

## Seed Sanitation: Practical Application at Eatmore Sprouts & Greens Ltd.

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- By Carmen Wakeling: Co-Owner and CEO



## Research Paper

## Disinfection of Alfalfa and Radish Sprouting Seed Using Oxidizing Agents and Treatments Compliant with Organic Food Production Principles

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## ABSTRACT

Antimicrobial seed treatments recommended by Canadian guidance for sprouted vegetable production (2,000 ppm of hypochlorite for 15 to 20 min or 6 to 10% hydrogen peroxide for 10 min at room temperature) are not fully compliant with organic production principles. We investigated the effect of a sequential treatment consisting of a 10-min soak at 50°C in water followed by exposure to a 2.0% H<sub>2</sub>O<sub>2</sub> plus 0.1% AcOH sanitizing solution against *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella enterica* inoculated onto alfalfa and radish seed. The sequential treatment was as effective as the recommended treatments and could reduce populations of all three species by a minimum of 3 log CFU/g using a reduced (1:2) ratio of seed to sanitizing solution and low concentrations of sanitizers approved for use in organic food production. However, the efficacy of all the treatments examined in this work was considerably reduced by storage of the seed for 4 weeks at either 11 or 75% relative humidity prior to treatment and assessment. None of the treatments could eradicate the target pathogens from seed, irrespective of time elapsed since inoculation. The results of this work suggest that the effect of storage should be considered in the assessment of antimicrobial treatments for sprouting vegetable seed.

## HIGHLIGHTS

- Recommended antimicrobial treatments for sprouting seed are not organic compliant.
- Alternatives based on mild heat and low chemical concentrations were investigated.
- Treatments were effective against three human pathogens on alfalfa and radish seed.

Key words: Alfalfa; Organic; Radish; Seed disinfection; Sprouts

Seeds from several plant species are used to produce sprouted vegetables, referred to herein as “sprouts,” which are defined as “the product obtained from the germination of seeds and their development in water or another medium, harvested before the development of true leaves and which is intended to be eaten whole, including the seed” (1). Sprouts can be produced on a small scale in the home or in commercial operations that provide consumers with a range of products year-round. Several of the more popular varieties, such as alfalfa sprouts, are good sources of nutrients, including vitamins, minerals, and phytochemicals with beneficial effects on human health (1, 2). Unfortunately, many outbreaks of foodborne illness associated with the consumption of sprouts have been reported worldwide, the majority of which were caused by enteric bacterial pathogens, including *Salmonella enterica*, *Shiga toxinogenic*

*Escherichia coli*, and, to a lesser extent, *Listeria monocytogenes* (5, 6, 20). Although the frequency and level of contamination with enteric bacterial pathogens in commercial sprouting seeds are typically very low, research has shown that temperatures maintained during germination (21 to 25°C) are conducive to rapid growth (15, 19). For example, populations of *S. enterica* and *E. coli* O157:H7 inoculated onto alfalfa seeds can increase by several logarithms after 1 to 2 days of germination at 25°C (17, 18). Accordingly, public health agencies in many jurisdictions now recommend the application of antimicrobial treatments immediately before sprouting to reduce the risks implied by latent seed contamination with enteric bacterial pathogens that could proliferate during germination. In the United States, the Food and Drug Administration (FDA) advises treatment with a 20,000-ppm calcium hypochlorite (Ca(OCl)<sub>2</sub>) solution prior to germination (21). Although antimicrobial treatments based on chemical sanitizers are a critical step in overall intervention strategies aimed at

This treatment is based on the link below. Eatmore Sprouts & Greens was the industry lead on this research and as trials were underway, we were doing real time trials at our facility.

We initiated this work as it was clear to us that validation was needed as regulation changes were imminent and we wanted to have alternative treatment for organic producers.

This has become our seed treatment and we want to continue to evolve the systems and processes.

Current methods will be discussed today.

We hope that others are interested in considering this and working to improve application and efficiency.

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- <https://www.sciencedirect.com/science/article/pii/S0362028X22102917>

A woman wearing a blue lab coat, a grey hairnet, and blue gloves is pouring a large amount of brown seed from a white bucket into a large black tub. The white bucket is sitting on a scale. The background shows a laboratory setting with various equipment and storage containers.

We start by weighing seed into bags.

