TOMATO SEED DISINFECTION WITH CHLORINE

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ABSTRACT

Chlorine is a commonly used disinfectant for tomato seed contaminated with the bacterial pathogens causing canker, spot, and speck diseases. Previous research has attempted to determine chlorine effectiveness by testing for the presence of the pathogen after treatment of infected seed lots. Even with the most advanced techniques, the accuracy of these methods is questionable, and can underestimate the potential for causing disease. In contrast, this research tested the effectiveness of chlorine for disinfecting tomato seed by plating treated seed onto potato dextrose agar to determine the percentage of disinfected seeds. The elimination of all bacterial growth from seed including bacteria such as Bacillus with highly resistant spores should indicate the efficacy of a treatment for the elimination of bacterial pathogens. Dry seed that was previously extracted by fermentation could be effectively disinfected after pre-soaking in water for 24 hours at a temperature inhibitory to germination, and then treating with 5000 mg/L chlorine at 20°C at pH 9.5 for 60 minutes with constant agitation. The threshold level for disinfection was 2500 mg/L of chlorine resulting in less than 1% infested seeds, but slightly more than 0%. Oxidation of the seed coat by the hypochlorite ion was the main factor resulting in disinfection, but an additional effect from the presence of low levels of hypochlorous acid was also found.

INTRODUCTION

Bacterial spot has been a persistent problem in Ontario and the U.S. despite considerable efforts to control the disease. As well, bacterial canker continues to occur sporadically but at sometimes very severe levels. Bacteria which cause these diseases are known to infest the seed coat of tomato seeds resulting in infected seedlings. This source of infection has been the focus of numerous attempts to devise effective seed treatments to eliminate bacteria. Bacterial spot bacteria (Xanthomonas spp.) produce spots on the surface of tomato fruit, and bacteria from these spots surface contaminate seed during the seed extraction process. Bacterial canker, in contrast, is a systemic disease infecting the stem of the plant. Bacteria can travel through the xylem into the fruit and thereby infect the seeds. In rare instances, canker bacteria have been found under the seed coat where they are difficult to eradicate by chemical means without damaging the embryo. The main problem with bacterial spot is in eliminating bacteria on the seed surface. Tomato seed has numerous epidermal cells which partially break down to form

"hairs" as shown in the photograph below. Pectinaceous material surrounds the seed and must be removed by fermentation, acid extraction, or pectinase prior to drying the seed. The extremely rough surface of the seed coat due to the presence of these hairs provides an ideal protected location for pathogenic bacteria to reside. Residual pectinaceous material may also cover the bacteria and protect them once the seed has dried. Canker bacteria have a capsule which provides additional protection from dessication.

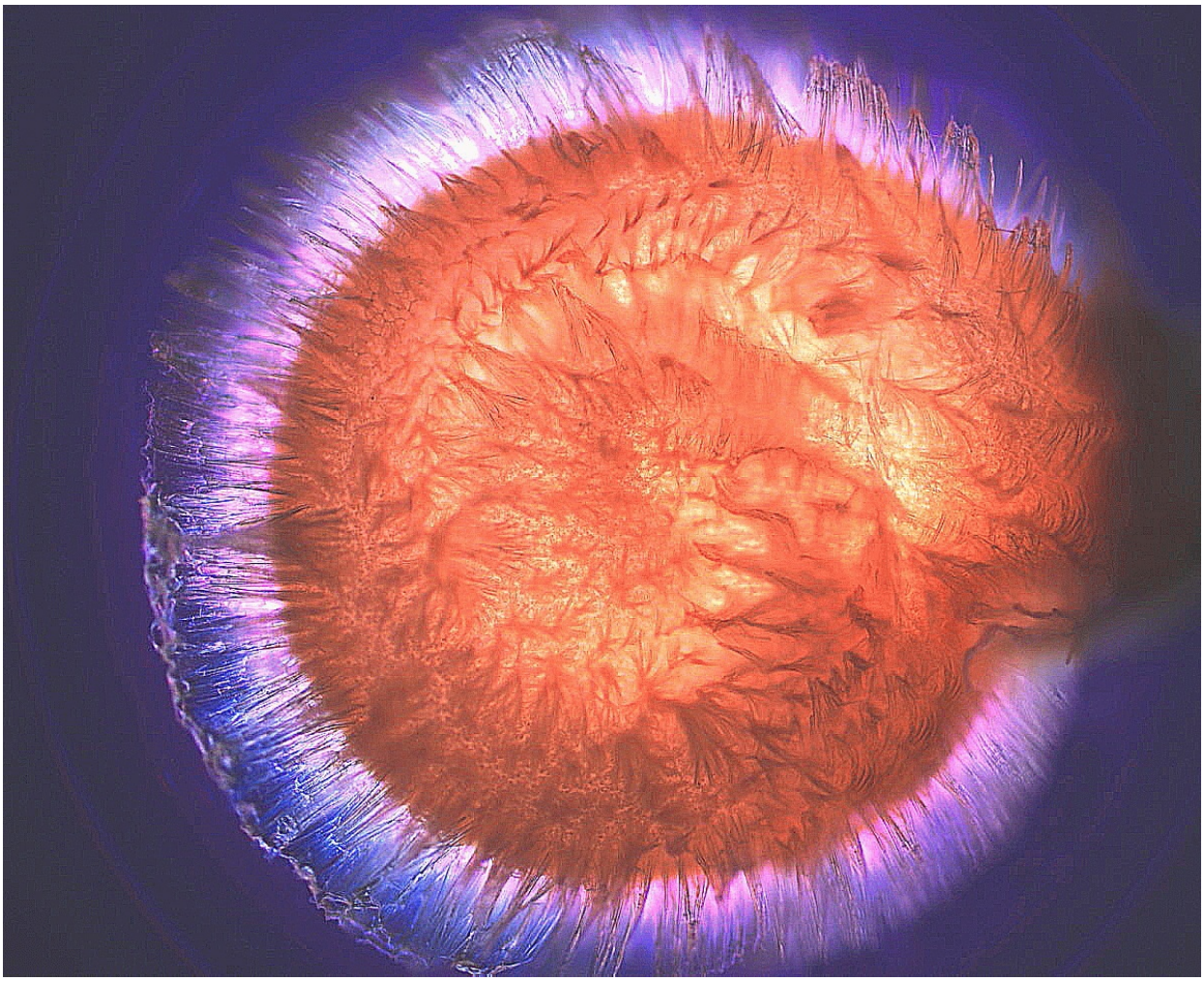

Figure 1. Tomato seed showing seed hairs encased in a pectinaceous substance. (Science magazine, Robert Rock Belliveau, retired plant pathologist).

Another term for seed hairs is "fuzz" and "defuzzing" is regularly done to make the seed surface smooth to aid in pelletizing and seeding. Since chlorine is an oxidizing chemical, the weight of the fuzz and the total weight of the seed coat affects the amount of chlorine required to disinfect seed, and the safety of the procedure. Once all of the organic matter in the seed coat has been oxidized, penetration of chlorine to the embryo will kill the seed.

Use of chlorine for disinfection of seed is common, but methods used often do not take into account complex factors which can affect results. One of the best sources for information on using chlorine as a disinfectant is "White's Handbook of Chlorination and Alternative Disinfectants" a 1062 page compilation of chlorine information. The most commonly used source of chlorine for disinfecting seed is sodium hypochlorite (NaOCl) which exists only in solution and dissociates into both the hypochlorite ion (OCl^-) and hypochlorous acid (HOCl), the relative amounts being determined by the pH of the solution (Figure 2). Very little HOCl is available at high pH levels.

There is a reservoir effect which results when HOCl is consumed. The OCl^- ion combines with H+ to form more HOCl keeping HOCl constant. Hence, when treating seed the amount of available HOCl will remain constant as long as the pH stays constant and the total amount of chlorine stays constant.

The hypochlorite ion is a strong oxidizing agent and readily reacts with organic matter to produce CO_2. It is also more effective than hypochlorous acid at inactivating virus particles. Hypochlorous acid is more effective at eliminating bacteria at low levels of chlorine such as in drinking water supplies, because it can penetrate cell walls easier than the hypochlorite ion which is electrostatically repelled from bacterial cell walls due to its negative charge. Disinfection is due not only to oxidation reactions but also substitution reactions that disrupt functions of proteins and nucleic acids. The important factor in treating seed is that chlorine reacts with organic matter resulting in a decrease in concentration, and must be continuously replenished to maintain a given amount of chlorine in solution.

Sources of sodium hypochlorite are variable in the actual concentration of chlorine due to the breakdown of sodium hypochlorite into sodium chloride and oxygen. The pH of commercial sources is kept very high, greater than pH 12, in order to reduce this breakdown. A 1% sodium hypochlorite (NaOCl) solution is equal to 10,000 mg/L free chlorine, but it cannot be assumed that the stated concentration of NaOCl on the label, for example 5.25%, can be used to make up a solution with a required concentration of chlorine.

There are several methods for measuring chlorine concentrations. Available chlorine is a measurement of the oxidizing power expressed in terms of an equivalent quantity of chlorine and was the method used in the following experiments.

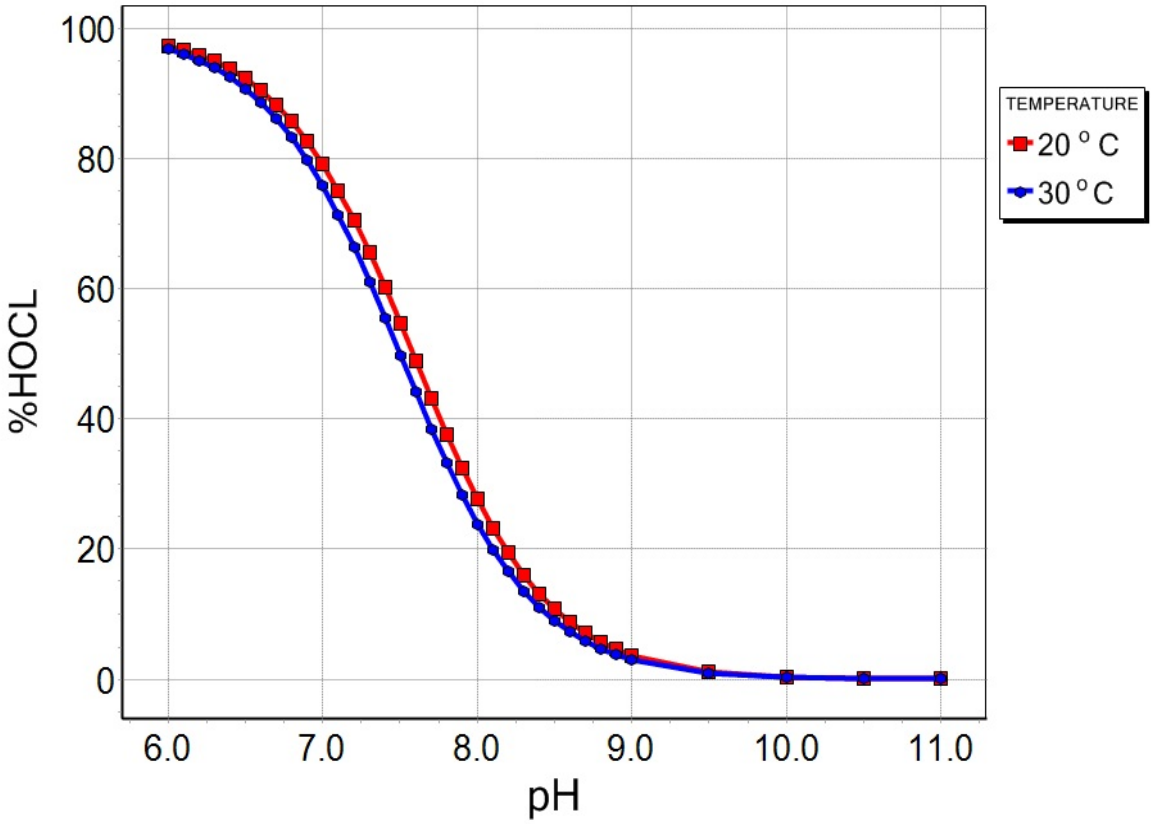

Figure 2. Percentage of chlorine present as HOCl (hypochlorous acid) as a function of pH at 20°C assuming zero ionic strength and considering HOCl and OCl^- (hypochlorite ion) as the only chlorine species present. (Adapted from Table 2.3, White's Handbook of Chlorination and Alternative Disinfectants).

