, data corresponding to that dilution were further analyzed to examine the source of the differences. The model for these latter analyses included the fixed effect of detergent treatment and the random effects of trial and trial*detergent. The trial*detergent term was included as an error term for the main effect of detergent. When an analysis indicated a significant effect of detergent treatment within a given dilution, each detergent treatment was compared with the negative control (water)

using the ADJUST=DUNNETT option of the LSMEANS statement. This option controlled the type I experiment-wise error rate at $\alpha = 0.05$ within each set of comparisons.

RESULTS AND DISCUSSION

Assays against conidia and chlamydospores in aqueous suspension. Colony counts per plate from the positive controls in the assays against conidia and chlamydospores were between 41-53 and 71-77, respectively (Table 2). No colonies were observed on the negative control (water only) plates. Spore suspensions used for the chlamydospore assays were composed of 38 to 61% chlamydospores per total number of infective propagules. Several treatments eliminated suspended conidia and chlamydospores of *F. oxysporum* f. sp. *vasinfectum* at the 1:100 dilution (Table 2). The interaction between dilution and detergent treatment for both conidia ($F = 3.16$; df = 29, 118; $P < 0.001$) and chlamydospores ($F = 22.02$; df = 29, 118; $P < 0.001$) at the 1:10 and 1:100 dilutions was significant. Slices of the interaction indicated significant differences among treatments for all dilutions and for both conidia and chlamydospore suspensions ($P < 0.001$). At the 1:10 dilution, CFUs corresponding to seven detergent treatments (Clorox, Simple Green d Pro 3, Trewax Nature's Orange, Formula 409 All-Purpose, Formula 409 Orange, Lysol, and Zep Industrial Purple) were not significantly different from those of the negative water control, which had 0 CFUs, for conidia or chlamydospores (Table 2). With the exception of Zep Industrial Purple, these same detergents remained effective at the 1:100 dilution.

When the six detergent treatments that were highly effective at the 1:100 dilution were tested at higher dilutions against spores in suspension, a significant interaction between dilution and detergent treatment for both conidia ($F = 87.81$; df = 14, 46; $P < 0.001$) and chlamydospores ($F = 21.29$; df = 14, 46; $P < 0.001$) indicated efficacy was not consistent among detergents at different dilutions. At the 1:1000 dilution, Lysol was the only treatment that was significantly different from the negative control in CFUs of both spore types ($P < 0.001$; Table 2). Formula 409 and Formula 409 Orange were similar to the negative control in numbers of conidia ($P = 0.973$ and 0.134, respectively) at the 1:1000 dilution, but were not as effective in reducing chlamydospores ($P = 0.004$ and 0.002, respectively). At the 1:10,000 dilution, Clorox was not significantly different from the negative control in conidial numbers. Although Clorox plates had 93% fewer CFU than the positive control chlamydospore plates, Clorox was significantly different from the negative control in numbers of CFUs ($P < 0.001$; Table 2). The mean number of CFU on the Simple Green d Pro 3 plates was also reduced relative to the positive control plates at the 1:10,000 dilution, but numbers of CFUs were significantly higher than the water control (conidia, $P = 0.009$; chlamydospores, $P < 0.001$). Other treatments were ineffective at this dilution. At the 1:100,000 dilution, none of the treatments appeared active against either spore type.

Assays against chlamydospores in soil. The number of CFU on the positive control plates averaged 28.39 (= 1514 CFU/g soil), which was considerably fewer than the desired number of CFU of 75 per plate (= 4000 CFU/g soil). One possible explanation for this reduction may be that some mortality of propagules, especially of conidia and mycelial fragments, occurred as the suspension was drying in the soil. The number of CFU on the positive control plates was similar to the estimated number of chlamydospores in the spore suspension added to the tubes of soil (data not shown). No colonies were observed on the negative control (water and soil only) plates. Although several treatments were highly effective against spores in suspension, few treatments were able to eliminate chlamydospores in soil. Although the analysis indicated a significant effect of detergent treatment ($F = 16.86$; df = 29, 58; $P < 0.001$), only Clorox produced counts of CFUs that were not different from those of the negative water control. Farmcleanse was the most effective of the alternative treatments, but it did not kill all propagules in the soil.

