Efficacy of Peanut Seed Treatments for Organic Management in Georgia

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ABSTRACT

One of the most critical and influential factors determining ultimate crop success is plant stand establishment. Because synthetic seed treatments are not allowed in organic production systems, alternatives are needed to assist in resistance of pathogens during germination and seedling growth. Several biological control materials were evaluated in laboratory assays and field trials to determine their potential for minimizing disease impact and maximizing yield in organic peanut production. These included *Bacillus subtilis* and several application methods of copper sulfate. *Bacillus subtilis* demonstrated excellent control of *Aspergillus* in the laboratory, but this was not confirmed in the field. Copper sulfate had no benefit against *Aspergillus*, but had some activity against *Rhizopus* in the laboratory. When applied dry to the seed, copper sulfate improved plant stands and reduced postemergence plant mortality (damping-off) compared to either *B. subtilis* or untreated seed. When applied in combination with cola as a sticking agent, copper sulfate did an excellent job of minimizing damping-off, but caused delayed emergence or a reduced plant stand compared to all other treatments. Because there was no evidence of direct toxicity against *A. niger* by the copper sulfate treatments in the lab assay, the field effect may be the result of enhanced host resistance to Aspergillus crown rot, or activity on another pathogen.

Key Words: *Bacillus subtilis*, captan, copper sulfate, organic peanut.

Advancements in organic production practices for peanut (*Arachis hypogaea* L.) have been made in the southeastern U.S., particularly for weed (Johnson *et al.*, 2012; Wann *et al.*, 2011b) and mid-season foliar disease control (Cantonwine *et al.*, 2008; Wann *et al.*, 2011a), but there are still serious obstacles to overcome. One of the key research needs is to improve plant stand without the use of synthetic seed treatments. Untreated peanut seed can result in 50% fewer established plants than seed treated with synthetic seed treatment (Melouk and Backman, 1995). Acceptable plant stands not only maximize yield potential but also minimize pest problems by reducing incidence of tomato spotted wilt virus (Branch *et al.*, 2003), and competition by weeds (Place *et al.*, 2010). Plant stands of around 11 to 12 plants/m of row maximized single row peanut production in conventional management in Georgia (Tubbs *et al.*, 2011), and yields were equal with plant stands of 9 to 11 plants/m using various weed control regimes with organic management in Georgia (Wann *et al.*, 2011b). Weed competition is a significant concern for organic growers since there are fewer options to control weeds in organic production systems than conventional systems.

There are several seedborne and soilborne pathogens that can attack peanut seed or seedlings, including *Rhizopus* spp., *Penicillium* spp., *Fusarium* spp., *Aspergillus niger*, and *A. flavus* (Sullivan, 1984). Rhizopus seed rot typically occurs during imbibition or just after germination, and is most common in cool-wet planting conditions (Melouk and Backman, 1995; Sullivan, 1984). Aspergillus crown rot, caused by *A. niger*, can attack the plant prior to emergence or after emergence before stems have hardened off significantly, and is more common in hot-dry conditions (Jackson and Bell, 1969; Melouk and Backman, 1995). A study conducted in North Carolina evaluating the efficacy of peanut seed or soil treated with organically acceptable seedling disease treatments, such as biocontrol organisms, activated charcoal, copper hydroxide, or hot water showed that seed treated with the biocontrol agent *Bacillus subtilis* showed the most promise among the other treatments tested (Ruark and Shew, 2010). Cantonwine *et al.* (2011) found that genotype and seed integrity were more important factors than *B. subtilis* seed treatment to improve plant stand and reduce Aspergillus crown rot, but the rate of the *B. subltilis* seed treatment used in the experiment was lower than that used by Ruark and Shew (2010).

The objectives of this research were to assess the direct toxicity of various seed treatments against *Rhizopus* and *Aspergillus* species, and relate these findings to the efficacy of these materials for improving plant stands and reducing postemergence plant mortality (damping-off) (Fig. 1). Effects on yield and time required for hand weeding were also investigated.

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Fig. 1. Peanut plants with symptomology of plant mortality (damping-off). Brown necrotic plant that emerged and died (far left); green but folded leaves from stressed and dying plant (center); and healthy fully expanded leaves from healthy plant (far right).

