Organic Oils as Seed Treatments for Soybeans to Inhibit Fungal Growth

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Abstract:

Producing organic crops has become essential to satisfy the desires of the end consumer. To completely fulfill this task and meet the requirements of the National Organic Program in the U.S., the seeds planted must be organic. Seeds succumb to fungal infections without seed treatments. Organic seed treatments are not common. The purpose of this study is to test the ability of three organic oils (tea tree, coconut, and lemon) to act as organic seed treatments to inhibit fungal growth on soybean seeds that were badly infected with fungal disease. Each oil treatment was tested with three separate concentrations 10%, 50%, and 100%. The treatments were observed for 10 days and the results were recorded as a percentage of seeds infected with fungi. Both lemon oil and coconut oil at all concentrations failed to inhibit fungal growth. Tea tree oil at all three concentrations successfully prevented fungal infection on 100% of the seeds. The effective treatments show promise as organic seed treatments. Because of the positive results from the tea tree oil, it was further tested for germination and fungal inhibition on live soybean seeds at 2%, 5% and 10% concentrations. The treatments were observed for 12 days, and the results were recorded as a percentage of seeds infected with fungi. The germination rate and final germination percentage were also recorded. The 10% concentration inhibited a high percentage of fungi, but inhibited germination completely. The 2% and 5% concentrations had low germination percentages and mediocre fungal inhibition. The results from this research project show that there are still many options to explore in terms of organic seed treatments. Tea tree oil should be further tested in field studies, viability and vigor tests, and the germination study should be replicated at the 2% and 5% concentrations. Tea tree oil is effective against certain seed fungal contaminants, but also displays some phytotoxicity at low concentrations. Research should be completed to find tea tree oil's effect on seedling emergence and early seedling growth. Tea tree oil could be effective in conjugation with other organic seeds treatments. Other essential oils that are produced organically should be tested against fungal control, and tea tree oil should be thoroughly tested and considered for cost effectiveness and possible health effects to farm workers.

Key Words:

Seeds, soybeans, organic, oils, treatment, fungus, fungi, mold, prevention, coconut, tea tree, melaleuca, lemon, citrus, essential oils, USDA organic, certified organic, germination, germination rate

Introduction:

Organic products are available in markets all over the world. Webster's Dictionary defines the adjective organic as "of, or relating to, yielding, or involving the use of food produced with the use of feed or fertilizer of plant or animal origin without employment of chemically formulated fertilizers, growth stimulants, antibiotics, or pesticides" (Merriam-Webster 2015). Many consumers are becoming increasingly informed and want their products, particularly their food, to be produced organically. According to the USDA, organic produce accounts for over 40% of the total organic product market and has significant economic importance (Greene 2014). To produce organic crops, organic seeds must be used according to

the National Organic Program (NOP), which provides the standards for certified organic production in the U.S. For farmers to qualify as producing organic food,

> "the producer must use organically grown seeds, annual seedlings, and planting stock: Except, that (1) nonorganically produced, untreated seeds and planting stock may be used to produce an organic crop when an equivalent organically produced variety is not commercially available: except, that, organically produced seed must be used for the production of edible sprouts; (2) Nonorganically produced seeds and planting stock that have been treated with a substance included on the National List of synthetic substances allowed for use in organic crop production may be used to produce an organic crop when an equivalent organically produced or untreated variety is not commercially available" (Electronic Code of federal Regulations – National Organic Program 205.204).

"The purpose of any seed treatment is to improve seed performance in one or more of the following ways: 1) eradicate seedborne pathogens or protect from soilborne pathogens, 2) optimize ease of handling and accuracy of planting (reduce gaps in stand or the need for thinning of seedlings, particularly when mechanical planters are used), and 3) improve germination percentages and rates" (Gatch 2014). Unfortunately, there are very few organic seed treatments that encourage seedling growth and inhibit fungal growth. According to the Organic Materials Review Institute (OMRI), which sets many of the standards used by the NOP, in order to be considered an organic seed treatment, the substance used for treating seeds must be certified by the USDA's National Organic Program (NOP) (NOP Rule 205, http://www.ams.usda.gov/AMSv1.0/nop).