PURPOSE

To determine the effect of chlorine on disinfection of tomato seed .

METHODS

In order to determine the amount of organic matter in the seed coat available to react with hypochlorite ion during seed treatment, the weight of the hairs on the seed coat and the total seed coat weight was determined. Seed was extracted from fresh tomatoes and the seed gel removed by overnight fermentation with pectic enzyme (0.1%). The washed seed was then air dried to approximately 5% moisture content. A total of 14 different seed lots were examined in this manner. For each seed lot, ten grams of seed was defuzzed by tumbling in fine nylon bags for 10 hours at room temperature until the exterior of the seed was smooth and polished. The polished seed was weighed to determine the percentage of seed weight lost due to the removal of the seed hairs (fuzz). The same seed lots were used to determine the weight of the seed coat before de-fuzzing. For each seed lot, 400 seeds were weighed and germinated until the seed coats were loose. The seed coats were then collected, dried and weighed.

For the seed treatment experiments, hybrid seed was produced in India, extracted using only fermentation and air dried to 8% moisture content or less. In most cases, only one seed lot with a very high level of germination was used (TSH16ID288). Three categories of seed were used. Unsorted seed was used in some of the earlier experiments (NS= not sorted); this seed consisted of about 90 % single seeds, and 10% seed in which 2 or more seeds were stuck together. The second category was single (S) seeds only, very carefully hand sorted to ensure only single seeds. The third category consisted of 2 or more seeds stuck together, doubles or clumps (DBL). In some cases, up to 9 seeds or more could be in one clump. Clumped seeds were initially screened out of the bulk seed lot, and then carefully hand sorted to ensure no single seeds were present. Doubles or clumps from the previously sorted seed were also used.

For all of the experiments, well water with a pH of approximately 7.5 was used. This water was not treated with chlorine in any municipal treatment system. A water analysis for various minerals is presented in Table 1.

Chlorine solution containing approximately 12% sodium hypochlorite was used in all the experiments. This solution can be obtained in bulk from swimming pool suppliers, and contains no additives such as fragrances as are sometimes found in small household containers. The actual concentrations were usually much lower than the labeled amount. Once the actual concentration in a container was measured(using the ExStik CL200A Waterproof Total Residual Chlorine Tester), the approximate concentration could be made up for any particular experiment, and adjusted in small increments to get as close as possible to the desired concentration. Chlorine solutions were made up and used immediately due to the unstable nature of sodium hypochlorite.

For the chlorine experiments, seed was either treated dry or after soaking for 18-24 hours in water at 6.4°C to prevent any germination. Small amounts of seed, 5 grams or less, were treated in large volumes of treatment solution, 1000 ml, so that the effect of the quantity of seed on the chlorine levels would be minimal. The pH levels were adjusted using concentrated solutions of either HCl or NaOH. The initial temperature of the treatment solution was adjusted to 20°C and the treatments were conducted in a room at 20°C . If the temperature required was higher than 20°C , then the room temperature was adjusted to match. The chlorine concentration, pH and temperature were measured at the start and end of the treatment period and averaged. The mixture of seed and solution was constantly stirred using a magnetic stirrer. All containers and instruments were autoclaved and all treatments were done in a laminar flow cabinet to maintain sterility. All treatments were for 60 minutes unless otherwise specified.

After treatment, the seed was collected in a small sterile collander and transferred to a 500 ml container of sterile water for an initial rinse (1-2 minutes) to remove most of the chlorine solution. A second rinse was then done in the same manner. Thirdly, the seed was aseptically transferred to a solution of sterilized sodium thiosulphate (20 g/L) in another 500 ml container and manually stirred for 30 minutes using a sterilized instrument. Following this, the seed was again transferred using sterilized small collanders and instruments to another 500 ml container of sterilized water to rinse off the sodium thiosulphate for 1-2 minutes. A final rinse in another container of sterilized water was done to make sure no residues remained.

The sodium thiosulphate solution was tested and shown to completely remove chlorine residues. It is a commonly used method for removing chlorine residues. The identity of the chlorine residue remaining on the seed prior to neutralization is unknown as the reaction products with chlorine are complex, but it is likely to be chloramine, a less reactive but still effective form of chlorine for disinfection. The freshly treated seed would also have some sodium hypochlorite solution remaining which would be neutralized by sodium thiosulphate. When seed is only rinsed with water, the chloramine residue remains embedded in the seed coat and is still present after drying. Any residual sodium hypochlorite solution converts to sodium chloride and sodium chlorate when the seed is dried. This method of neutralization eliminated any residues on the seed which could interfere with the assessment of disinfection. If this neutralization step was not done, the chloramine residues on the seed could prevent the growth of any remaining bacteria when the seed was plated out on potato dextrose agar (PDA). Commercially, chlorine treated seed is just rinsed and dried so that the chlorine residue is still present on the finished seed.

Total available chlorine was measured on treatment solutions with the ExStik CL200A Waterproof Total Residual Chlorine Tester which measures total chlorine from 0.01 to 10.00 ppm (mg/L). Samples were taken from each solution and diluted to obtain a reading in the mid-range of the meter for accuracy. Two readings were taken and averaged for each sample. The reading was then multiplied by the appropriate factor depending on the dilution ratio. The total of all chlorine present in all forms, including dissolved free chlorine, chloramines, hypochlorous acid (HOCl) and hypochlorite ion (OCl^-) is measured with this meter. In these experiments, since only sodium hypochlorite was used, most if not all of the chlorine would be present as either

hypochlorous acid or hypochlorite ion. The relative amount of these two molecules is determined by the pH of the solution. The pH of each solution as well as the temperature was measured. Temperature was not a factor in HOCl concentrations at the pH levels used in these experiments. Hence, a formula to calculate HOCl based on the total chlorine concentration and pH at 20°C could be used. This formula was obtained from White's Handbook and is as follows:

Concentration of HOCl in mg/L at 20°C = (1/(1+((2.62*10^-8)/(10^-C))))*A
where A= Total Chlorine in mg/L, and C = pH

The amount of hypochlorite ion in solution can be calculated by subtracting the hypochlorous acid concentration from the total chlorine (chlorine).

In some recommendations regarding the use of chlorine, use of a surfactant is recommended. To determine the effectiveness of using a surfactant with chlorine, seed was pre-soaked for 25 hours in 0.1 % Ag-surf, a commercially available non-ionic agricultural surfactant.

PDA, a medium that supports a wide range of microorganisms, was prepared using fresh potatoes and other ingredients. Plates (10 mm petri dishes) were poured and allowed to dry in the laminar flow cabinet overnight to prevent any surface moisture from interfering with the assessment of seed disinfection. The freshly treated, chlorine neutralized seed was placed on the plates using aseptic techniques with 25 seeds per plate, on 8 plates, for a total of 200 plated seeds per treatment. The remaining seed was placed in a nylon bag and tumble dried for 3 hours at approximately 35°C and used for germination tests. In cases where germination results were required, 200 seeds were germinated on moistened cotton pads using 50 seeds per plate, with % germination recorded after 7 days. The PDA plates with treated seed were examined after 24, 48 and 72 hours of incubation at 27°C to record the number of seeds with bacterial growth. Seed was examined for several more days, but no change in percentage of infested seeds occurred after 3 days of incubation. Germination on the PDA occurred from 3-7 days after plating, although the fairly hard PDA was not conducive to good germination. No germination occurred from infested seeds. The bacterial growth around these seeds totally inhibited germination.

Figure 3. Set-up for chlorine disinfection experiments in laminar flow cabinet showing the seed treatment beaker on the magnetic stirrer, and the rinsing and neutralization containers.

Figure 4. Aseptically placing treated seed on PDA media to determine disinfection.

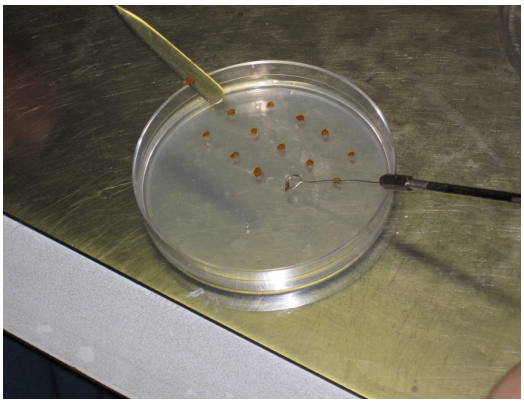

Figure 5. Close-up of plating procedure.

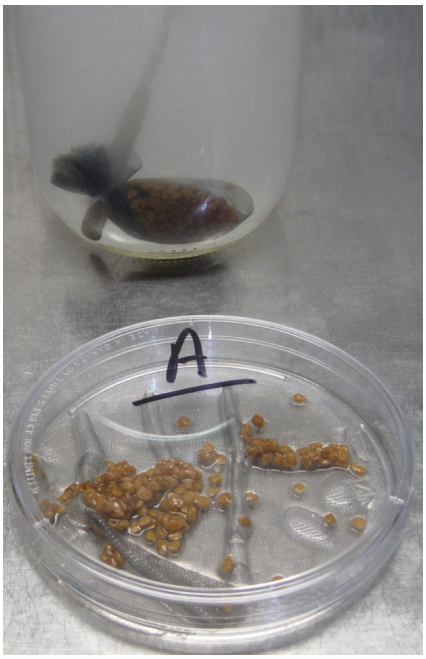

Figure 6. Nylon seed bag in HCl treatment solution, and treated seed in foreground (Exp. 31A).

For the hydrochloric acid experiments, commercial grade muriatic acid containing 31.45% HCl was used. As a guide to the amount of HCl to use, we referred to published papers in which a concentration of 0.6M HCl was used to treat tomato seed (Dhanvantari, 1989; Dhanvantari and Brown, 1993; and Fatmi, Schaad and Bolkan, 1991). In these experiments, muriatic acid was diluted to make up 3.33% and 6.66% muriatic acid solutions, corresponding to 0.333 and 0.666 Molar HCl solutions respectively. Seed was not pre-soaked for any of these experiments since previous experiments had shown that this was extremely detrimental to germination. All utensils and containers were either autoclaved or flame sterilized, and all treatments and procedures were conducted in a laminar flow cabinet to ensure sterility. Two grams of dry seed inside a porous nylon bag were treated in each experiment in 1000 ml of solution and stirred manually during treatment. The treated seed was removed from the solution, aseptically transferred to a 500 ml container of sterile water and rinsed with stirring for 5 minutes to remove most of the acid solution. Seed was transferred to a second 500 ml container of sterile water and rinsed again. Following this, seed was transferred to a sterile sodium hydroxide solution (0.634 g dry NaOH/L of water), and manually stirred for 30 minutes to completely neutralize any acid residues. The seed was then aseptically transferred to another 500 ml container of sterile water and stirred manually for 15 minutes to remove any sodium hydroxide residues. Finally, seed was rinsed again in sterile water to ensure no sodium hydroxide residues remained. The pH level of the final rinse water was monitored to

be sure it was between pH 7 and 8. The treated seed in the nylon bag was transferred to a sterile petri dish, aseptically cut open, and the seeds were placed individually on plates of PDA, using the same aseptic technique as used for the chlorine treated seed.