# Treatment capture sheet

Date :			FM: 9.2.006 Daily Seed Start Record 2023						Your Name:				
MORNING TITRATION													
Date	Time	Initials	Type (Titration or Strip)			Reading			Satisfactory Range?			Comments (i.e. if reported)	
Seed Type Used	Amount to Start today : Qty (1,2,3,4) x Unit of Measure (cup, 15#, etc)		CCP - 1 Hot Water Start temp°C >=50°C	CCP - 1 Hot Water finish temp°C >=50°C	CCP - 1 Hot Seed Rinse start	CCP - 1 Hot Seed soak finish time (= 10mins)	CCP-2 Seed Sanitation Start Time(s)	CCP-2 Record Sanitation Finish time (= 10mins)	Volume used (H2O2) in Litres	Volume Used (Acetic Acid) in ml	Sanitation Initials	Soak Time	Transfer Time / Drum or Cooler / Initials
Alfalfa	x	#'s	°C	°C	:	:	:	:					No. Buckets / Transfer Time / Drum or Cooler / Initials
													To Drum: _____/_____/_____/_____
													To Drum: _____/_____/_____/_____
													To Drum: _____/_____/_____/_____
Clover	x	#'s											To Drum: _____/_____/_____/_____
Broccoli	x	#'s											To Drum: _____/_____/_____/_____
Deli Blend	x	#'s	°C	°C	:	:	:	:					
Garlic	x	#'s	°C	°C	:	:	:	:					
Mixed Bean												BIN #	Combined, Drained, and Transferred
Stainless 1	x Buckets		°C	°C	:	:	:	:					
Stainless 2	x Buckets		°C	°C	:	:	:	:					
Sunflower	x		°C	°C	:	:	:	:					
Pea Shoots (Sneekled			°C	°C	:	:	:	:					

**PREPARE FOR TOMORROW:**

Alfalfa	No. of Buckets	_____ X _____
Deli Blend	No. of Buckets	_____ X _____
Garlic	No. of Buckets	_____ X _____
Mixed Bean	Total No. of Large Buckets (20 gal. size): _____	_____ X _____    _____ X _____    _____ X _____

AFTERNOON TITRATION						
Date	Time	Initials	Type (Titration or Strip)	Reading	Satisfactory Range?	Comments (i.e. if reported)
Required concentration for our use is a 2% solution. ppm = 20,000						
Manual dilution rate is 600 ml of 35% Hydrogen Peroxide to 10L of water.						
Strip: 1 minute 40 seconds with blue around the edges (2 min 40 secs)						

TRIAL SEED STARTS		
Product	Lot Code	Comments

Manager's Signature:	Date:	Data Entry Signature:	Date :
HACCP Coordinator/QA (or Designate) Signature:			Date :

Comments:

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## SOP: Alfalfa, Broccoli and Clover Seed Starting Procedures

Form #:	1.8.005
PCP:	1. Control of Operation
Revision #:	2.7
Revision Date:	28-Dec-2022
Revised By:	V. Garcia
Supersedes:	18-Jan-2022

### **THIS PROCESS CONTAINS CRITICAL CONTROL POINTS**

**IMPORTANT NOTE:** Remember that seed starting procedures might change depending on variables like seed lots or yields. Check Seed Start whiteboard for unusual situations. Management will inform you at the start of any changes. Please feel free to ask questions.

**Time Required:** 10-20 Minutes, started when you know that the equipment will be ready, but needs to be completed by 1pm.

**Frequency:** Daily as required by Production Plan

**Person assigned:** Individual's trained to do the task (see Training Manual for confirmation)

**Rationale:** Seed starting is a very important part of sprouting. The inspection of dry seed is the main way to reduce Physical Contamination and the Seed Sanitation is the one of the main tools to reduce Biological contamination in our facility. Good hygienic practices throughout the procedure reduce the potential for an individual to introduce Biological hazards during the process.

**Dilution rates for chemicals:** Refer to [1.8.023 SOP Seed Start Chemical Table](#) and [SOP 4.1.1.002 Chemical Usage Chart](#).

**Equipment needed: both to do the job and for safety:** Gloves, Blue Apron, Boots, Protective Eyewear, respirator, large blue, white bin, paddle.