According to a research project by Schmitt (2006) called Seed Treatments for Organic Vegetable Production (STOVE), there are two main organic seed treatments currently used: hot water treatments and hot air treatments. These treatments only provide moderate to good sanitation control of seed-borne diseases. STOVE optimized these physical treatments and combined these treatments with thyme oil. This combination provided good inhibition to seed-borne diseases in both field and greenhouse tests (Schmitt 2006). Christian (2007) from Iowa State University tested 18 essential oils on corn seed to see if they would inhibit fungal growth as an organic fungicide. He found that 5 essential oils, cinnamon, clove, oregano, savory, and thyme, completely controlled the three most common corn seed pathogens in vitro. Although these 5 essential oils controlled the fungi, the seeds when treated and planted showed much lower field emergence than those seeds treated with a conventional fungicide (Christian 2007). This is a concern for further research. Fungal inhibition is crucial, but seed vigor must not be impaired.

This research project is designed to test three NOP certified organic oils, coconut oil, tea tree oil, and lemon oil on soybean seeds to see if they will inhibit fungus that would prevent strong seedling germination, growth, and establishment. Fungal diseases on soybean seeds are traditionally a major problem so if a seed treatment is effective on soybeans, it will likely be effective on other crops as well. Coconut oil can kill some types of viruses, bacteria, and fungi, thus allowing this oil to be a good candidate for seed treatment research (Coconut Research Center 2015). Tea tree oil has antiseptic and antimicrobial properties (Carson 2006), and lemon oil has antiseptic and antifungal properties (Organic Facts – Lemon Oil) thus also allowing these oils to be good candidates for this project. The oils used in this research project are 100% pure USDA Certified Organic oils produced by NOW Foods in the United States. The coconut (*Cocos nucifera)* oil is described as virgin organic coconut oil and is cold pressed and unrefined. The tea

tree (*Melaleuca alternifolia*) oil was extracted through steam distilling from leaves. The lemon (*Citrus limon*) oil was extracted by being cold pressed from fresh fruit peel.

Materials and Methods:

The purpose of this study is to test the ability of three organic oils to inhibit fungal growth on nonviable soybeans and to test tea tree oil's effect on germination of viable soybean seed ('Midori' Wannamaker Seed Company, Saint Matthews, South Carolina). Nonviable soybeans were selected for fungus inhibition tests because they leach electrolytes during imbibition and produce consistently large and diverse fungal populations after hydration.

For fungal inhibition, each oil was tested at three concentrations: 10%, 50%, and 100% in water, approximately 50 mL per treatment. Each concentration had three replications of 20 seeds per replication. Prior to treating with the oil, the seeds were rinsed with approximately 100 mL of distilled water to ensure a clean surface. The seeds were dipped in the oil for 5-10 seconds and placed in the treatment plate containing germination blotter paper (Anchor Paper Co, St, Paul, MN) saturated with approximately 11mL of tap water. In order to have a negative-fungal control treatment, three replications of a bleach (NaClO – 5.25%) treatment were performed (OSU Biological Safety 2011). Bleach has been shown to effectively surface sterilize seeds and decrease microbial growth (Sauer 1986). The bleach treatment was a 10% bleach solution in tap water, and the seeds were submerged for 7 minutes before being placed in the treatment plate with saturated blotter paper. Also, a positive-fungal control was implemented with no treatment and placed only in the plate with the saturated blotter paper. These treatments were observed for 10 days at 24.4 ^{O}C, and the percentage of seeds infected per replication was calculated.

Because of the results of the first experiment, the tea tree oil was tested at three different concentration: 2%, 5%, and 10% in water (approximately 50 mL per treatment) to see if the oil inhibits germination and to see if tea tree oil inhibits fungi on live soybean seeds. Each concentration had three replications of 20 seeds per replication. Prior to treating with the oil, the seeds were rinsed with approximately 100 mL of distilled water to ensure a clean surface. The seeds were dipped in the oil for 5-10 seconds and placed in the treatment plate containing germination blotter paper (Anchor Paper Co, St, Paul, MN) saturated with approximately 11mL of tap water. Also, a positive-germination control was implemented with no treatment and placed only in the plate with the saturated blotter paper. These treatments were observed for 12 days at 25 ^{O}C. The germination rate and the final germination percentage were calculated, and the percent of fungal infection was recorded.

The experimental designs were completely randomized. All the treatments were numbered and placed randomly in the treatment area. This randomization ensured there was no bias due to the location of the treatments and ensured that variables were distributed at chance levels across the treatments.