Figure 7. Close-up of seed treated with 0.666 M HCl for 2 hours (Exp. 31A)

For the trisodium phosphate (TSP) treatment, a solution containing 120 g/L of TSP was prepared resulting in a pH of 12.73. TSP is commonly used to treat seed to inactivate the tomato mosaic virus (TMV). The mechanism of inactivation has been shown to be due only to the pH of the solution. Both HCl and chlorine solutions have also been shown to inactivate TMV on the seed coat of tomato seed. However, the effect of TSP on disinfecting tomato seed has never been reported.

Table 1. Water analysis for water used to make up all solutions.		
Parameter measured		
pH	7.50	
EC (mmhos/cm)	0.68	
Nitrate Nitrogen	1.00 ppm	0.07 mmol/L
Ammonium - N	< 0.50 ppm	
Phosphorus	< 1.00 ppm	
Potassium	< 1.00 ppm	
Calcium	104.50 ppm	2.61 mmol/L
Magnesium	28.65 ppm	1.19 mmol/L
Bicarbonate	369 ppm	6.05 mmol/L
Alkalinity	295.2 ppm	
Chloride	7.00 ppm	0.20 mmol/L
Sulphates	48.29 ppm	0.50 mmol/L
Sodium	8.96 ppm	0.39 mmol/L
Zinc	0.55 ppm	8.52 mmol/L
Manganese	0.29 ppm	5.22 mmol/L
Copper	<0.01 ppm	
Iron	0.51	9.02 mmol/L
Boron	0.07 ppm	5.91 mmol/L
Molybdenum	0.02 ppm	0.19 mmol/L
Silicon	7.20 ppm	256.39 mmol/L
Cations	8.00	
Anions	7.32	
Report# 428201 SGS Agrifood Laboratories, 1-503 Imperial Road North, Guelph, Ontario, N1H 6T9, Canada (519-837-1600)		

RESULTS

Section 1. Determination of seed coat weight

Seed Lot	Seeds per gram	Total weight of seed coat %	% Hairs removed by de-fuzzing	Percentage of Seed Coat Composed of Seed Hairs
193ID141	336	25.21	14.2	56.3
218ID142	396	22.77	11.4	50.1
390ID144	404	22.22	9.8	44.1
833ID145	404	25.25	13.4	53.1
218ID199	360	21.62	9.3	43.0
329ID202	412	23.71	11.7	49.3
390ID203	392	20.59	8.8	42.7
390ID204	408	21.43	9.3	43.4
501ID205	367	22.02	7.9	35.9
833ID206	421	25.26	10.8	42.7
836ID207	385	22.12	9.9	44.8
1045ID208	381	21.74	11.5	52.9
1045ID209	354	22.12	9.6	43.4
1077ID210	354	21.24	9.9	46.6
AVERAGES		22.66	10.54	46.3

The seed coat comprised 20-26% of seed weight depending on the seed lot. Variations may occur due to the amount of handling during drying in commercial production. Seed is rubbed while drying on screens in order to separate the seeds, and depending on the way this is done, some of the seed hairs can be rubbed off. On these seed lots, 35-56% of the seed coat was composed of "hairs" that can easily be removed by "de-fuzzing". This fuzz or finely divided organic material is present during seed treatment, and reacts vigorously with the hypochlorite ion (an oxidizing agent), producing a significant amount of heat due to the chemical reaction. Bacteria can reside deep within these hairs thus requiring a very good treatment method for their elimination. The amount of organic matter in the seed coat constantly reacts with chlorine thus reducing chlorine concentrations unless the chlorine is continuously replenished.

Section 2. Assessment of % double or clumped seeds.

Untreated dry tomato seed contains a percentage of seeds that are stuck together. A representative sample of seed lot TSH16ID288 was hand sorted to grade out all of the single seeds. The percentage of seeds with 2 or more seeds stuck together was found to be approximately 10%.

Figure 8. Sample of tomato seeds before treatment showing from 2 to several seeds stuck together.

Figure 9. Close-up of clumps of seed prior to seed treatment.

Double or clumpy seeds are more difficult to treat because they must be separated before or during the treatment process in order to have the entire seed coat exposed to the treatment chemical. During the course of treating tomato seed with chlorine, a double seed was noticed after treatment and carefully separated, aseptically plated on PDA, and examined later for bacterial growth. Only these two seeds had bacterial growth on them, showing that the seed treatment had not penetrated between the two seeds. This treatment was only at 2902 mg/L chlorine, much lower than normally used for commercial treatment. Seeds that separate late in the treatment process may also not be properly treated, and may account for a few infested seeds in some of the experiments.

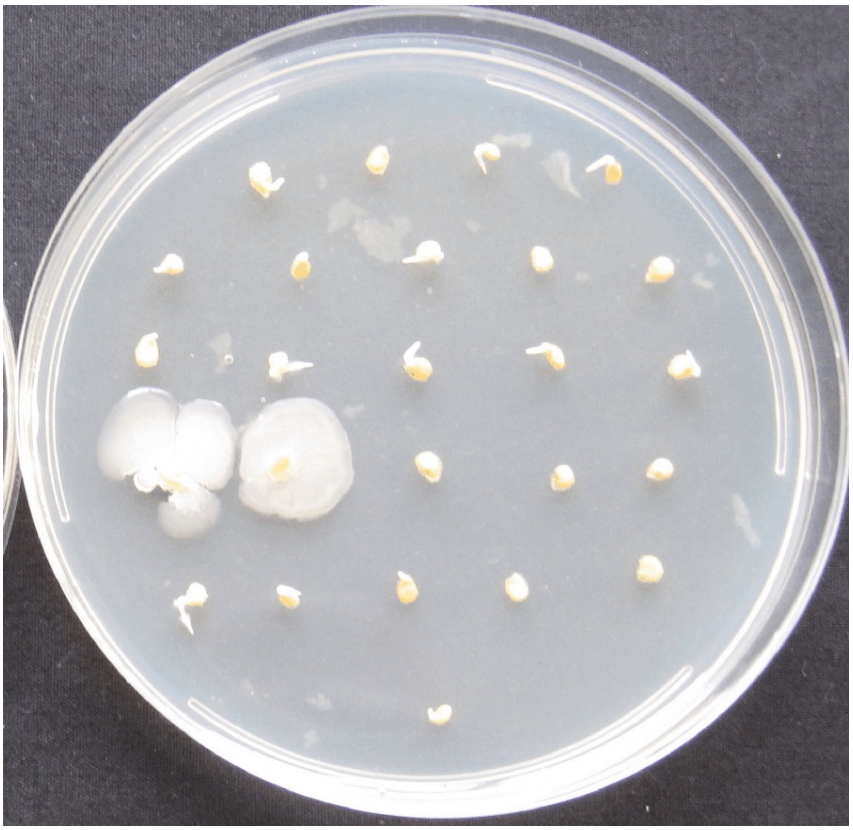

Figure 10. Unsorted tomato seed treated for 60 minutes with 2902 mg/L chlorine at pH10.6 showing 2 infested seeds that had remained stuck together during treatment. (Exp. 10)

Section 3. Chlorine neutralization procedure with sodium thiosulphate ($Na_2S_2O_3$).

2.5 grams of TSH16ID288 seed was soaked for 29 hours prior to treatment for 60 minutes in 1000 L of solution containing 5180 mg/L chlorine (avg of 5430 at start, and 4930 at end). Temperature averaged 20.8°C and pH was 9.5. Half of the treated seed was rinsed twice with sterile water and dried aseptically in petri dishes in the laminar flow cabinet overnight. The other half of the treated seed was neutralized with sodium thiosulphate and also dried overnight. Portions of the water rinsed and neutralized seed, both fresh and dried, were placed on chlorine test paper (Hydrion Sanitizer Test Paper, Micro Essential Laboratory Inc.) to demonstrate the presence of chlorine residues. These paper strips turn various shades of purple depending on the amount of chlorine present in a liquid solution. The range varies from 10 - 200 ppm chlorine. When wet treated seed is placed on the strips, the purple color indicates a chlorine residue. For dry seed, the strip was moistened prior to placing the seed on it. The dried seed was also placed on PDA seeded with bacteria just prior to pouring the plate and incubated for 5 days to determine the effect of chlorine residues on bacterial growth.

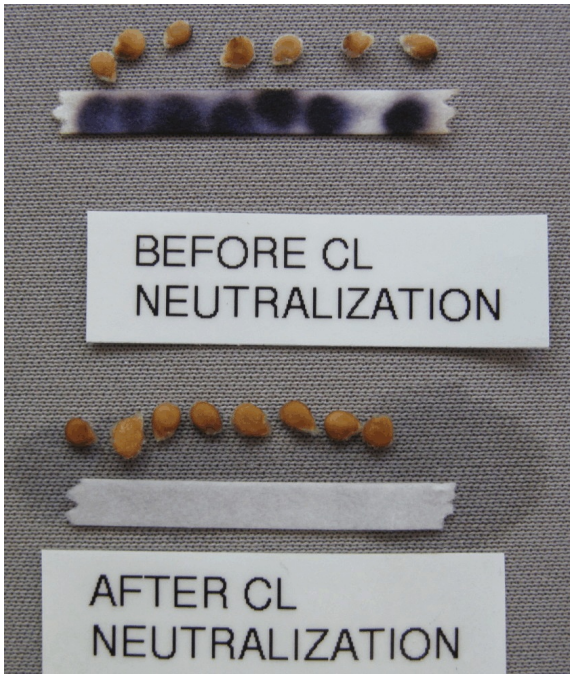

Figure 11. Effect of treated seed on chlorine residue paper before drying. Seed was placed on paper and then moved to show the purple color indicating a strong chlorine residue.

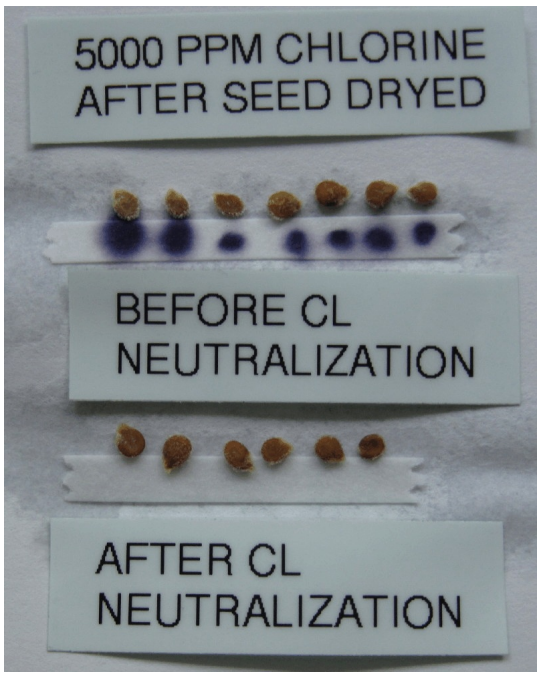

Figure 12. Strong chlorine residue shown to be present after drying seed; not present when chlorine neutralized with sodium thiosulphate.