Our results indicate that several readily available, inexpensive, and non-corrosive cleaning products are suitable alternatives to bleach for disinfecting surfaces contaminated with spores of *F. oxysporum* f. sp. *vasinfectum*. The alternative cleansers, Simple Green d Pro 3, Formula 409, Formula 409 Orange, Trewax Nature's Orange, and Lysol Disinfectant Antibacterial Kitchen Cleaner Citrus Scent, range in price from $3.50 to $5.55 per liter. Four of these products, Simple Green d Pro 3, Formula 409, Formula 409 Orange, and Lysol Disinfectant Antibacterial Kitchen Cleaner Citrus Scent contain quaternary ammonium compounds (Table 1). Quaternary ammonium compounds are widely used as disinfectants, primarily against bacteria (Ridenour and Armbruster, 1948; Dixon et al., 1976). However, the effectiveness of quaternary ammonium products against fungi appears

to be species-dependent. Benzalkonium chloride and cetrimide were able to disinfect some human pathogens (*Candida* spp., *Trichophyton* spp.) but not others (*Aspergillus ochraceus* K. Wilh.) at a 0.5% concentration (Gupta et al., 2001, 2002). Quaternary ammonium-containing treatments at concentrations as low as 0.008% effectively killed the chytrid fungus, *Batrachochytrium dendrobatidis* Longcore, Pessier & D.K. Nichols, a pathogen implicated in the mass die-off of tropical amphibians (Johnson et al., 2003).

Table 2. Mean number of colony forming units of *F. oxysporum* f. sp. *vasinfectum* per plate after conidia and chlamydospores in suspension (v/v, product/water), or chlamydospore-infested soil, were exposed to detergent treatments for 5 minutes.

	Conidia					Chlamydospores					Soil
	Dilution[a]					Dilution					Dilution
Treatment	1:10	1:100	1:1000	1:10,000	1:100,000	1:10	1:100	1:1000	1:10,000	1:100,000	1:10
Water only (negative control)	0	0	0	0	0	0	0	0	0	0	0
Spores + Water (positive control)	53.28*	41.33*	48.56*	43.11*	41.33*	70.72*	77.28*	76.94*	74.11*	74.28*	28.39*
Clorox	0	0	0	0	41.55*	0	0	0.17	5.5*	60.50*	0
Simple Green d Pro 3	0	0	0	1.17*	41.05*	0	0	0	7.78*	64.50*	31.33*
Trewax Nature's Orange	0	0	0	43.17*	43.11*	0	0	9.56	65.67*	72.28*	33.56*
Formula 409 Antibacterial All Purpose	0	0	0.44	40.11*	40.89*	0	0	17.00*	66.67*	78.17*	27.06*
Formula 409 Orange Cleaner	0	0	2.39	40.72*	44.44*	0	0	17.00*	69.89*	74.17*	27.44*
Lysol Antibacterial Kitchen Cleaner	0	0	46.72*	40.78*	42.11*	0	0	59.67*	76.61*	74.67*	31.00*
Zep Industrial Purple	0.06	35.44*	-	-	-	1.17	66.89*	-	-	-	23.94*
Clean-X, Unelko	37.61*	41.33*	-	-	-	52.67*	75.83*	-	-	-	31.17*
Farmcleanse	16.06*	38.44*	-	-	-	10.22*	71.50*	-	-	-	7.89*
Greased Lightning Auto & Shop	22.61*	41.89*	-	-	-	24.44*	79.67*	-	-	-	24.06*
Greased Lightning Multipurpose	26.67*	35.95*	-	-	-	26.11*	77.22*	-	-	-	28.89*
OilEater	28.67*	35.89*	-	-	-	29.50*	69.33*	-	-	-	29.94*
Palmolive Oxy	31.72*	40.17*	-	-	-	56.00*	77.33*	-	-	-	21.55*
Pine-Sol	8.72*	26.33*	-	-	-	4.89*	34.72*	-	-	-	14.72*
Power Clean 101	24.17*	40.00*	-	-	-	16.50*	69.72*	-	-	-	28.17*
Purple Power Citrus	41.05*	37.50*	-	-	-	60.94*	72.06*	-	-	-	27.44*
Purple Passion	39.22*	39.67*	-	-	-	46.28*	76.17*	-	-	-	30.94*
Purple Power	20.33*	38.78*	-	-	-	13.94*	78.17*	-	-	-	31.33*
RHCD- Special	27.95*	32.44*	-	-	-	25.39*	49.67*	-	-	-	27.50*
Simple Green	42.61*	38.33*	-	-	-	53.83*	74.50*	-	-	-	34.61*
Simple Green Max	46.00*	39.83*	-	-	-	55.72*	72.61*	-	-	-	41.00*
Superclean	29.05*	33.44*	-	-	-	28.39*	68.50*	-	-	-	32.06*
Super C	34.72*	33.56*	-	-	-	21.94*	65.72*	-	-	-	26.67*
Zep 505	38.89*	42.89*	-	-	-	60.67*	74.74*	-	-	-	33.39*
Zep All Purpose	45.28*	33.94*	-	-	-	62.39*	72.22*	-	-	-	30.72*
Zep Citrus	36.11*	35.28*	-	-	-	47.78*	74.11*	-	-	-	27.22*