Table 1a. Treatment numbers for fungal inhibition. Each treatment was assigned a number in order for randomization.

1,2,3	Bleach – 10% Concentration
4,5,6	No Treatment
7,8,9	Lemon Oil – 10% Concentration
10,11,12	Lemon Oil – 50% Concentration
13,14,15	Lemon Oil – 100% Concentration
16,17,18	Tea Tree Oil – 10% Concentration
19,20,21	Tea Tree Oil – 50% Concentration
22,23,24	Tea Tre Oil – 100% Concentration
25,26,27	Coconut Oil – 10% Concentration
28,29,30	Coconut Oil – 50% Concentration
31,32,33	Coconut Oil – 100% Concentration

Table 1b. Treatment numbers for tea tree oil germination inhibition. Each treatment was assigned a number in order for randomization.

1,2,3	No treatment
4,5,6,	2% Concentration
7,8,9	5% Concentration
10,11,12	10% Concentration

Table 2a. Experimental Design of Seed Treatments for fungal inhibition. Seed treatments were randomized, and seeds were observed in sealed plastic boxes on germination blotter paper saturated with 5 mL of treatment solutions for 10 days. Degree of fungal infection was assessed and values assigned in Table 3a below.

21	28	24	31	3	4	15	20	17	32	1
2	26	19	25	14	13	29	12	9	2	10
30	23	18	22	11	33	7	5	16	6	8

Table 2b. Experimental Design of Seed Treatments for tea tree oil germination inhibition. Seed treatments were randomized, and seeds were observed in sealed plastic boxes on germination blotter paper with 5 mL of treatment solutions for 7 days. Germination rate and final germination percentage were calculated and recorded in Table 3b.

11	12	1	3
8	7	2	10
4	6	9	5

Results:

The results of the fungal inhibition experiment are shown in Appendix A and Table 3a. The bleach treatments had 0% fungal infection (Table 3a). The untreated seeds had 95.0% fungal infection (Table 3a). Lemon oil had fungal infection ranging from 48.3%-98.3% for all the concentrations (Table 3a). Tea tree oil had 0% fungal infection at all concentrations (Table 3a). Coconut oil had fungal infection ranging from 95%-98.3% for all the concentrations (Table 3a).

The results of the tea tree oil germination experiment are shown in Appendix B and Table 3b. The untreated seeds had 100% fungal infection, 0.53 seeds/day germination rate and 3.3%

germination (Table 3b). The 2% concentration had 68.3% fungal infection, 5.5 seeds/day germination rate, and 38.3% germination (Table 3b). The 5 % concentration had 60.0% fungal infection, 3.9 seeds/day germination rate, and 23.3% germination (Table 3b). The 10% concentration had 25.0% fungal infection, 0.0 seeds/day germination rate, and 0.0% germination (Table 3b).

Table 3a. Quantitative scoring of fungal infection based on the pictures in Appendix A. Percentages represent a subjective assessment of the degree of fungal infection for each seed treatment after 10 days of incubation. Values are means of three replications and are shown along with the standard error of the mean.

Treatments	**% Fungal Infection Mean ± SE**
Bleach	0.0
Untreated Control	95.0 ± 5.0
Lemon Oil 10% Concentration	96.7 ± 3.3
Lemon Oil 50% Concentration	98.3 ± 1.7
Lemon Oil 100% Concentration	48.3 ± 19.7
Tea Tree Oil 10% Concentration	0.0
Tea Tree Oil 50% Concentration	0.0
Tea Tree Oil 100% Concentration	0.0
Coconut Oil 10% Concentration	95.0 ± 2.9
Coconut Oil 50% Concentration	98.3 ± 1.7
Coconut Oil 100% Concentration	98.3 ± 1.7

The means of the fungal infections above show the effectiveness of each oil and concentration. The standard errors show the variability around means (Table 3a). All concentrations of tea tree oil treatments showed no symptoms of fungal infection. This shows that tea tree oil is very consistent in fungal prevention on soybean seeds that were badly contaminated. Seeds treated with all concentrations of coconut oil were consistently infected with fungi with low variability in the means (Table 3a). Lemon oil showed extreme fungal infection at the 10% and 50% concentrations and low variability in the means and showed some fungal control at the 100% concentration with high variability (Table 3a). The bleach treatment showed no fungal infection, and the untreated control showed 100% fungal infection (Table 3a).