Results of the test for chlorine residues using the paper indicator clearly showed that sodium thiosulphate was effective in removing residues from seed. Hence, assessing seed for disinfection should not be influenced by chlorine residues. Drying the seed did not eliminate chlorine residues. The dark purple colour on the paper test strips indicated residual chlorine concentrations of at least 200 ppm or more.

When chlorine treated seed was dried and plated on PDA inoculated with bacteria as shown below, the seed in which the chlorine residues were not eliminated clearly showed a small clear area (halo) around them, indicating that the chlorine residue prevented bacterial growth. Neutralization of the chlorine residues removed any effect of the seed on bacterial growth.

Removal of chlorine residues from treated seed eliminates the possibility that they could interfere with the assessment of disinfection, and result in erroneous estimates of % disinfection. It is not known if chlorine residues would be effective in eliminating pathogens when seed is placed in a growing medium. Assuming that the chlorine treatment was effective, the additional effect of a chlorine residue would be redundant.

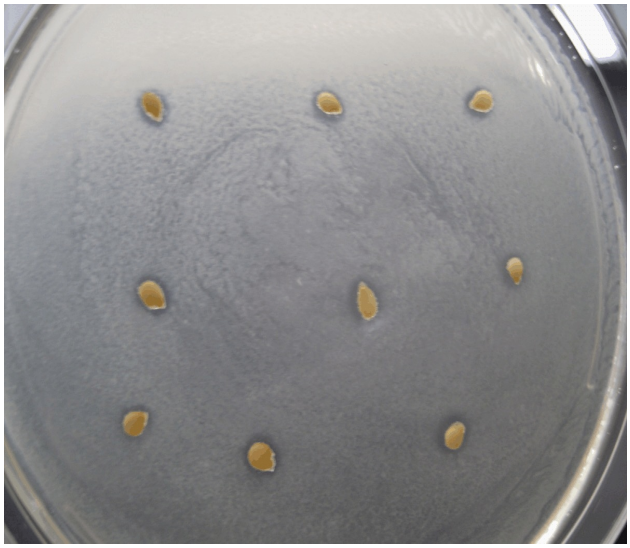

Figure 13 Halos around chlorine treated seed.

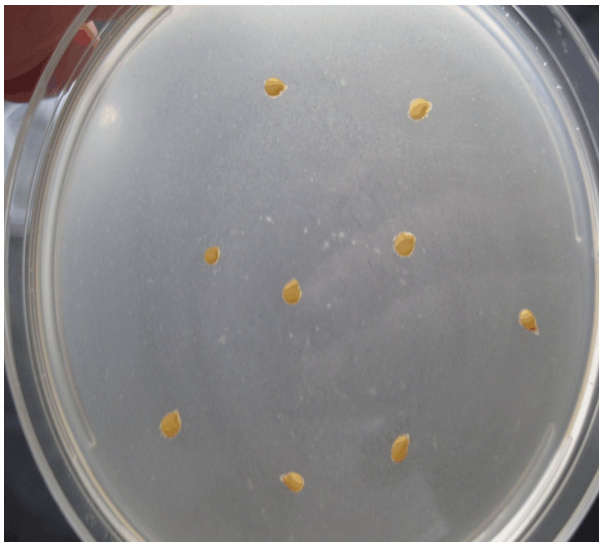

Figure 14 No effect of treated seed on bacterial growth after neutralization of chlorine residues with $Na_2S_2O_3$

Section 4. Observation of possible pectinaceous material after seed treatment.

On several occasions while plating seed from HCl seed treatments, small amounts of clear, soft, jelly like material was noticed. This material was not identified, but may have been pectinaceous in nature. Incomplete fermentation would not destroy all the pectins in the seed pulp, and this material could help to glue the seed together, making it more difficult to treat. Pectinase is not often used in hybrid seed production areas after seed is extracted, but might be helpful in alleviating this problem. HCl obviously did not destroy this gel like substance (probably pectin), whereas the gel was never noticed in any of the treatments using chlorine. It is possible that the hypochlorite ion is capable of oxidizing and destroying the gel; this would assist in more effective seed treatments.

Figure Possible pectinaceous material found with seed after HCl seed treatment.

Section 5. Effect of pre-soaking seed on efficacy of chlorine seed treatments.

EXP #	SEED LOT	SEED TYPE	PRE SOAK TIME (HR)	Total Chlorine mg/L	HOCl mg/L	pH	Temp °C	% INFEST-ED SEED	% GERM
5	T4/427	NOT SORTED	0	4825	1	11.28	20.6	2	100
12	T16/288	DOUBLE	19.5	4878	1	11.35	20.4	0	99.0
18A (control)	T16/288	NOT SORTED	0	0	0	7.8	20.0	100	99.0

The seed that was not pre-soaked (0 hours) resulted in 2% infested seeds compared to 0% for the soaked seed, even though the soaked seed consisted of 100 % double seeds or clumps. The unsoaked seed was not sorted so contained approximately 10% doubles or clumps. This was a good test to show the efficacy of a level of chlorine around 5000 mg/L, even though the pH was not adjusted to a lower level to enhance availability of HOCl. Soaking seed helps to separate the doubles and clumps of seed to allow the treatment access to the entire seed surface. The chlorine treatment had no detrimental effect on germination and was identical to that of the check treatment (Exp. 18A).

Section 6. Efficacy of various levels of chlorine in disinfecting tomato seed.

EXP. #	SEED LOT	SEED TYPE	PRE SOAK TIME (HR)	Total Chlorine mg/L	HOCl mg/L	pH	Temp °C	% INFESTED SEED
19	T16/288	SINGLE	25	465	0	12.09	20.4	86
20	T16/288	SINGLE	22.5	421	35	8.62	20.2	69
15	T16/288	NOT SORTED	19.2	852	11	9.46	20.0	1
33A	T16/288	SINGLE	24	1408	18	9.46	20.0	1.5
6	T4/427	NOT SORTED	19	2850	2	10.68	20.0	1.7
8	T4/427	NOT SORTED	19.5	2310	48	9.30	21.2	1.5
10	T16/370	NOT SORTED	18	2902	3	10.61	30.3	2.2
11	T4/427	NOT SORTED	15.5	4978	0	12.1	20.2	0.5
16	T16/288	NOT SORTED	29	4930	59	9.50	20.8	0

Chlorine concentrations averaging 443 mg/L resulted in 69-86% infested seed, but when increased to 850 mg/L to 3000 mg/L, percentage of infested seed ranged between 1 and 2.2% . Raising the level of chlorine to 4978 was more effective resulting in only 0.5% infested seed (1/200). The high pH in experiment 11 (pH was not adjusted) would have resulted in almost no hypochlorous acid, a more effective disinfectant, and may have been the reason 1 seed out of 200 was infested. The lower pH in experiment 16 resulted in 0% infested seeds, and may have been due to the presence of significant amounts of hypochlorous acid (59 mg/L).

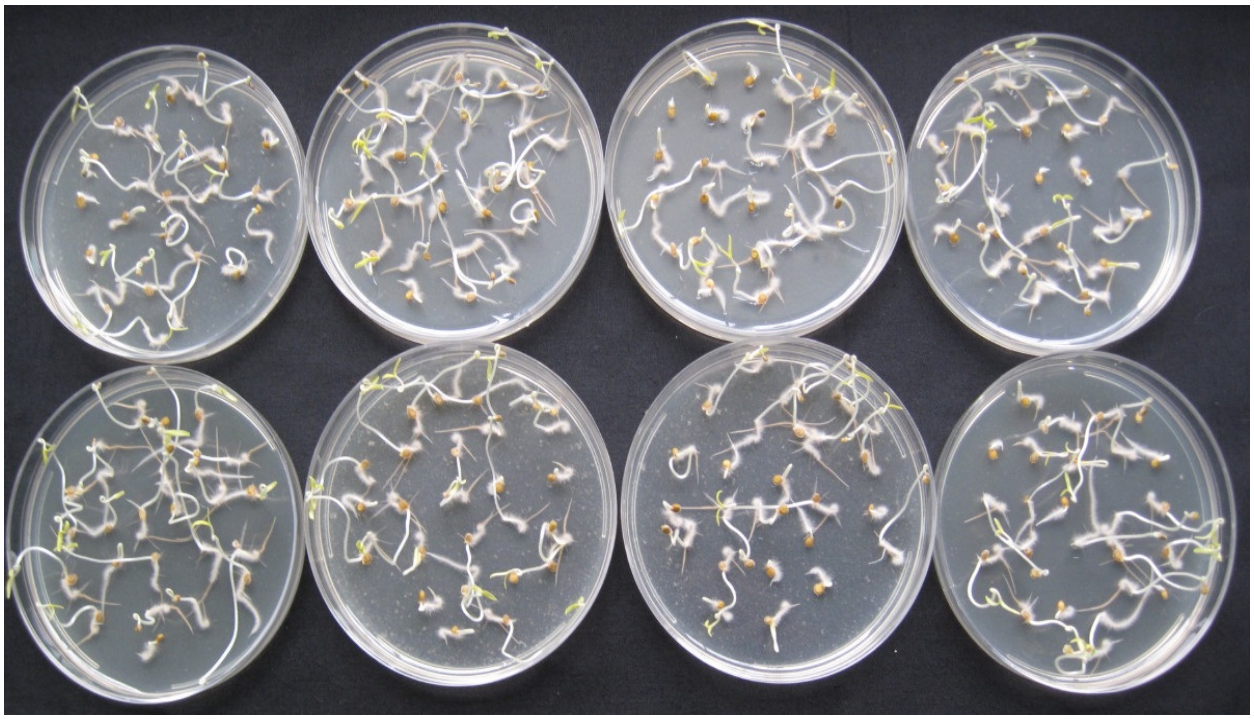

Figure 16. 100% disinfected seed after treatment with 4930 mg/L chlorine at pH 9.5 for 60 minutes (Exp. 16).

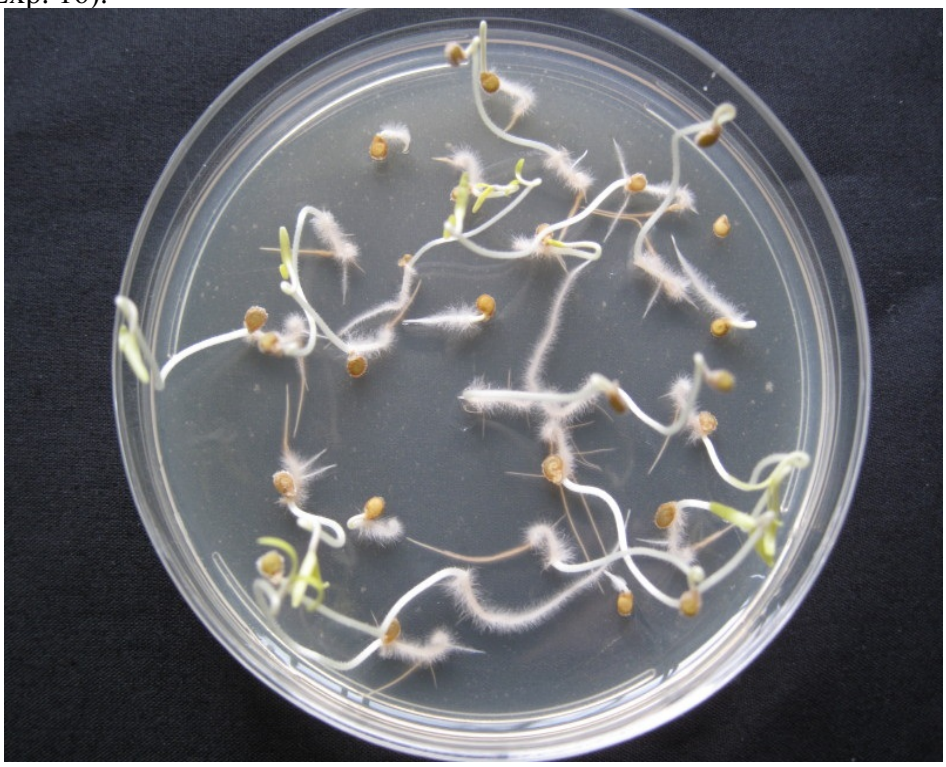

Figure 17. Close-up of disinfected seeds from Exp. 16 after 7 days of incubation.