**WARNING:** Handle Hydrogen Peroxide with appropriate caution.

#### **Step by step process:**

- Before beginning confirm that the information on the Seed Starts & Harvests Sheet and the FM 9.2.006 Daily Seed Start Record are the same, and that the Daily Seed Start Record has been signed by a Manager.
  - All seed starts will be recorded on FM 9.2.006 Daily Seed Start Record.
1. Weigh seed into each mesh bag (can be prepared night before). Bags with Clover or Broccoli are to be marked with ribbon tags accordingly.
  2. While pouring seed look for physical contamination. Refer to [SOP: 1.8.611 Product Inspections and Discards](#). Report any unusual findings immediately.
  3. Transfer starting bags to seed start area. Add **hot** water to large bin (122°F/50°C target temp). Do not let the temperature go over 54°C or fall below 50°C. – **CCP 1**. Remember max 14 bags in the bin at a time.
  4. Place mesh bags into the large blue bin with hot water. Record start temperature and time. – **CCP 1**.
  5. Agitate seeds by rocking the blue bin.
  6. Let seed bags sit in the hot water for 10 minutes. Monitor the temperature during the 10 minute period. Do not let the temperature drop below 50°C. If temperature starts to drop, bring it up by adding hot water as needed. After 10 minutes, record finish time and temperature. – **CCP 1**



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7. While the seed is soaking, put on goggles and a respirator and fill the white bin with H<sub>2</sub>O<sub>2</sub> and vinegar. (Refer to [1.8.023 Seed Start Chemical Table](#)).
8. Once the required time of 10 minutes for the hot water soak has passed, open the cap on the bin with the hot water soak and let the bin and the seeds drain.
9. Once the bin and the seed bags have drained, transfer the seed bags directly to the bin containing H<sub>2</sub>O<sub>2</sub>.
10. Check the start time and record it into the log. – **CCP 2** Remember max 14 bags in the bin at a time.
11. **Record** the volume of H<sub>2</sub>O<sub>2</sub> used. – **CCP 2**.
12. **Record** the volume of vinegar used. – **CCP 2**.
13. Agitate the seeds or use a paddle to ensure that they are completely submerged.
14. **Using** paddle, continually ensure all bags are submerged.
15. **Let** sit for 10 minutes. **Record** finish time. – **CCP 2**
16. Open valve on bin and let drain. Make sure to block exit of bin so no seed bags slip through.
17. **Rinse** bags thoroughly with **WARM** water.
18. Inspect drum(s) that the seed will be loaded into. Confirm visually that the drum(s) and the drum room are clean. If the drum(s) or the room does not appear clean, do not load the drum(s). Communicate this with the Indoor Growing Team and management.
19. Designate a person to take the white mesh bags, in the big blue bin, to the drum rooms. They will load the seed directly into a sanitized drum immediately

OR

If there is any reason why the seeds cannot be transferred into the drums, they MUST be watered every half hour until they are loaded. If over 1 hour, report to management.

### Documentation Requirements:

Record all information on FM [9.2.006 Daily Seed Start Record](#).

### Corrective Actions:

1. If it is observed that the sanitizer is not working then seed needs to be treated as un-sanitized and steps are to be followed to complete the sanitation of the seed. Report to a manager immediately.
2. If contamination occurs at any time between sanitizing and loading, seed needs to be re-sanitized or discarded. Notify a manager immediately and hold off on taking any more steps.
3. Report and broken or damaged equipment to management.
4. Any Incident involving CCPs will be documented following [SOP 1.10.001 Incident & Corrective Action Handling](#) and recorded on [FM 7.2.5.002 CCP Incident Report Log](#).



### Hot Water Provision:

- We use a Navian Boiler to heat the water for the 50 C Hot water needed. We set it at 54 C or 145 F because it must travel from the boiler room to the seed start room before use.
- Boiler maintenance is essential
- Any hot water source will work



# We pre-fill insulated totes with hot water

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- We monitor with a good old-fashioned thermometer.
- We take temperature before we begin filling
- We take temperature of bin before we begin transfer of seed into it
- The starting temperature is taken at the time that all the seed bags have been transferred into bins and the seed bags have been stirred.
- We use good old-fashioned kitchen timers to record times.
- Temperatures are taken at the beginning, mid-way and end of process.
- We stir on a regular basis during the process
- Beginning and end temperatures are recorded





# Preparation and monitoring of the Hydrogen Peroxide

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- We use 35% Food Grade Hydrogen Peroxide for this portion of the process.
- We pre-mix it using a Super Dos mixing pump
- This is pumped through chemical resistant hoses to a second bin (we use insulated, but you don't have to)
- For large batches of seed we begin pre-filling bin right after hot water start.
- This solution is stable until the vinegar is added so we can have it staged to work time wise.