Table 3b. Quantitative scoring of fungal infection based on the pictures in Appendix B. Percentages represent a subjective assessment of the degree of fungal infection for each seed treatment after 12 days of incubation. Germination rate in seeds/day and final germination percentage. Values are means of three replications and are shown along with the standard error of the mean.

Treatments	% Fungal Infection Mean ± SE	Germination Rate (seeds/day)	Final Germination Percentage
Untreated Control	100.0	0.53 ± 0.3	3.3 ± 1.7
Tea Tree Oil 2% Concentration	68.3 ± 14.8	5.5 ± 2.1	38.3 ± 16.4
Tea Tree Oil 5% Concentration	60.0 ± 20.2	3.9 ± 1.5	23.3 ± 9.3
Tea Tree Oil 10% Concentration	25.0 ± 2.9	0.0	0.0

The means of the fungal infections above show the effectiveness of each concentration. The standard errors show the variability around means (Table 3b). The untreated control showed 100% fungal infection. Tea tree oil at 10% concentration showed the least amount of fungal infection and the 2% and 5% concentrations had similar fungal inhibition percentages.

Discussion:

Soybean seeds were selected for this study because fungal infection is often a problem, and untreated seeds germinate poorly due to fungal diseases particularly in cold soils. With increased planting and production of organic soybean seeds, an organic seed treatment is needed to meet this growing demand. The 'Midori Giant' seeds used in the fungal-inhibition study were nonviable and had a heavy load of fungal inoculum making them ideal for screening different substances for fungal inhibition (Table 3a). The inexpensive organic compounds tested were compared to a standard bleach treatment that is frequently used for seed disinfection. Some of the treatments were more effective than others.

Lemon oil was included in this study because it does not have any human health concerns and is commonly used as a home remedy for common illnesses suggesting antimicrobial effects (Health Benefits of Lemon Oil 2015). Lemon oil did not produce encouraging results and is not a good candidate as an organic seed treatment (Table 3a). At the 100% concentration lemon oil inhibited fungi by slightly more than 50%. This oil should be tested further to see its effects on seed germination, but it will be less cost effective than tea tree oil because 100% concentration will be needed. The relative cost of each treatment of lemon oil was $0.95 per 20 seeds.

Coconut oil was included in this study because there are not any human health concerns connected to the use and consumption of coconut oil, and it is has shown positive results treating common ailments and killing viruses (WebMD – Coconut Oil 2015, Coconut Research Center 2015). Coconut oil did not prevent fungi to any reasonable degree at any concentration and is not a good candidate as an organic seed treatment, and thus it should not be tested further (Table 3a). The relative cost for each treatment of coconut oil was $0.56 per 20 seeds.

Tea tree oil was included in this study because it has antifungal properties and has low health risks (Derrer 2014). Tea tree oil successfully prevented fungal growth on all seeds at all concentrations and all replications and shows promise as an organic seed treatment with further research. Lower concentrations should have less of an effect on germination and vigor and will be less of a cost constraint. The relative cost for each tea tree oil treatment was $0.95 per 20 seeds. Tea tree oil does have some human health concerns. According to WebMD, this oil is safe

for topical application but should not be taken by mouth because it can cause confusion, inability to walk, unsteadiness, rash and coma and ingestion of tea tree oil can be toxic (WebMD – Tea Tree Oil 2015). In order for tea tree oil to be applied commercially, it will need to be diluted and applied to the seeds on site at the farm. Because this oil does not mix well with water, the solution will need to be constantly agitated during application and the seeds will need to be planted quickly after application. Research should be completed to find a way to make tea tree oil into a coating so the seeds can be ordered with the treatment ready. According to an article in Clinical Microbiology Reviews by Carson (2006), many different bacteria are susceptible to tea tree oil at concentrations of 1.0% or less (Carson 2006). Tea tree oil has been tested against many different types of fungi and has shown good inhibition at concentrations of 2% or less (Carson 2006). In this study completed by Carson (2006), tea tree oil was tested against *Candida albicans* to find the mode of action in inhibiting fungal growth. Tea tree oil altered the permeability of *C. albicans*, membrane fluidity of this fungus was increased, and respiration of *C. albicans* was inhibited causing its subdued growth (Carson 2006). Also, tea tree oil obstructs the formation of germ tubes in *C. albicans* (Carson 2006). These are the likely modes of action for fungal inhibition in this research project. The results of this research project are consistent with other studies completed on inhibition of fungi and contribute to the possibilities of organic seed treatments. According to a study completed by Hammer (2004), tea tree oil's antifungal properties alter fungal membrane properties and compromise membrane-associated functions (Hammer 2004). An article from the American Journal of Infection states that tea tree oil has antimicrobial properties and demonstrated promising efficacy in treating *Staphylococcus aureus* (Halcon 2004).