Materials and Methods

Laboratory Assay. Media-based assays were conducted in the laboratory at Valdosta State University from 2008 to 2012 to evaluate the potential of organically acceptable seed treatments to suppress growth of *Aspergillus* species or *Rhizopus* on peanut seed. Experimental units consisted of 100 mm diameter potato dextrose agar (PDA) plates with four peanut seed (cv. Tifguard [Holbrook *et al.*, 2008]). Seed treatments included 1) untreated, 2) conventional (Dynasty [azoxystrobin + fludioxonil + mefenoxam], Syngenta Crop Protection, Greensboro, NC), 3) copper sulfate applied dry, 4) copper sulfate plus water, 5) copper sulfate plus cola, 6) *Bacillus subtilis* (Kodiak or Kodiak HB; Bayer CropScience, Research Triangle Park, NC), and 7) *Streptomyces lydicus* WYEC-108 (Actinovate STP, Natural Industries, Inc., Houston, TX, or ActinoGrow ST, SipcamAdvan, Research Triangle Park, NC). In the treatments with copper sulfate, the material was ground into a fine powder using a mortar and pestle prior to application to seed, and the water or cola sprays were used as sticking agents. All seed treatments were standardized at 2.5 g product per kg seed. Ten experimental trials were conducted using a randomized complete block design with 6 to 10 replications each. Blocking was done due to differences in light across the lab bench. Two of the trials used seed that were inoculated with spores of *R. stolonifer*; all others relied on inocula occurring naturally on the seed. Inoculations with *R. stolonifer* spores were done by tapping a sporulating culture of the fungus into a bag of seed and mixing the seed well to evenly distribute the spores. The experiments were maintained on laboratory benches at room temperature (20–22.5°C). Treatment effects were evaluated as percent seed affected per plate at 3 d for *Rhizopus* and at 5 and 7 d for *Aspergillus*. *Aspergillus niger* and green-spored *Aspergillus*, presumed to be *A. parasitica* and *A. flavus*, were recorded separately. For a seed to be recorded as affected, the fungal colony had to originate at the seed and grow on both seed and agar.

Field Assessment. Field experiments were conducted near Tifton, GA at the University of Georgia (UGA) Lang-Rigdon and Blackshank Farms in 2010 and Ponder Farm in 2011. The trials were conducted on Alapaha loamy sand at Lang-Rigdon, and Tifton loamy sand at Blackshank and Ponder Farms. Peanuts were planted on 27 May 2010, 28 April 2010, and 13 June 2011 at Lang-Rigdon, Blackshank, and Ponder, respectively. In 2010 at both locations, cv. Georgia Green (Branch, 1996) was planted, while Tifguard was planted in 2011. A change in seed was required because Georgia Green seed was no longer available from seed distributors starting in 2011, and Tifguard was identified as a cultivar with very good potential in organic production systems (Wann *et al.*, 2011a). A change in seed genetics likely caused a differentiation in disease resistance, although resistance to *A. parasitica* and *A. flavus* are not well known for most cultivars since they are not usually tested for resistance to a pest that is normally easily controlled with fungicidal seed treatments under conventional production conditions, so quantification of this change in resistance potential is not readily available. However, Tifguard had better plant stands than any other tested cultivar in two years of organic management in South Georgia (Wann *et al.*, 2011a), so it was considered the most optimal cultivar of choice for this experiment. All seed used in these trials were Foundation seed with germination exceeding 90 percent. Trials were planted at 20 seed/m of row at approximately 5 cm deep into a conventionally tilled seedbed (moldboard plow followed by rototiller) using a 2-row Monosem precision air planter (Monosem, Inc., Edwardsville, KS). Plots consisted of two rows, spaced 91 cm apart, and were 9.1 m in length at the Lang-Rigdon location, and 7.6 m in length at the Blackshank and Ponder locations. At the Lang-Rigdon and Ponder Farm locations, plots were managed under organically acceptable practices using a flex-tine cultivator (Aerostar, Einböck GmbH & CoKG, Austria) twice per week for the first five weeks after planting for weed control followed by hand weeding by rep on 19–22 July 2010 and 19–22 July plus 10 Aug. 2011. No additional disease or insect control was

used. Both of these sites were irrigated to ensure at least 3 cm water per week (irrigation + rainfall). At the Blackshank location, plots were not irrigated, and conventional peanut production practices were used for in-season pest control following UGA Cooperative Extension Service recommendations (Beasley *et al.*, 1997). Peanuts were dug on 15 Oct., 20 Sept., and 14 Nov. followed by harvest on 19 Oct., 24 Sept., and 22 Nov. at Lang-Rigdon, Blackshank, and Ponder locations in their respective years.