Section 7. Effect on germination of excessive chlorine treatment

EXP. #	SEED LOT	SEED TYPE	PRE SOAK TIME (HR)	Total Chlorine mg/L	HOCl mg/L	pH	Temp °C	% GERM
13	T4/427	NOT SORTED	19.5	8275	304	9.00	24.9	99.3
24	T16/288	SINGLE	26	8045	338	8.94	33.1	99.0

In experiment 13 conducted at 24.9°C, germination was very good and the seed did not appear to be damaged. Although the germination in experiment 24 conducted at 33.1 °C was good, the seed was over treated based on external appearance in that it was very white and the seed coat appeared to be thin. The germination was weaker (slower) although the final germination was good. The very high temperature of 33.1 °C would have increased the oxidation rate considerably, based on the rule that the chemical reaction rate doubles for every 10°C rise in temperature. The long term viability of seed treated this way may not be good. However, there is considerable variability allowable when treating seed so that higher chlorine levels, lower pH's, and higher temperatures can be tolerated as long as all of these do not converge at the same time. It is important to have a wide margin of safety when commercially treating seed.

Figure 18. Bleached appearance of tomato seed treated at 8275 mg/L NaOCl at pH 9.00 and 24.9°C for 60 minutes (Exp. 13).

Section 8. Determination of threshold of toxicity of chlorine due to low Ph

EXP. #	SEED LOT	SEED TYPE	PRE SOAK TIME (HR)	Total Chlorine mg/L	HOCl mg/L	pH	Temp °C	% GERM
25A	T16/288	SINGLE	24	6165	248	8.96	22.0	100
25B	T16/288	SINGLE	26	5720	1688	7.96	24.2	99.5

Germination was not affected by high levels of HOCl resulting from low pH levels. While HOCl is more effective at penetrating bacterial cells than the hypochlorite ion, it may not penetrate the seed coat in the same way. In fact, converting a significant portion of chlorine to HOCl by reducing the pH will reduce the oxidizing potential of the disinfecting solution, and could reduce seed treatment effectiveness, depending on whether pathogens are eliminated by direct oxidation of the cell wall by the hypochlorite ion, or by penetration of the cell wall by hypochlorous acid.

Section 9. Efficacy of chlorine on 100% double or clumped seed

EXP. #	SEED LOT	SEED TYPE	PRE SOAK TIME (HR)	Total Chlorine mg/L	HOCl mg/L	pH	Temp °C	% INFESTED SEED	% GERM
30D	T16/288	Double	18	4572	52	9.52	21.4	0	100
14	T16/288	Double	20	5292	38	9.72	20.5	0.33*	100
30E	T16/288	Double	18	4792	19	9.99	21.3	0	100
12	T16/288	Double	19.5	4878	1	11.35	20.4	0	NA
* 1 infested seed out of 300 plated out (normal number plated was 200)									

Although non-sorted seed only contained 10% double or clumped (seeds stuck together after drying), this comparison used 100% double seeds to create an extreme case to see if approximately 5000 mg/L chlorine would be effective for disinfection. With the pH ranging between 9.52 and 11.35, a non-disinfected seed was only found once. This indicates that double seeds may result in the occasional non-disinfected seed. In the above series of tests, a total of 900 seeds were plated. If it is assumed that single seeds would have been fully disinfected, then the 1 infested seed in 900 doubles would represent 1 seed in 9000 seeds in a normal non-sorted seed lot. This would make the task of finding such an infested seed by plating non-sorted seed lots extremely difficult.

Section 10. Effect of pH on efficacy of chlorine on single seeds at low chlorine levels.

EXP. #	PRE SOAK TIME (HR)	Total Chlorine mg/L	HOCl mg/L	pH	Temp °C	% INFESTED SEED	% GERM
19	25	465	0	12.09	20.4	86	96.5
20	22.5	421	35	8.62	20.2	69	98.5
Seed lot T16/288, single seeds							

Single seeds were used in this experiment to ensure that any infested seeds would be due strictly to the effect of chlorine on the total seed surface, and not due to escapes where the area in which seeds stick together would not get treated properly. Chlorine at 421 and 465 mg/L did a very poor job of disinfecting seed, but reducing the pH from 12.09 to 8.62 where there would be some hypochlorous acid present reduced the number of infested seeds by 20%. Thus pH could have some effect in maximizing the effect of chlorine if low levels of chlorine are used. Obviously, for a 60 minute treatment, chlorine at approximately 450 ppm was insufficient for seed disinfection.

Section 11. Effect of pH on efficacy of chlorine on single seeds at 1454 mg/L chlorine

EXP. #	PRE SOAK TIME (HR)	Total Chlorine mg/L	HOCl mg/L	pH	Temp °C	% INFESTED SEED	% GERM
22	21	1454	19	9.45	20.6	1	99.0
23	23	1454	0	11.88	20.5	58.5	98.0
Seed lot T16/288, single seeds							

This comparison showed the importance of pH in improving the efficacy of chlorine due to the higher level of HOCl, resulting in only 1% infested seed at a pH of 9.45. In this experiment, at pH 9.45 the concentration of HOCl was 19 mg/L, much higher than that used for treating swimming pools (2 mg/L). However, as shown in Section 10, HOCl at 35 mg/L was ineffective at disinfecting seed when the hypochlorite level was low. Hence, it is the combined effect of hypochlorite and hypochlorous acid that resulted in the low levels of infested seed in experiment 22.

Section 12. Effect of pH on efficacy of chlorine on single seeds at 2400 mg/L chlorine.

EXP. #	PRE SOAK TIME (HR)	Total Chlorine mg/L	HOCl mg/L	pH	Temp °C	% INFESTED SEED	% GERM
27A	21	2450	40	9.36	20.8	0	99.5
27B	23	2406	0	11.72	21.3	5.5	99.5
Seed lot T16/288, single seeds							

At a total chlorine level of 2450 ppm, reducing the pH to 9.36 from 11.72 reduced the percentage of infested seed from 5.5% to 0%. The reduction in pH resulted in 40 mg/L of HOCL which improved the effectiveness of the chlorine treatment at this level of total chlorine. This may indicate that at higher levels of chlorine it would be advantageous to keep the pH at around 9.5 to ensure the presence of some HOCl to improve the effectiveness of the chlorine treatment.

Section 13. Effect of pH on efficacy of chlorine on double or clumped seeds at approximate chlorine levels of 2200-2500 mg/L.

EXP. #	PRE SOAK TIME (HR)	Total Chlorine mg/L	HOCl mg/L	pH	Temp °C	% INFESTED SEED
29F	22	2484	0	12.99	20.8	0.5
29A	22.6	2349	0	11.86	20.5	8.0
29B	23.5	2186	2	10.73	21.1	0.5
29C	24.5	2380	12	9.89	21.1	1.5
29D	25.0	2228	86	8.98	21.2	0.5
29E	23.5	2456	634	8.04	21.6	0.0
Seed lot T16/288, double seeds						

With double seeds and lower levels of chlorine, the effectiveness of chlorine treatment may be more dependent on when the seeds split apart; this could determine the length of time the newly exposed surface of such seeds is treated. In this series of treatments, experiment 29A had the highest amount of infested seeds. This could be due to just one large clump of seeds that came apart late in the treatment process. The use of 100% double seeds allows potential weaknesses in the chlorine seed treatment process to be expressed. The lowest pH which would have the highest concentration of HOCl had 0% infested seeds. pH levels in the range of 9 to 11 reduced the percentage of infested seeds to about 1%. The effectiveness of using total chlorine levels in the 2000 - 2500 mg/L range is questionable even with pH reduction, and higher levels are probably necessary to have a more consistent disinfection effect. Seeds that are stuck together may not be fully disinfected even if they come apart during treatment. Depending on when they separate, the length of time that the newly exposed seed surface is treated may determine the potential for disinfection. It should be noted that unless clumped seeds had been used in this series of treatments, it is likely that no infested seeds would have been found.

Section 14. Effect of pH on efficacy of chlorine on double or clumped seeds at approximate chlorine levels of 4462-4792 mg/L.

EXP. #	Total Chlorine mg/L	OCl^- mg/L	HOCL mg/L	pH	% INFESTED SEED	% GERM
30G	4692	4691	1	11.08	0	99.5
30F	4702	4693	9	10.3	0	99.5
30E	4792	4764	28	9.81	0	100
30D	4572	4507	65	9.42	0	100
30C	4668	4489	179	8.98	0	100
30B	4590	4085	505	8.49	0.5	100
30A	4462	3229	1233	8.00	0	99.5
Seed lot T16/288, DOUBLE seeds, pre-soak time of 18 hours Temperature ranged between 20.8 and 22.2 °C and would not have influenced results very much.						

Efficacy of chlorine for disinfecting double seeds significantly improved at higher levels of chlorine. Only 1 infested seed was found at a pH of 8.49 (experiment 30B). This may be the result of a double seed that split apart late in the treatment process. Alternatively, the conversion of OCl^- to HOCl at the low pH of 8.49 would have resulted in less oxidation activity, and possibly slightly less disinfection efficacy. Overall, excellent disinfection of double seeds resulted from treatment with approximately 4500 mg/L chlorine at a pH between 9 and 11, at 21-22°C, for 60 minutes with continuous stirring. As well, germination was excellent at all levels of chlorine tested. A high level of oxidation potential may be important in helping to separate seeds that are stuck together, and could be more important than the presence of HOCL in disinfection when this factor is considered.

Figure 19. Appearance of seed treated with 4702 mg/L chlorine at pH 10.3 for 60 min.

Section 15. Effect of pre-soaking seed in 0.1% Ag-surf surfactant on % infested seeds after chlorine treatment.

EXP. #	PRE SOAK TIME (HR)	Total Chlorine mg/L	HOCl mg/L	pH	Temp °C	% INFESTED SEED
33A NO AG-SURF	24	1408	18	9.46	20.0	1.5
33B 0.1% AG-SURF	25	1359	19	9.43	20.8	3.5
Seed lot T16/288, single seeds						

The low concentration of chlorine employed in this pair of treatments resulted in enough infested seeds to show that the surfactant did not enhance the ability of chlorine to disinfect seed when used with pre-soaked seed. There were more infested seeds present when the surfactant was used although due to the low levels of infestation, the significance of this is questionable. Since the previous treatments were completely effective without the use of surfactant, there is no reason to use a surfactant. Some chlorine treatments in the literature have recommended using a surfactant, but it is doubtful that there was any experimental evidence to justify their use. Surfactants may in fact interfere with the activity of chlorine.