Hot water seed treatments are well established for preventing seedborne disease, but these treatments only work well for certain crops like brassica, carrots, tomatoes and peppers (Gatch 2014). Hot water treatments can also cause a reduction of vigor over time (Gatch 2014). Bleach disinfection can be used to eliminate pathogens on the seed coat but not beneath it, and there is some debate as to whether bleach is considered organic or not (Gatch 2014). "Biological seed treatments, alone or in conjunction with priming and pelleting processes, may have potential in some situations for improving seedling health…but the results have been inconsistent"(Gatch 2014). The tea tree oil treatments in this study consistently prevented fungal growth and were very effective compared to other research done on mainstream organic seed treatments. Tea tree oil needs to be tested against various seedborne and soilborne inoculum to see its effectiveness compared to conventional seed treatments. Seed treatments generally protect the crop for 10-14 days before breaking down (Paulsrud 2001). Tea tree oil would need to be tested in a field study to see how quickly it would break down and if it would last long enough to be an effective seed treatment. Research should also be completed testing tea tree oil in conjunction with other oils or substances as organic seed treatments.

Tea tree oil was further tested to see its effects on preventing fungal growth on live seeds and its effects on germination. The seeds tested had a reported >80% germination. All the treatments had germination percentages much lower than this. Because of the low germination percentages, field studies need to be completed and the 2% and 5% concentration treatments should be repeated. The inhibition effect of the tea tree oil at 10% concentration on fungal growth on nonviable seeds (0% infection) is greater than this on viable seeds (23% infection). These results are unfortunate. The fact that there was fungal infection on all germination treatments showed that tea tree oil can prevent fungi that grows on dead and dying tissue but does not prevent fungal growth as well on live seeds. There was great variability within the

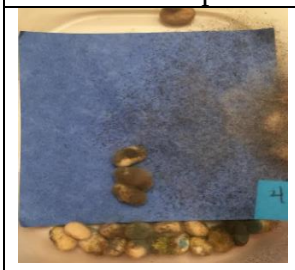
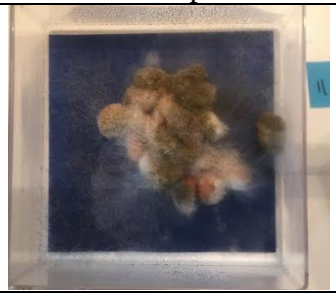
germination percentage results of each replication. This may be because some of the seeds in the lot were much more contaminated with fungi than other. Even with the random sampling of seeds, it is possible for some of the seeds to have been more contaminated than others. This also shows the value of doing replicated experiments. Many of the germinated seedlings were abnormal and had stunted growth after the 12 days. The seedlings could grow out of this over the course of several weeks, but this initial abnormality is not good for commercial treatment. This experiment should have had a bleach treatment as a control on the viable seeds to see the potential germination of the seeds. Bleach treatment or a fungicide like captan or thiram are the industry standard for disinfection of seeds and would work well for this control. Tea tree oil does not dissolve in water; this may make developing commercial product more difficult because oil is more challenging to work with.

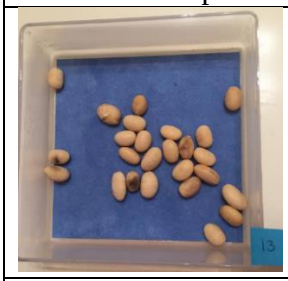
Conclusion:

The effective treatments from this experiment show promise as organic seed treatments. The results from this research project show that there are still many options to explore in terms of organic seed treatments. Tea tree oil should be further tested in field studies, viability and vigor tests, and the germination study should be replicated at the 2% and 5% concentrations. Tea tree oil is effective against certain seed fungal contaminants, but also displays some phytotoxicity at low concentrations. Research should be completed to find tea tree oil's effect on seedling emergence and early seedling growth. Tea tree oil could be effective in conjugation with other organic seeds treatments. Other essential oils that are produced organically should be tested against fungal control, and tea tree oil should be thoroughly tested and considered for cost effectiveness and possible health effects to farm workers.