A randomized complete block design was used for all three field experiments, using seven, six, and five replications at Lang-Rigdon, Blackshank, and Ponder locations, respectively. The independent variable consisted of five seed treatments including 1) untreated, 2) conventional (Trilex Star [captan + trifloxystrobin + thiophanate-methyl], Bayer CropScience, Research Triangle Park, NC), 3) copper sulfate applied dry, 4) copper sulfate plus cola, and 5) *Bacillus subtilis* (Kodiak HB). Copper sulfate treatments were ground as described for the lab assay and all materials were applied at 2.5 g product per kg of seed. Stand counts were conducted by counting all emerged seedlings in a 1 m section in six random locations in each plot. These counts were assessed at 1, 2, 3, and 4 wk after planting at the Lang-Rigdon and Ponder locations, and at 2 and 3 wk after planting at Blackshank. In addition, a plant mortality count was conducted by counting all seedlings that had emerged but died. These data were collected at 2, 3, and 4 wk after planting at Lang-Rigdon and Ponder locations, and at 3 wk after planting at Blackshank. The amount of time spent hand weeding was recorded on a per plot basis in both organically managed locations. Yield was collected at all three locations, and grade factors were evaluated for the Lang-Rigdon and Ponder sites.

Data Analysis. For the laboratory assay, records of *A. niger* and green-spored *Aspergillus* were pooled for analyses because incidence values of *A. niger* alone were not high enough to significantly distinguish treatments, and the responses to treatments were similar for all *Aspergillus* species observed (data not shown). General linear model univariate analysis of variance (IBM SPSS statistics 20, Armonk, NY) was used to analyze percent incidence data, with seed treatment classified as a fixed factor, and trial as a random factor. Tukey's honest significant difference tests ($P<0.05$) were conducted to distinguish significant differences among seed treatments. For the field experiments, data were statistically analyzed using PROC GLIMMIX in SAS 9.2 software (SAS Institute, 2009), with mean separation by pairwise t-tests. Data were analyzed individually for each experiment instead of combining over year × site locations to account for treatment differences (or lack thereof) observed in the experiments. Correlation analyses were performed using PROC CORR in SAS.

Results and Discussion

In the lab assay, three experimental trials with 26 replications total were used to evaluate treatment effects for each pathogen. A total of 10 trials were conducted, however four trials were excluded due to low pathogen pressure (<25% in untreated controls). Trends observed in these trials were consistent with the trials included in the analyses (data not shown). One trial was excluded due to high pressure (100% of untreated seed affected by *Rhizopus*), which was the result of adding too much inoculum. The trend observed in this trial differed from the trials included in the analysis and is discussed later. Two of the trials used to evaluate *Rhizopus* did not include all seed treatments. Therefore, only those treatments that were common to all trials were compared.

There were no interactions between seed treatment and trial, and seed treatment significantly affected incidence of *Aspergillus* and *Rhizopus* ($P<0.01$). Compared to the untreated control, *Bacillus subtilis* and *S. lydicus* seed treatments suppressed *Aspergillus* ($P<0.01$), with *B. subtilis* completely suppressing pathogen establishment (Table 1). The dry and cola copper sulfate treatments resulted in similar incidences of *Aspergillus* to the untreated control ($P=0.299$ and 0.154, respectively), while incidence was higher than the untreated control when copper sulfate was applied with water ($P<0.01$) (Table 1). The effectiveness of *B. subtilis* against *Aspergillus* is in agreement with other studies (Kimura and Hirano, 1988; Zhang *et al.*, 2008), while the lack of response by copper sulfate was unexpected, since similar copper containing compounds provided fungicidal activities against *A. carbonarius* in a lab study using grape-like media (Belli *et al.*, 2006). Copper sulfate did suppress the incidence of *Rhizopus*, with similar levels of suppression to that of Dynasty ($P=0.161$) (Table 1). However, the activity of copper sulfate was almost completely overcome when pathogen pressure was high (data not shown), which suggests limitations to this seed treatment. Compared to the untreated control, *B. subtilis* had no effect on *Rhizopus* ($P=0.926$), while *S. lydicus* caused higher incidence ($P=0.021$) (Table 1). *Streptomyces lydicus* has been shown to reduce *Pythium* and

Table 1. Seed treatment effects on incidence of Aspergillus and Rhizopus in lab assay.