Section 16. Replication of chlorine seed treatment conducted by Dhanvantari and Brown, 1993

This experiment repeated the seed treatment with chlorine reported in the above paper. This was a batch type treatment in which 100 grams of dry seed were treated at 25°C in 1 liter of solution containing 0.96% sodium hypochlorite for 60 minutes with constant stirring. The actual chlorine level was never measured or reported. The source of sodium hypochlorite was not specified but was probably household bleach containing approximately 5.25% sodium hypochlorite according to the label. Concentrations can vary considerably from the label due to the breakdown of the chemical, so the actual concentration of chlorine in the treatment solution was unknown.

A 0.96 % solution of NaOCl was prepared from household chlorine with a labeled concentration of 5.25 % by dilution to exactly 9640 mg/L of total chlorine using a chlorine meter, rather than by diluting by the labeled concentration. Chlorine concentration, pH, and temperature were recorded at the start of the treatment and at 5 and 10 minute intervals for 60 minutes.

EXP. #	TIME (MIN)	Total Chlorine mg/L	OCl^- mg/L	HOCl mg/L	pH	Temp °C	% INFESTED SEED	% GERM
28	0	9640	9592	48	9.88	25.5	100	
	5	4800	4784	16	10.07	27.6		
	10	3310	3262	48	9.41	28.7		
	20	1535	1461	74	8.88	29.0		
	30	1190	1062	128	8.5	28.6		
	40	860	708	152	8.25	27.9		
	50	748	555	193	8.04	27.1		
	60	701	481	220	7.92	26.3	1.5	100
Seed lot TSH16/288, dry, non-sorted								

Chlorine levels dropped to 4800 mg/L within 5 minutes of the initiation of the treatment. Organic matter (seed coat) rapidly reduced the chlorine level in solution resulting in less effective concentrations of chlorine. Due to the rapidly decreasing pH, the concentration of HOCl actually increased over the 60 minute treatment. However, this also resulted in a faster decrease in the concentration of OCl^- resulting in less oxidizing potential. The proportion of infested seed was reduced to 1.5 %. It is unknown how closely this treatment came to duplicating the original experiment in which chlorine levels could have been significantly lower. The problem with this method is that the level of chlorine was below the effective level of approximately 5000 mg/L for at least 50 minutes of the total 60 minute treatment. The level of hypochlorous acid increased to a fairly high level due to the drop in pH, but this was insufficient to totally disinfect the seed.

Hence, if there were pathogens on the seed, they were probably not eliminated. The seed was not pre-soaked in water and this would also have decreased the effectiveness of the treatment.

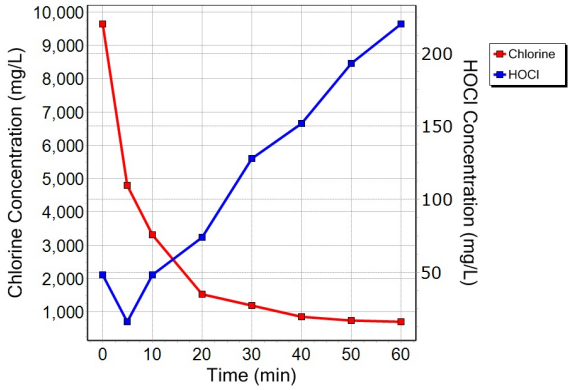

Figure 20. Changes in chlorine and HOCl concentrations over time during a batch treatment of 100 grams of seed in 1L of 0.96% NaOCl or 9640 mg/L chlorine (initially).

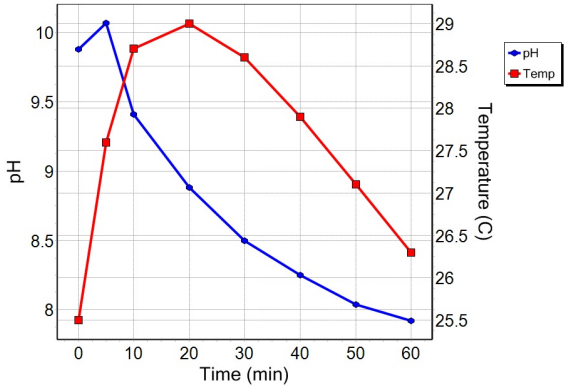

Figure 21. Changes in pH and temperature during a batch treatment of 100 g of seed in 1L of 0.96% NaOCl (initially).

When using a ratio of seed to solution as above, the increase in temperature due to the oxidation reaction of hypochlorite ion with organic matter is very noticeable. In this particular instance, most of this reaction was over within 20 minutes as evidenced by the cooling of the solution. The pH continued to decrease over the course of the treatment due to the accumulation of H^+ ions. This always occurs when treating seed with chlorine due to release of CO_2 into solution and must be countered with addition of NaOH to maintain a particular pH level. It is not an important factor if the amount of seed is small relative to the volume of solution.

Section 17. Replication of chlorine treatment by Fatmi, Schaad and Bolkan (1991)

This experiment repeated the seed treatment with chlorine reported in the above paper. This was a batch type treatment in which 100 grams of dry seed were soaked at room temperature in 600 ml of solution containing 0.5% sodium hypochlorite for 20 minutes. The chlorine concentration was not reported. The source of sodium hypochlorite was not specified but was probably household bleach containing approximately 5.25% sodium hypochlorite according to the label. As stated before, actual concentrations can vary considerably from the label due to the breakdown of the chemical, so the actual concentration of chlorine in the treatment solution was unknown. Sodium hypochlorite does not exist as a dry chemical and can only be obtained in solution, so that exact concentrations cannot be prepared as would normally be done in most chemical treatments.

A container of household bleach was procured and the above concentration of NaOCl was made up using the concentration stated on the label. This resulted in less than the 0.5% NaOCl stated in the original experiment; namely 0.4375% NaOCl due to the lower amount of NaOCl than stated on the container. Chlorine concentration, pH and temperature were recorded at the start of the treatment and at 5 and 10 minute intervals for 60 minutes.

EXP. #	TIME (MIN)	Total Chlorine mg/L	OCl^- mg/L	HOCl mg/L	pH	Temp °C	% GERM
26	0	4375	4326	49	9.53	19.8	
	5	998	996	2	10.38	21.6	
	10	499	498	1	10.20	22.1	
	20	281	278	3	9.51	22.1	
	30	280	269	11	8.98	22.0	
	40	291	256	35	8.44	22.1	
	50	275	223	52	8.21	22.0	
	60	246	186	60	8.07	22.2	98.5
Seed lot T16/288, dry, non-sorted							

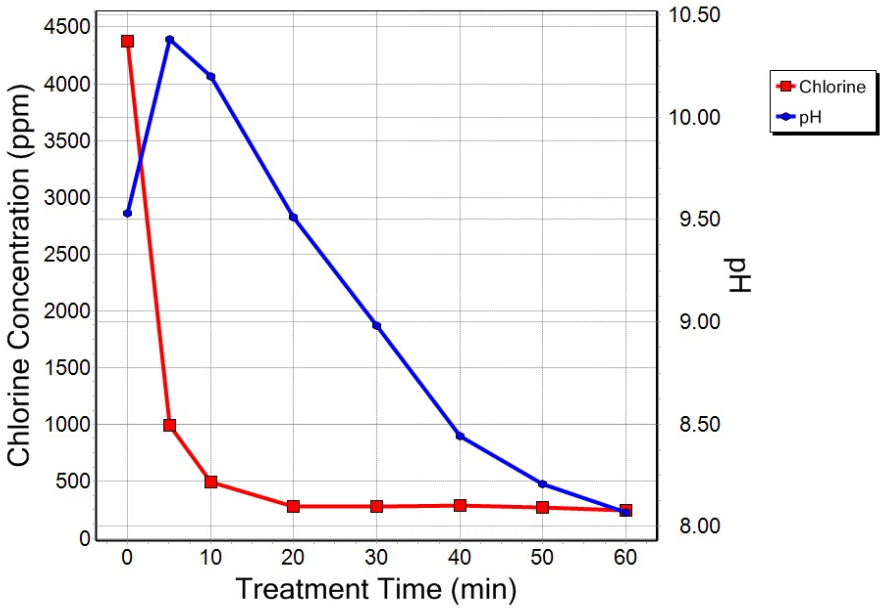

Figure 22. Changes in pH and chlorine concentration during treatment of 100 grams of seed in 600 ml of chlorine solution with an initial concentration of 4375 mg/L.

The chlorine concentration decreased rapidly due to the high amount of seed relative to solution and the oxidation reaction was essentially over after 20 minutes, the actual duration of the treatment by Fatmi, et al. Although Fatmi only soaked the seed in the solution, in the duplicated experiment shown here, the seed slurry was stirred with a magnetic stirrer, but it required a powerful stirrer to keep the very thick slurry moving. Disinfection of the seed at the end of the 60 minute treatment was not determined, but in the original experiment the seed had a very high population of bacteria. This is not surprising because chlorine concentration dropped to an ineffective level (based on the previous experiments) within 5 minutes of the initiation of treatment.

Section 18. Effect of increasing chlorine concentrations on seed disinfection and germination in batch treatments.

Exp #	Chlorine concentration mg/L		pH		Temperature		grams of Cl used per gram of seed	% Germ.	% Infest -ed Seed
	Initial	Final	Initial	Final	Initial	Final			
39A	4990	363	11.37	9.09	22.7	23.4	0.046	99.5	14.5
39B	6420	452	11.76	8.59	23.3	23.8	0.060	99.0	5.5
37	8040	527	12.14	8.83	21.0	24.7	0.075	99.0	0.0
28*	9640	701	9.88	7.92	25.5	26.3	N.A.	100.0	1.5
36A	10450	733	12.22	8.66	20.9	25.7	0.097	98.5	1.0
36B	11385	837	12.51	8.19	20.7	25.6	0.105	99.5	0.0
36C	12420	989	12.59	8.14	19.9	25.9	0.114	99.0	0.5
34	13300	730	12.60	7.98	20.8	27.5	0.126	100.0	0.0
35A	15780	1226	12.55	8.36	21.1	23.8	0.146	99.5	NA
35B	16665	1742	12.66	8.18	21.6	26.4	0.149	99.5	NA
35C	19180	2310	12.75	8.05	21.6	27.4	0.168	95.0	NA
35D	22360	3310	12.84	8.18	21.6	27.7	0.190	88.0	NA
35E	23859	4330	12.88	8.30	21.6	28.8	0.196	69.0	NA
Seed lot TSH16/288, 6% clumped seed, pre-soaked in water for 24 hours, 10 grams of seed per 100 ml of solution. Seed was treated for 60 minutes.									

*28= data from Section 12 for comparison only.

Experiment 35A had a chlorine concentration of 10,450 mg/L which was similar to the level of 9640 in experiment 28 as reported in Section 12. Some infested seeds came through the treatment in both cases. The initial level of 13,300 mg/L using 10 grams of seed per 100 ml of chlorine solution resulted in no infested seed, and germination was still excellent. Although data on disinfection was not collected on treatments using more than this, use of up to 16,665 mg/L chlorine still resulted in excellent germination but germination was detrimentally affected above this level.

Section 19. Effect of extended treatment times on disinfection of seed treated with chlorine at various concentrations using the batch method.