Appendix A:

Visual assessment of seed treatment effectiveness.

Seed Treatments		
Bleach Treatment 10% Concentration		
Rep #1	Rep #2	Rep #3
		
Untreated Control		
Rep #1	Rep #2	Rep #3
		
Lemon Oil 10% Concentration		
Rep #1	Rep #2	Rep #3
		
Lemon Oil 50% Concentration		
Rep #1	Rep #2	Rep #3
		

Lemon Oil 100% Concentration		
Rep #1	Rep #2	Rep #3
		
Tea Tree Oil 10% Concentration		
Rep #1	Rep #2	Rep #3
		
Tea Tree Oil 50% Concentration		
Rep #1	Rep #2	Rep #3
		
Tea Tree Oil 100% Concentration		
Rep #1	Rep #2	Rep #3
		

Coconut Oil 10% Concentration		
Rep #1	Rep #2	Rep #3
		
Coconut Oil 50% Concentration		
Rep #1	Rep #2	Rep #3
		
Coconut Oil 100% Concentration		
Rep #1	Rep #2	Rep #3
		

Appendix B:

Visual assessment of the tea tree oil effectiveness on germination inhibition and fungal inhibition.

Untreated Control		
Rep #1	Rep #2	Rep #3
		
Tea Tree Oil 2%		
Rep #1	Rep #2	Rep #3
		
Tea Tree Oil 5%		
Rep #1	Rep #2	Rep #3
		
Tea Tree Oil 10%		
Rep #1	Rep #2	Rep #3
		

Works Cited

Carson, C.F, Hammer, K.A., and Riley, T.V. 2006. *Melaleuca alternifolia* (Tea Tree) Oil: a Review of Antimicrobial and Other Medicinal Properties. Clinical Microbiology Reviews. 19(1): 50-62. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1360273/.

Christian, Erik J. 2007. Plant Extracted Essential Oils as a Contact Fungicide Seed Treatment for Organic Corn. Iowa State University. http://lib.dr.iastate.edu/cgi/viewcontent.cgi?article=15548&context=rtd.

Coconut (Cocos Nucifera) The Tree of Life. 2004. Coconut Research Center. http://www.coconutresearchcenter.org/.

Coconut Oil. 2009. WebMD – Therapeutic Research Faculty. http://www.webmd.com/vitamins-supplements/ingredientmono-1092-coconut%20oil.aspx?activeingredientid=1092 &activeingredientname=coconut%20oil.

Colley, M., McKenzie, L., Stone, A., Brewer, L., and Baker, B. 2014. Organic Seed Resource Guide: Introduction and Table of Contents. Extension Organic Agriculture. http://www.extension.org/pages/18340/organic-seed-resource-guide:-introduction-and-table-of-contents#.VaJzQ19Vikp.

Derrer, David T. 2014. Tea Tree Oil. WebMD – Vitamins and Supplements Lifestyle Guide. http://www.webmd.com/vitamins-and-supplements/lifestyle-guide-11/supplement-guide-tea-tree-oil.

Electronic Code of Federal Regulations. 2015. Title7: Agriculture, Part 205 – National Organic Party. http://www.ecfr.gov/cgi-bin/text-idx?rgn=div5&node=7:3.1. 1.9.32#se7.3.205_1204.

Gatch, Emily. 2014. Organic Seed Treatments and Coatings. Washington State University. http://www.extension.org/pages/18952/organic-seed-treatments-and-coatings#.VaJzTV9Vikp.

Generic Materials Search: seed treatments. 2014. OMRI. http://www.omri.org/simple-gml-search/results/%22Seed%20Treatments%22.

Goggi, Susana. 2004. Integration of Natural Seed Treatments in Organic and Open-pollinated Corn Systems. Leopold Center for Sustainable Agriculture – Iowa State University. https://www.leopold.iastate.edu/sites/default/files/grants/E2004-28.pdf.

Greene, Catherine. 2014. Organic Market Overview. USDA Economic Research Service. http://www.ers.usda.gov/topics/natural-resources-environment/organic-agriculture/organic-market-overview.aspx.