Seed Treatment	*Aspergillus* species	*Rhizopus* species
	Incidence (%)[a]	
Untreated	61.5 c	37.5 b
Copper sulfate	74.1 cd	---
Copper sulfate + water	90.4 d	13.5 a
Copper sulfate + cola	76.0 cd	---
Bacillus subtilis	3.5 a	32.7 b
S. lydicus	25.0 b	55.8 c
Dynasty	0.0 a	0.0 a
HSD[b]	17.0	16.4
MSE[c]	0.042	0.046

[a]Means within a column followed by the same letter are not significantly different according to Tukey's Honestly Significant Difference test.

[b]Honest Significant Difference value from the Tukey's post-hoc test.

[c]Mean square error used in Tukey's post-hoc test.

Rhizoctonia solani on pea and cotton seed (Yuan and Crawford, 1995). *Rhizopus* is not listed as target pathogens for *S. lydicus* (Cao *et al.*, 2010), and may not have activity against this fungus.

In the field experiments, plant stands were not improved on any sample date with *B. subtilis* compared to the untreated check (Figs. 2 and 3). There was also equal or greater levels of plant mortality after emergence compared to untreated seed (Figs. 4 and 5), although final totals of both stand counts and dead plants were equal to the untreated in all three field experiments (including the Blackshank location in 2010 – data not shown). Other studies have suggested that *B. subtilis* has the potential to aid in germination and emergence for improved stand establishment (Cantonwine *et al.*, 2011; Ruark and Shew, 2010; Turner and Backman, 1991). The most favorable responses to *B. subtilis* have been under stressed conditions, such as

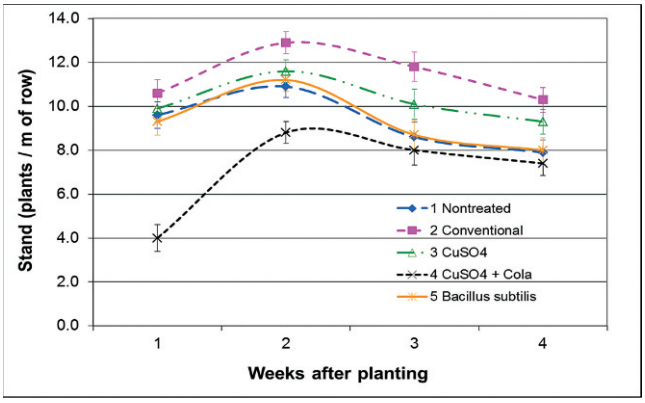

Fig. 2. Organic seed treatment plant stands, Lang-Rigdon Farm Tifton, GA, 2010. Peanut planted on 27 May 2010 at 20 seed/m of row. Error bars represent ± standard error of the mean.

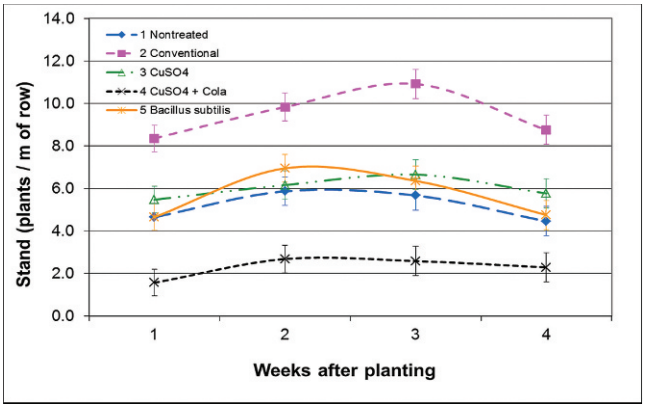

Fig. 3. Organic seed treatment plant stands, Ponder Farm Tifton, GA, 2011. Peanut planted on 13 June 2011 at 20 seed/m of row. Error bars represent ± standard error of the mean.

seed that were mechanically shelled (Cantonwine *et al.*, 2011), or unfavorable planting conditions from limited water, poor rotation, or cool soils (Turner and Backman, 1991). Yet, in the previous research from South Georgia, only one out of 15 genotypes in each year of the trial had a positive plant stand response that was solely related to *B. subtilis* seed treatment (Cantonwine *et al.*, 2011). Warm soil temperatures at the 10 cm depth in the Lang-Rigdon-2010 experiment (25 C) and very hot and dry conditions at the Ponder-2011 experiment (36 C) may have caused greater pressure from *A. niger* to overburden the *B. subtilis* treatment, or poor establishment conditions for *B. subtilis* where it could not suppress the pathogen.