## Heating and cooling at initial CCP

- Seed is soaked in Hot water for 10 minutes at 50-53 C
- Then it is cooled or transferred to Hydrogen Peroxide quickly.
- Important note: Do not let seed go over time and cool quickly or germination will be impacted





This is the barrel of H2O2 prior to dilution. HANDLE WITH CARE AND USE PPE. We dilute to 2%

	SOP: Hydrogen Peroxide Barrel Change Procedure	Form #:	1.8.031
		PCP:	1. Control of Operation
		Revision #:	1.7
		Review Date:	28-Dec-2022
		Reviewed By:	V. Garcia
		Supersedes:	18-Jan-2022

**Time Required:** Approximately 20-30 minutes.

**Frequency:** Dependent on usage, but every 12-16 days (approximately).

**Person assigned:** Individual's trained to do the task (see Training Manual for confirmation)

**Rationale:** Hydrogen Peroxide is used as a Seed sanitising agent and purchased at 35% Food Grade Concentration. This is diluted onsite to 2% Concentration and as such extreme care must be exercised when changing a Barrel.

**Dilution rates for chemicals:** Hydrogen Peroxide in Barrel is at 35% Concentration and should be treated with extreme care.

**Equipment needed for the job and safety:** Gloves, Apron, Boots, Smock, Long length production gloves, Elbow length heavy Gloves, Protective Eyewear, Chemical Respirator, Full Face mask, Yellow carrying trolley, Barrel Cap Remover.

**WARNING:** Handle Hydrogen Peroxide with appropriate caution.

#### Step by step process for Changing Barrel:

1. There should be a full barrel of Hydrogen Peroxide (H2O2) waiting, pre-loaded, onto the yellow carrying Trolley. If this is not the case, see a [Manager](#) for assistance.
2. Get Barrel Cap remover from outside the Seed Bay Entrance.
3. Equip with appropriate PPE for the task. Use standard work gloves, Apron, Boots, Smock, (as per usual seed start processes), then ensure that chemical respirator, protective eyewear, face mask, and elbow length gloves on top of regular gloves, are being worn before proceeding.
4. Take the full barrel of H2O2 using the Trolley and park this close to the Drum that is to be changed.
5. Hook up the cold water and hose Nozzle to the hose by the Barrel.
6. Ensure H2O2 valve is in the 'Off' position.
7. Undo 'in-pipe' to old Barrel.
8. Take out the 'in-pipe' to the H2O2. **CAUTION – This step needs to be performed very carefully as there is the possibility to damage the ~~Drum~~ equipment by mishandling or Splashing and Spilling H2O2.**
9. CAREFULLY remove pipe from Barrel and rest inside sanitized sink. Ensure this is stable and will not flex out of Sink.
10. Remove old Barrel from area by sliding across to the side.
11. Place white cap on old barrel.
12. Hose down and scrub the area where the Barrel was resting to ensure there is no residual marking.
13. Remove seals from New Barrel (Using Cap remove, straight end) exposing screw tops (one tight thread <white>, one loose thread <Yellow>).
14. Hose down top of new Barrel to remove any contamination. You can encourage this water to drain around the side of the Barrel as there are drainage gaps.
15. Take some blue towel and dry off the top of the barrel to avoid unintentionally diluting the hydrogen





## Important! Testing and monitoring tools

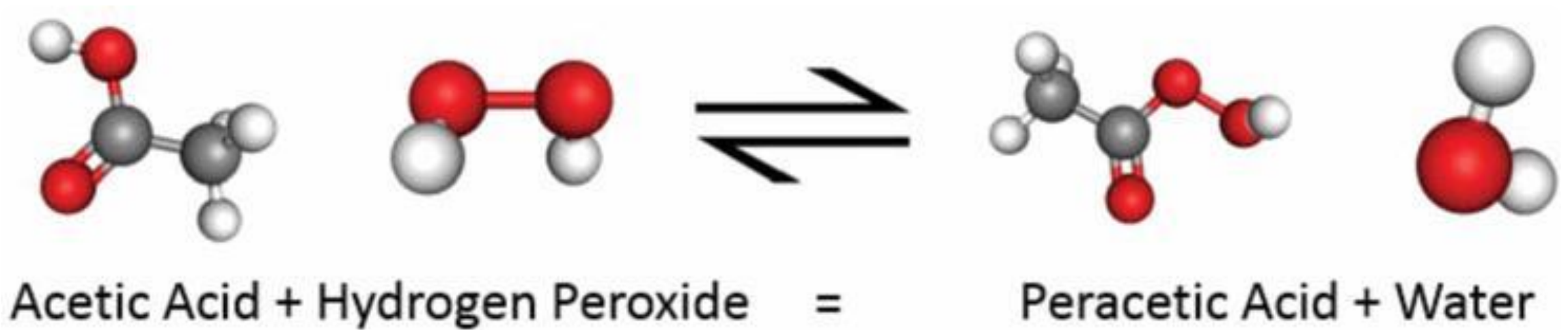
- We use test strips and titration to test concentration
- Generally, test strips are used morning and night
- Titration is used when barrel is changed or if we have concerns about strips