Halcon, Linda and Milkus, Kelly. 2004. *Staphylococcus aureus* and wounds: A review of tea tree oil as a promising antimicrobial. American Journal of Infection Control. 32(7): 402-408. http://www.ajicjournal.org/article/S0196-6553(04)00365-7/abstract?cc=y=.

Hammer, K.A., Carson, C.F., and Riley, T.V. 2004. Antifungal effects of *Melaleuca alternifolia* (tea tree) oil and its components on *Candida albicans, Candida glabrata*, and *Saccharoyces cerevisiae*. Journal of Antimicrobial Chemotherapy. 53: 1081-1085. http://jac.oxfordjournals.org/content/53/6/1081.full.pdf+html.

Health Benefits of Lemon Oil. 2015. Organic Facts – Organic Information Services Pvt Ltd. https://www.organicfacts.net/health-benefits/essential-oils/health-benefits-of-lemon-oil.html.

Merriam-Webster. 2015. Organic. Dictionary.com. http://www.merriam-webster.com/dictionary/organic.

Miller, Sally A., and Lewis Ivey, Melaine L. Hot Water and Chlorine Treatment of Vegetable Seeds to Eradicate Bacterial Plant Pathogens. The Ohio State University Extension. http://ohioline.osu.edu/hyg-fact/3000/pdf/3085.pdf.

National Organic Program. 2014. USDA. http://www.ams.usda.gov/AMSv1.0/nop.

Organic Seed Alliance. 2015. http://seedalliance.org/.

OSU Biological Safety. 2011. Fact Sheet Disinfection Using Chlorine Bleach. Oregon State University Research Office. http://oregonstate.edu/dept/larc/sites/default/files/pdf/chlorine-fact-sheet.pdf.

Paulsrud, Bruce E., Martin, Drew, and other authors. 2001. Oregon Pesticide Applicator Training Manual Seed Treatment. Cooperative Extension Work University of Illinois. http://www.oregon.gov/oda/shared/documents/publications/pesticidesparc/pesticideapplicatorseedtreatmenttrainingmanual.pdf.

Raudales, Rosa E. and McSpadden Gardener, Brian B. 2008. Microbial Biopesticides for the Control of Plant Diseases in Organic Farming. The Ohio State University Fact Sheet Agriculture and Natural Rsources. http://ohioline.osu.edu/hyg-fact/3000/pdf/HYG_3310_08.pdf.

Sauer, D.B., and Burroughs, R. 1986. Disinfection of Seed Surfaces with Sodium Hypochlorite. Phytopathology. 76 (7): 745-749. https://www.apsnet.org/publications/phytopathology/backissues/Documents/1986Articles/Phyto76n07_745.pdf.

Seed Treatment: A Tool for Sustainable Agriculture. 2007. Seed Treatment and Environment Committee. International Seed Federation. CropLife International.

Schmitt, A., Jahn, M., Kromphardt, C., Krauthausen, H.J., Roberts, S.J., Wright, S.A.I, Amein, T., Forsberg, G., Tinivella, F., Gullino, M.L., Wikstrom, M., Van der Wolf, J., Groot, S.P.C., Werner, S., and Koch, E. 2006. STOVE: Seed Treatments for Organic Vegetable Production. European Joint Organic Congress. http://edepot.wur.nl/116019.

Tea tree oil (*Melaleuca alternifolia*). 2013. Drugs and Supplements: Mayo Clinic. The Natural Standard Research Collaboration. http://www.mayoclinic.org/drugs-supplements/tea-tree-oil/background/hrb-20060086.

Tea Tree Oil. 2009. WebMD. Therapeutic Research Facility. http://www.webmd.com/vitamins-supplements/ingredientmono-113-tea%20tree%20oil.aspx?activeingredientid=113&activeingredientname=tea%20tree%20oil.

Van Der Wolf, JM., Birnbaum, Y., Van Der Zouwen, P.S., and Groot, S.P.C. 2007. Disinfection of Vegetable Seed by Treatment with Essential Oils, Organic Acids and Plant Extracts. Plant Research International. Seed Science % Technology. 36: 76-88. http://www.researchgate.net/profile/Steven_Groot/publication/40099038_Disinfection_of_vegetable_seed_by_treatment_with_essential_oils_organic_acids_and_plant_extract/links/02e7e51f1221911e8b000000.pdf.