The only seed treatment that provided some level of efficacy in stand establishment was copper sulfate. When applied dry to the seed, there was an 18% increase in plant stand compared to the untreated seed at 4 wk after planting (Fig. 2), and half as many plants that died after emergence by week 4 at Lang-Rigdon in 2010 (Fig. 4). When copper sulfate was applied after the seed were sprayed with cola, the number of plants that died after emergence was reduced even further, with 79% fewer dead plants than the untreated seed. This is presumably due to greater adherence of the material to the seed. However, emergence of seedlings from the soil was severely delayed compared to all other treatments in both years, and never fully recovered in 2011 at the Ponder location, resulting in a plant stand that was nearly 50% of the untreated seed (Fig. 3). Although care was taken when wetting the seed prior to application of the copper sulfate, it is possible that there was some damage to the seed testae from this process. Such damage is detrimental to seed health, and is one reason the seed industry does not use liquid seed treatments. It is also possible that the

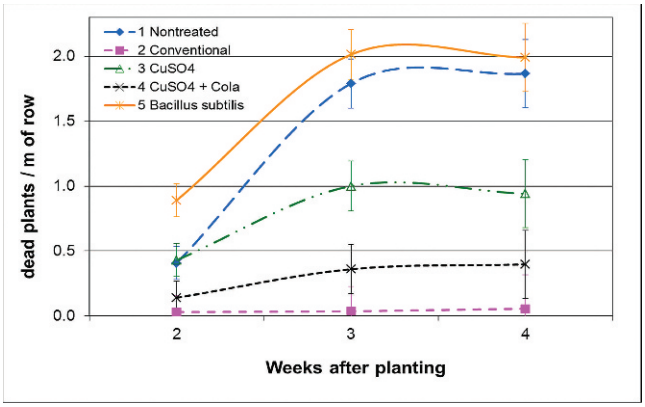

Fig. 4. Plant mortality counts (seedlings that died after emergence), Lang-Rigdon Farm Tifton, GA, 2010. Peanut planted on 27 May 2010 at 20 seed/m of row. Error bars represent ± standard error of the mean.

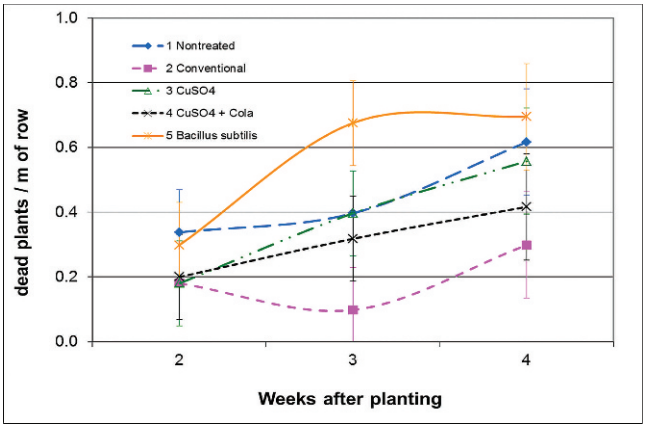

Fig. 5. Plant mortality counts (seedlings that died after emergence), Ponder Farm Tifton, GA, 2011. Peanut planted on 13 June 2011 at 20 seed/m of row. Error bars represent ± standard error of the mean.

additional sugars from the cola attracted facultative pathogens leading to slowed or limited emergence. There was no evidence in the laboratory study of phytotoxicity from a higher rate adherence in the treatments including cola.