We use  
White  
Vinegar in  
addition to  
the H2O2

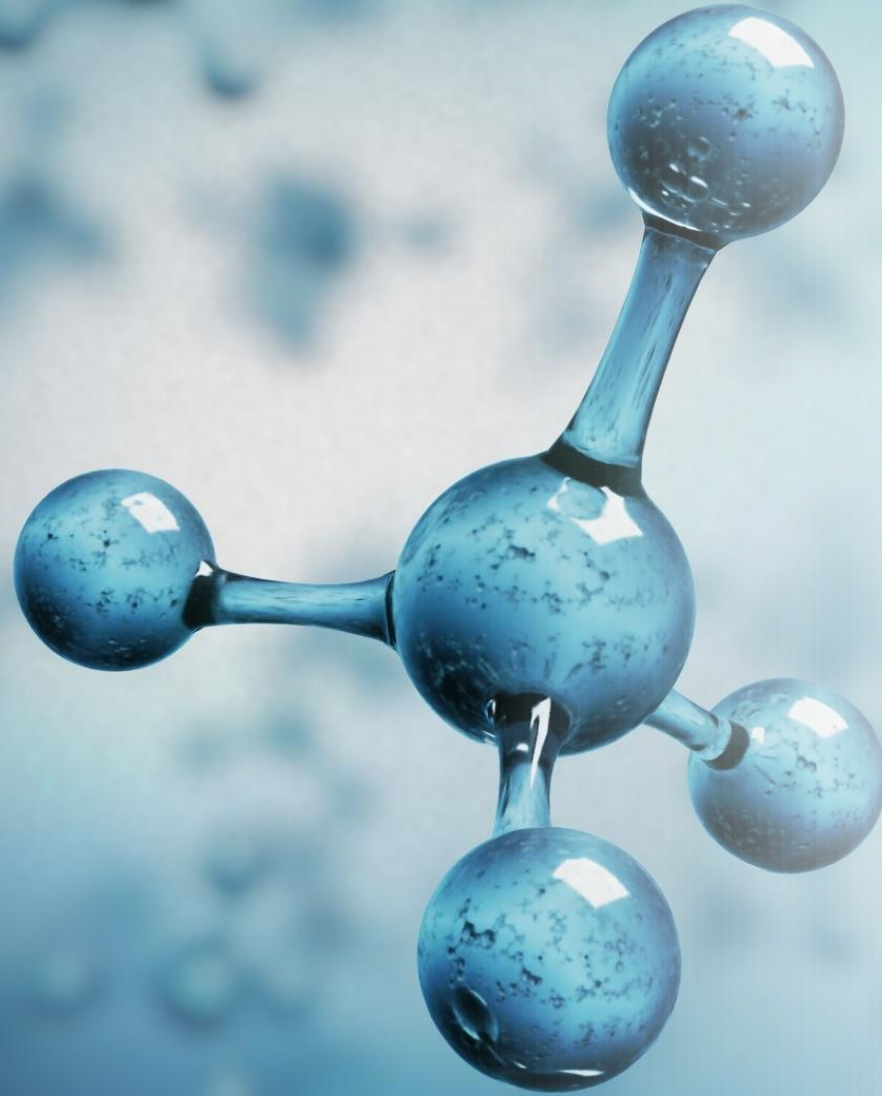
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Seed goes into  
Hydrogen peroxide  
then vinegar is  
added

- Wear PPE
- Stir well
- Let bubble for 10 minutes (we use old-fashioned timer)
- Drain and rinse
- It is ready for transfer to growing vessel now



- Peracetic acid is usually produced in concentrations of 5-15%. When peracetic acid dissolves in water, it disintegrates to hydrogen peroxide and acetic acid, which will fall apart to water, oxygen and carbon dioxide. Peracetic acid degradation products are non-toxic and can easily dissolve in water.



A background image showing wooden blocks arranged to spell out "DON'T FORGET" in two rows. The top row has four blocks (D, O, N, T) and the bottom row has five blocks (F, O, R, G, E, T). The text "Things to keep in mind" is overlaid in white outline font, with a vertical line separating it from a list of bullet points on the right.

# Things to keep in mind

- Seed almost doubles in size.
- We put a maximum of 14 bags in the large Saeplast bins
- Train your team to use PPE for the chemical portion of this process

Thank you and questions

Happy to  
share more  
with anyone  
interested