[a] Dilutions are v/v of formulated product in reverse osmosis water. All experiments were conducted three times.

* CFU significantly different from the negative control at $P = 0.05$ using Dunnett's adjustment for multiple comparisons.

One of the most effective treatments for sanitizing spores in suspension, Trewax Nature's Orange, lists no hazardous compounds in its list of ingredients. Information from the manufacturer indicates the cleaner contains essential oils and organic solvents derived from citrus. Two treatments, RHCD-Special and Zep Citrus also contained citrus-derived material (limonene). Without knowledge of the compositions of these products, it cannot be determined which compounds are responsible for the antifungal activity of Trewax Nature's Orange.

In contrast to results from assays against spore suspensions in water, assays in soil against chlamydospores of race 4 indicated only Clorox was effective. These findings are consistent with an earlier report that effectiveness against spores in aqueous suspension is not a good predictor of effectiveness in soil (Moore and O'Neill, 2000). In the soil assays, the only non-bleach treatments that appeared to affect chlamydospores were Farmcleanse and Pine-Sol. Neither of these treatments was effective against spores in suspension. While the basis for the differences in results between assays against spores in suspension compared with assays in soil are unknown, the germicidal properties of some disinfectants including sodium hypochlorite and quaternary ammonium compounds are known to be influenced by factors such as temperature, the presence of organic matter, water mineral content, and pH (Ridenour and Armbruster, 1948; McDonnell and Russell, 1999). Based on our results and these earlier reports, the observed differences in results between assays in soil and against spores in suspension suggest opportunity for additional study.

In summary, results from assays against spores in suspension indicate at least five commonly available non-bleach cleansers, Simple Green d Pro 3, Trewax Nature's Orange, Formula 409, Formula 409 Orange, and Lysol Disinfectant Antibacterial Kitchen Cleaner Citrus Scent, are viable alternatives to bleach for surface sterilization of farm implements, tools, and clothing. Additionally, the generally poor results of evaluated cleansers against chlamydospores in soil emphasize the importance of complete removal of contaminated soil from equipment. Finally, practical application of these results requires care in the selection of non-bleach cleanser because of the similarity among brand names of distinctly different products. With this caveat in mind, our results identify useful alternatives to bleach in situations where corrosion or other undesirable properties of bleach are of concern.

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DISCLAIMER

Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture or the University of California. The U.S. Department of Agriculture is an equal opportunity provider and employer.

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