Exp #	Time min.	Chlorine concentration mg/L		pH		Temperature		grams of Cl used per gram of seed	% Germ.	% Infested Seed
		Initial	Final	Initial	Final	Initial	Final			
39A	60	4990	363	11.37	9.09	22.7	23.4	0.046	99.5	14.5
40A	90	4725	322	11.11	8.39	22.8	23.0	0.044	99.0	16.5
40B	120	4725	285	11.11	8.33	22.8	23.6	0.044	96.5	18.5
36B	60	11385	837	12.51	8.19	20.7	25.6	0.105	99.5	0.0
41A	60	11526	832	12.34	8.71	20.9	24.2	0.107	100.0	0.0
38	90	11000	600	12.46	8.24	20.4	24.9	0.104	99.5	0.0
41B	90	11526	676	12.34	8.22	20.9	25.2	0.108	100.0	0.0
41C	120	11526	590	12.34	8.35	20.9	24.5	0.109	100.0	0.5
Seed lot TSH16/288, 6% clumped seed, pre-soaked in water for 24 hours, 10 grams of seed per 100 ml of solution.										

In these batch treatments, initial chlorine levels of 4700 - 5000 mg/L were ineffective in disinfecting seed, but increasing the level to over 11,000 mg/L resulted in virtually no infested seeds. Extending the treatment time did not seem to have any effect, probably because most of the oxidation reaction and treatment would have been complete in about 20 minutes.

Section 20. Effect of hydrochloric acid on disinfection of tomato seed for 30 and 60 minute treatments

EXP. #	SEED LOT	MURIATIC ACID CONC. %	HCl CONC. MOLAR	TREAT-MENT TIME (MIN)	TEMP °C	% INFESTED SEED	% GERM
1	T4/427	3.33	0.333	30	20.0	100	84
4	T4/427	6.66	0.666	30	22.8	100	95.0
7	T4/427	6.66	0.666	60	20.0	100	74
Seed was not sorted or pre-soaked.							

Concentrations of HCl used in this series of treatments were in the range used in previous experiments by Dhanvantari (1989), Dhanvantari and Brown (1993) and Fatmi, Schaad and Bolkan (1991). In all cases, 100% of the seeds remained infested after treatment. While the 30 minute treatment with 0.666 M HCl resulted in good germination, doubling the treatment time to 60 minutes decreased germination to 74%. In the previous chlorine treatments with the same seed lot, germination was 100% in several of the treatments using medium and high levels of chlorine.

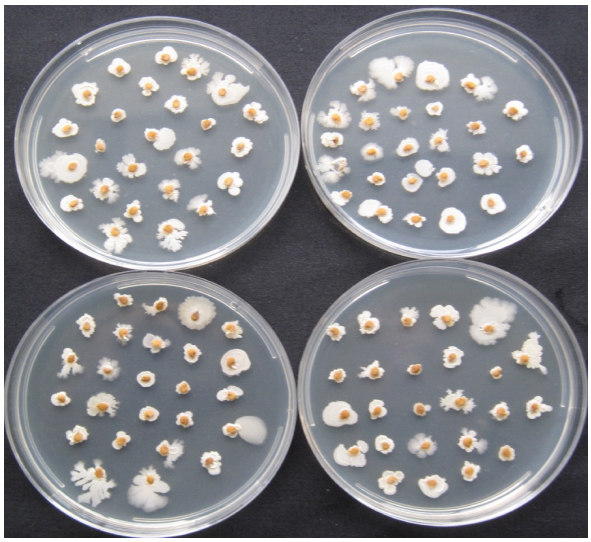

Figure 23. 100% infested seed after treatment with 0.666 M HCl for 60 minutes (Exp 7).

Section 21. Effect of 6.66 % muriatic acid (0.666 M HCl) on germination for various treatment times.

EXP. #	SEED LOT	MURIATIC ACID CONC. %	HCl CONC. MOLAR	TREAT-MENT TIME (MIN)	TEMP °C	% INFESTED SEED	% GERM
18A	T16/288	0	0	60	20	100	99.0
18B	T16/288	6.66	0.666	30	20	NA	98.0
18C	T16/288	6.66	0.666	60	20	87.5	99.0
18D	T16/288	6.66	0.666	90	20	NA	99.0
18E	T16/288	6.66	0.666	120	20	NA	98.5
18F	T16/288	6.66	0.666	180	20	NA	98.5
18G	T16/288	6.66	0.666	240	20	NA	97.0
18H	T16/288	6.66	0.666	300	20	NA	99.0

With this seed lot, no detrimental effects on germination were found with treatment times up to 5 hours using 0.666 M HCl. A few disinfected seeds were found in the 60 minute treatment.

Section 22. Effect of 0.666 M HCl on disinfection of double seed treated for 2 - 5 hours.

EXP. #	SEED LOT	MURIATIC ACID CONC. %	HCl CONC. MOLAR	TREAT-MENT TIME (MIN)	TEMP °C	% INFESTED SEED	% GERM
18C*	T16/288	6.66	0.666	60	20	87.5	99.0
31A	T16/288	6.66	0.666	120	20	32	NA
31B	T16/288	6.66	0.666	180	20	7	NA
31C	T16/288	6.66	0.666	240	20	1	NA
32	T16/288	6.66	0.666	300	20	2	98.0
Seed was all doubles or clumps and was not pre-soaked. Data not presented here indicated that pre-soaking seed followed by HCl treatment was very detrimental to germination.							

* also reported in Section 19

The percentage of infested seeds was reduced to 1-2% when the treatment with 0.666 M HCl was extended to 4-5 hours, with no detrimental effect on germination with this seed lot. This was still less effective than chlorine treatments at 5000 mg/L, pH 9.5, for 60 minutes. With seed lot T4/427 germination was detrimentally affected by HCl applied for only 60 minutes at 0.666 M.

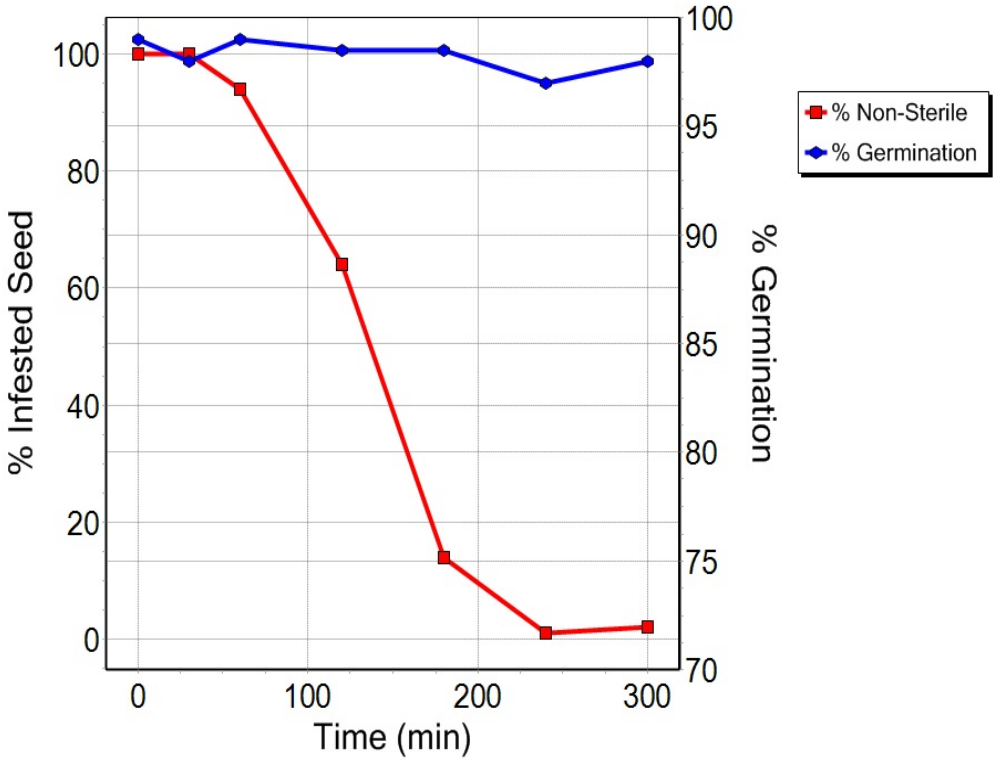

Figure 24. Effect of 0.666 M HCl on % infested seed and germination for the seed lot T16/288.

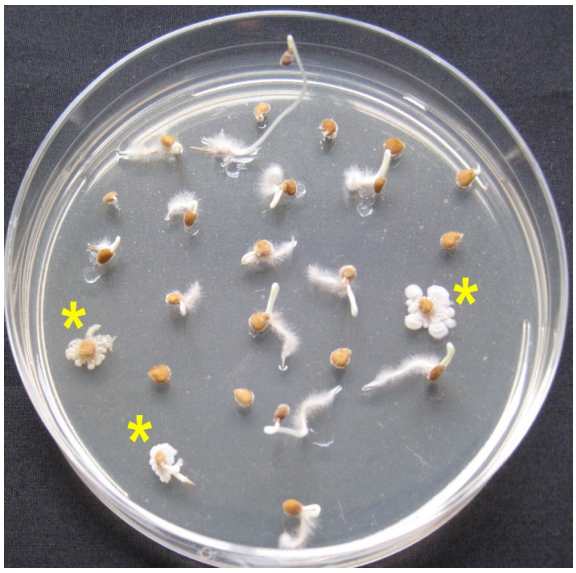

Figure 25. Exp 31B showing 3 infested seeds out of 25 after HCl treatment for 180 minutes.

21.) Effect of trisodium phosphate (TSP) Na_3PO_4 on disinfection and germination

EXP. #	SEED LOT	TSP CONC. G/L	pH	TREAT-MENT TIME (MIN)	TEMP °C	% INFESTED SEED	% GERM
9	T4/427	120	12.73	60	19.2	100	39.5

TSP did not disinfect the seed and was very detrimental to germination at this concentration and treatment time.

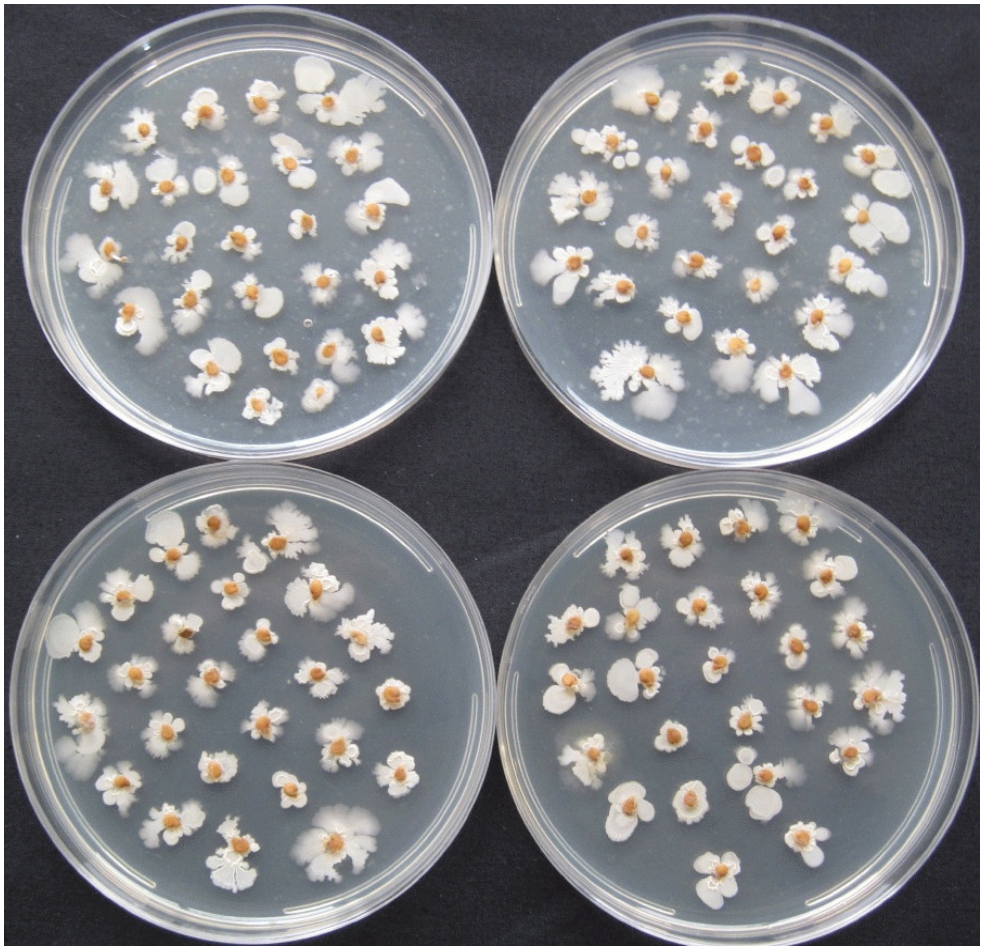

Figure 26. Infested seed after treatment in 12% trisodium phosphate solution for 60 minutes.