Since the lab assays showed good activity from *B. subtilis* and no benefit from copper against *A. niger*, yet the field trials displayed no effect from *B. subtilis* and a positive response where copper sulfate was applied, this would indicate that there were additional variables affecting seedling response to the treatments in this research. It is possible that *Rhizopus* or other pathogens sensitive to copper sulfate contributed to seedling deterioration during germination, although the hot and relatively dry soil environment in these field studies would have been expected to be very suitable for *A. niger*. It can also be speculated that there may be some supplemental micronutrient benefit from the copper as well, aiding in growth and a resulting healthier seedling that had greater resistance to damping-off. Peanut seed soaked in low concentrations (10^{-4}M) of copper sulfate, and other inorganic salt solutions, were shown to reduce Aspergillus collar rot (crown rot) in greenhouse and field studies in India (Dasgupta *et al.*, 2000). The authors attributed this effect to enhanced defense responses of the plant rather than direct toxicity to *A. niger* due to the low concentration of the material. Copper is one of the 13 essential mineral elements needed for plant growth, and copper sulfate is a common micronutrient fertilizer with a nutrient content of 25–35% copper (Brady, 1974). In previous research, copper has increased seed germination for several plant species (Ouzounidou, 1995) and aided in root and shoot growth in alfalfa (*Medicago sativa* L.) (Peralta *et al.*, 2001), although it can also inhibit germination and growth at high concentrations. The phenomena that caused higher plant stands and reduced mortality with the inclusion of copper in this study was not confirmed; however, it does open opportunities for supplemental research on the effect of copper on germination and stand establishment, and also in reducing damping-off.

Despite variations in plant stand in 2010 at both locations, there were no resulting yield differences (Table 2). However, there were yield differences at the 2011 Ponder location, and this was highly correlated with plant stand (Pearson coefficient = 0.86, $P < 0.0001$). Plant stand and yield were also highly correlated in another organically managed trial in Georgia (Wann *et al.*, 2011a), which proves the importance of establishing an optimum plant stand in organic production. Although there were no yield differences in 2010, there was a difference in the amount of time required for hand weeding the plots (Table 3). There was likewise a strong correlation between plant stand and time spent hand weeding in the Lang-Rigdon 2010 experiment for all four stand count dates (Pearson coefficient ranging from −0.39 to −0.51, $P < 0.025$ for all dates). Therefore, whether plant stand affects yield or amount of time spent hand weeding, either way affects net revenue for the organic peanut farmer, such that improved plant stands result in increased income.

Summary and Conclusions

Research from the lab assay experiment showed promising results for multiple biological control materials depending on the seed or seedling pathogen in question. However, these effects were not consistent with field results. Based on field results, copper sulfate appeared to have more consistent potential as an organically acceptable material to assist with stand establishment in an

Table 2. Peanut pod yield under organic management as influenced by seed treatment, Tifton, GA, 2010–2011.

Seed Treatment	2010 Lang-Rigdon	2010 Blackshank	2011 Ponder
	kg/ha		
Untreated	2870 a[a]	1715 a[a]	1195 b[a]
Conventional	3070 a	1905 a	1860 a
Copper sulfate	2940 a	1975 a	1325 b
Copper sulfate + cola	3090 a	1745 a	655 c
Bacillus subtilis	2970 a	1690 a	1215 b
SE[b]	± 150	± 385	± 210

[a]Means within a column followed by the same letter are not significantly different according to pairwise t-tests at P = 0.05.
[b]Standard error of the mean.

organic peanut production system compared to the other materials investigated. The fungicidal activity of *B. subtilis* against *Aspergillus* species in vitro did not translate to the field in this study. These data also suggest that copper sulfate may provide an indirect mode of action against the development of Aspergillus crown rot. More research related to this effect would be valuable. Evaluating other particle sizes in combination with an organically acceptable sticking agent that does not damage the seed testae could result in even better activity of copper sulfate on seed and seedling disease.

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Table 3. Time requirement for hand weeding plots as influenced by seed treatment, Tifton, GA, 2010–2011.

Seed Treatment	2010 Lang-Rigdon	2011 Ponder
	hrs/ha	
Untreated	249 a[a]	342 ab[b]
Conventional	121 b	323 b
Copper sulfate	225 a	425 ab
Copper sulfate + cola	249 a	487 a
Bacillus subtilis	258 a	355 ab
SE[c]	± 39	± 90

[a]Means within a column followed by the same letter are not significantly different according to pairwise t-tests at P = 0.05.
[b]Means within a column followed by the same letter are not significantly different according to pairwise t-tests at P = 0.10.
[c]Standard error of the mean.

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