CONCLUSIONS

1.) Tomato seed pre-soaked in cold water for 18-24 hours at a temperature to inhibit germination was optimally disinfected using 5000 mg/L of chlorine for 60 minutes at 20°C at pH 9.5. The treatment solution must be vigorously agitated or stirred to ensure that all seed surfaces are equally exposed to the treatment solution.

2.) Tomato seed disinfection is more effective if seed is soaked for 24 hours prior to treatment at a temperature that will inhibit germination. Soaking allows seed that is stuck together to come apart so that all the surfaces of the seed will be treated equally well. It may also allow the chlorine solution to penetrate the many crevices on the seed surface more effectively. The elimination of bubbles in the seed hairs of the seed coat is also important. Freshly extracted seed can be treated with chlorine immediately without drying and re-soaking.

3.) To be effective, chlorine must be maintained at 5000 mg/L during the 60 minute treatment by continuously adding chlorine as required based on monitoring the concentration with a chlorine meter. The maximum ratio of seed to treatment solution should not exceed 20 kg of dry seed per 200 L of solution. If treating freshly extracted seed, the ratio would be 40 kg of seed per 200 L of solution. As the ratio of seed to volume of solution increases, temperature will rise proportionately resulting in a greater oxidation reaction. As long as the average chlorine concentration is 5000 mg/L or more and the average temperature is 20°C at a pH of 9.5, optimum disinfection will be achieved. Higher temperatures and higher chlorine levels will result in even more oxidation and disinfection effect. Since effective disinfection is achieved at lower chlorine levels than those required to detrimentally affect germination, there is considerable latitude allowed for higher chlorine and temperature levels.

4.) At the concentration of chlorine required for effective disinfection (5000 mg/L), oxidation of the seed coat surface by the hypochlorite ion appears to be the main factor resulting in disinfection. Hypochlorous acid which is present in significant amounts at a pH of 9.5 or less enhances disinfection. Depending on the ratio of seed to treatment solution, pH will decline during the course of treatment but can be kept at pH 9.5 by the addition of HCl or sodium hydroxide as required. If pH is reduced much below 9.5, the amount of hypochlorite ion will be proportionately reduced, and the amount of oxidation could be reduced resulting in less effective disinfection. The amount of hypochlorous acid available at pH 9.5 (approximately 60 mg/L) is sufficient to eliminate any bacteria that escape oxidation due to the hypochlorite ion.

5.) Maintenance of chlorine at 5000 mg/L for 60 minutes ensures that the seed coat is oxidized deep enough to eliminate any bacteria buried in the seed hairs. A reduction in time would result in less oxidation of the seed coat and increase the chance of bacteria

deep within the exterior of the seed coat surviving. Conversely, excessive oxidation by increasing exposure time could penetrate through the seed coat to the embryo and injure it. The threshold level of chlorine for almost complete disinfection was 2500 mg/L, so using twice this level at 5000 mg/L was very effective. Also, concentrations of chlorine up to 7500 mg/L or more were not harmful to germination, so there is a good safety factor when treating seed. It is likely that there is a time*concentration variable that could be considered when treating seed. Hence, it is suggested that 60 minutes * 5000 ug/L = 300,000 minute-mg/L would give the same amount of disinfection when variable concentrations of chlorine and time are used. For instance, if the chlorine concentration happened to be averaging 6000 mg/L, the time exposure could be reduced to 50 minutes without any decrease in the effectiveness of disinfection.

6.) Temperature of the treatment solution is important since chemical reactions proceed at a rate that is dependent on temperature. The reaction rate doubles for every 10°C rise in temperature. Most of these experiments were conducted at a standard temperature of 20°C. If treatments are conducted at a lower temperature, the amount of oxidation will be reduced and the effectiveness of disinfection will be lessened. Conversely, higher temperatures will increase oxidation of the seed coat and could be detrimental to germination if temperatures are too high. In most cases starting the treatment process at 20°C is satisfactory providing the ratio of seed to treatment solution is 10 kg/200 L or less. Temperature will rise slightly during the course of treatment by 2-3 degrees but this is still safe. Even with the maximum amount of 20 kg/200 L, the temperature will only rise to about 26°C which is acceptable. However in the latter case it is safer to start the treatment at about 17°C so that the final temperature is not too high.

7.) In all of these experiments the treatment solution was stirred vigorously so that all of the seed surfaces would be continuously exposed to chlorine. This is very important since if seed settles to the bottom of the treatment container, oxidation of the seed coat will reduce chlorine levels rapidly in the mass of seed and ineffective disinfection will result.

8.) Batch treatments using a specific ratio of seed to treatment solution may be effective in disinfecting tomato seed, but further research would be necessary before recommending this approach for large quantities of seed. For small quantities of seed, if the volume of treatment solution is high relative to the amount of seed, then the concentration of chlorine will not be reduced very much by the organic matter of the seed coat. For instance, about 200 grams of tomato seed can be safely treated in 60 liters of 6000 mg/L chlorine solution and the final chlorine level will still be above 5000 mg/L. This method is useful for treating small batches of research seed or selections in a breeding program. 100-200 selections with 1 gram of seed of each selection can be treated in small nylon bags in a washing machine using this method. In related work to control bacterial canker in a breeding program several years ago, elimination of bacterial canker from research plots was achieved using this method. This method can also be used to treat test samples of commercial seed lots to check germination prior to treating the entire seed lot.

9.) In these experiments, the chlorine residue was removed from treated seed so that the true amount of disinfection could be determined. It is not necessary to remove chlorine residue when treating seed for practical purposes. The chlorine residue on the seed is persistent and not detrimental to germination. It cannot be removed by rinsing with water. The significance of the residue in additionally protecting the seed from any surviving pathogens on the seed is unknown. Since the seed is essentially disinfected anyway, any further action by the residue would not seem to be necessary.

10.) Chlorine residues on seed may consist of chloramine which has good disinfectant properties. The effect of chlorine residue on bacterial growth was shown in experiments in which treated seed was plated on PDA plates seeded with a fast growing bacterium. Normally, chlorine residue is not removed and would be concentrated in the seed coat as seed is being dried. During drying which takes about 4-5 hours at temperatures ranging from 25-35°C the moisture content is reduced from 50% to 5% concentrating the chlorine residue in the seed. This drying step constitutes an additional treatment step which would be detrimental to any bacteria remaining on or in the seed coat. During germination, seed imbibes water over a 24 hour period and this step could also expose any pathogenic bacteria to another round of exposure to chlorine residues. Because these additional steps were not done in the experiments reported on here, the efficacy of chlorine to disinfect tomato seed and eliminate pathogens may have been underestimated. If some of the work were to be repeated, seed should be dried after chlorine treatment, soaked in sterile water for 24 hours to simulate the initial steps in germination and then soaked in sodium thiosulphate solution to remove chlorine residue prior to plating on PDA.

11.) Chlorine treated seed should be rinsed thoroughly with water before drying to allow the seed to be dried without sticking together. The seed coat of chlorine treated seed is soft and sticky due to chemical degradation of organic matter, and rinsing alleviates the stickiness. If seed is not dried using specific procedures, some seeds will be glued together, and in extreme cases with larger seed batches, large seed balls can be formed during drying. If using a small amount of seed in a porous nylon bag, good seed singulation will result with tumble drying in a clothes dryer modified to dry at a low temperature. The methods used for commercial seed treatment are shown in a video available on the website of Tomato Solutions - www.tomatosolutions.ca .

12.) An important additional benefit of chlorine seed treatment is enhanced germination. Because much of the seed coat is oxidized, any germination inhibitors such as abscisic acid are eliminated and the seed coat is made more permeable to water intake. Chlorine treated seed germinates faster and more uniformly than untreated seed. This is an important factor in establishing uniform stands of seedlings in plug trays for commercial tomato production.

13.) Chlorine treated seed can be stored for long periods of time provided the seed is kept cool and dry. Temperatures in the 6-7°C range are adequate, and if the seed is kept in sealed

plastic containers, humidity is not an issue. If seed is kept in non-sealed containers, humidity should be controlled so that the sum of the temperature in degrees Fahrenheit and the % relative humidity is 100 or less. Chlorine treated seed that has been pelletized and kept in sealed plastic pails at 6-7°C has been stored for 20 years with germination maintained at 99%. Chlorine treated seed that is kept at higher temperatures and in high humidity will decrease in germination faster than raw untreated seed lots because the seed coat is more permeable.

14.) Bacterial colonies found on tomato seed treated with lower levels of chlorine were uniform and similar in appearance. In other seed treatment work (Dick, 1981), both yeasts and bacteria were found on the seed, and the presence of spore forming bacteria such as Bacillus spp. was confirmed. In the present study, the identification of bacteria found on the seed was not attempted but they may have been spore forming bacteria with significant resistance to chlorine disinfection. Non-spore forming pathogens would be eliminated prior to the destruction of spore forming bacteria. The percentage of infested seeds is an indication of the relative effectiveness of various disinfection procedures. It should also be noted that "infested seeds" as determined by the growth of bacteria around the seed on a PDA plate could result from just one surviving saprophytic bacterium, possibly a spore former, on the seed. Hence, even when infested seeds are found, the actual number of surviving saprophytic or pathogenic bacteria in a given quantity of seed could be extremely low.

15.) Pre-soaking seed in cold water for 18-24 hours to separate seeds was shown to be an effective initial step in seed disinfection with chlorine. It is possible that some seeds may not separate in time to be effectively treated, and hence 100% disinfection may never be achievable. Pathogens will be 100% eliminated if exposed for sufficient time to chlorine, but if the seed separates just a few minutes prior to the end of the seed treatment, pathogenic bacteria may not be eliminated. The greatest risk would occur if a few seeds did not separate completely at all. With bacterial diseases, a minimum number of bacteria are required to initiate disease, and the objective is to reduce seed contamination below this number.

16.) Although hydrochloric acid was shown to be a poor disinfectant, it may still be an effective seed treatment chemical. Bacterial pathogens are susceptible to very low pH levels and can be eliminated from seed while allowing saprophytic bacteria to survive, especially if they happen to be spore formers.

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