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**Research Project Report for the Ohio Vegetable
and Small Fruit Research and Development Program**

Project Title: Effectiveness of Seed Treatments in Eliminating Bacterial Pathogens on Seed and their Effects on Germination and Seedling Vigor

Principal Investigators

Sally A. Miller, Professor and State Extension Specialist/Vegetable Crop Disease Management, Department of Plant Pathology, OSU/OARDC, Wooster;
Phone 330-263-3678; FAX 330-263-3841
e-mail: miller.769@osu.edu

Mark Bennett, Professor, Department of Horticulture and Crop Science, OSU, Columbus
V. M. Balasubramaniam, Assistant Professor, Department of Food Science and Technology, OSU, Columbus

Project Participants

Melanie L. Lewis Ivey, Research Associate, Katie Gerber Undergraduate Intern, Maria Berg, Graduate Student, and Jhony Mera, Research Assistant, Department of Plant Pathology, OSU/OARDC, Wooster; Elaine Grassbaugh, Department of Horticulture and Crop Science, OSU, Columbus

Objectives of the Research

The goal of this research project was to identify seed treatments that reduce or eliminate bacterial pathogens but also do not harm the seeds and/or reduce seedling vigor. The specific objectives were: **1) To evaluate the effectiveness of seed treatments in killing bacteria on or in seed, 2) To evaluate the vigor of seeds exposed to these treatments and 3) To conduct on-farm studies in which the performance of plants from treated and untreated seed are compared.**

The effectiveness of hot water seed treatments in eliminating bacterial spot and canker in tomatoes was demonstrated in trials carried out in 2003. However, there is a concern that hot water treatment can damage low-vigor seed lots. There is a need to further evaluate hot water treatment as well as alternative treatments for tomatoes and other vegetable crops. For the first part of the study (Objective 1), we evaluated hot water and other treatments, including Clorox and acid, for their ability to kill bacteria on and in seeds of tomatoes, mustard greens and squash. For the second part of the study (Objective 2), treated and untreated seeds of each crop were assessed for germination and seedling vigor. Finally (Objective 3), seedlings from commercial lots of hot treated and untreated seeds of mustard greens were planted in separate fields on cooperator farms and evaluated during the season for the presence and severity of bacterial diseases.

Effect of treatments on pure cultures of bacterial pathogens

Treatments (Tables 1) were applied to suspensions of *Xanthomonas campestris* pv. *vesicatoria* 767, *Pseudomonas syringae* pv. *maculicola* Buurma5ri, and *Pseudomonas syringae* pv. *lachrymans* JSF1-04. All liquid treatments were performed by mixing the bacterial suspension and treating agent 1:1 (v:v). Treated suspensions were serially diluted and plated onto YDC (*Xanthomonas*) or PF (*Pseudomonas*) media. Colonies were counted after 72 hr and CFU/ml were determined. There were three replicates per treatment. Data were analyzed by ANOVA and means separated using Fisher's least significance difference test.

Table 1. Effects of treatments on survival of the tomato bacterial spot pathogen, *Xanthomonas campestris* pv. *vesicatoria*, the mustard greens peppery spot pathogen, *Pseudomonas syringae* pv. *maculicola*, and the angular leaf spot pathogen, *Pseudomonas syringae* pv. *lachrymans*.

Treatment	<i>Xanthomonas campestris</i> pv. <i>vesicatoria</i> (CFU/ml)	<i>Pseudomonas syringae</i> pv. <i>maculicola</i> (CFU/ml)	<i>Pseudomonas syringae</i> pv. <i>lachrymans</i> (CFU/ml)
Untreated	1.3 x 10 ⁸ ab*	4.7 x 10 ⁷ ab*	2.7 x 10 ⁸ a*
Hot water (37C 10 min, then 50C 25 min)	0 c	3.7 x 10 ⁴ b	5.0 x 10 ⁵ b
Hot water (37 C for 10 min, 50 C for 15 min) **	-	3.3 x 10 ⁵ b	-
Clorox (diluted 1:4 + silwet; 1 min)	0 c	0 b	0 b
Hydrochloric acid (1.24%, 30 min)	0 c	0 b	0 b
Sonication (high, Aquasonic 50HT sonicator)	1.8 x 10 ⁸ a	6.9 x 10 ⁷ a	2.7 x 10 ⁸ a
Microwave (high, 30 sec)	7.8 x 10 ⁷ bc	8.6 x 10 ⁷ a	2.0 x 10 ⁶ b

*Values are the means of three replicates; means followed by the same letter within a column are not significantly different at p<0.05.

**Data from a separate experiment.

Treatment with dilute Clorox and hydrochloric acid (1.24%) killed all bacteria in the suspensions in the time allotted. However, hot water treatment completely killed only *Xanthomonas*. *Pseudomonas* bacterial counts were reduced by hot water treatment but not eliminated. Neither sonication nor microwaving at recommended times reduced populations of any of the bacteria more than approx. two orders of magnitude.

Effectiveness of treatments in eliminating bacteria from infested seed

Seeds were artificially infested with the bacterial pathogens described above. Approximately 125 seeds per lot from infested seed lots were placed in cheesecloth bags and treated (Table 2a), then the seeds were removed, placed on labeled filter papers and dried overnight. Fifty tomato or mustard greens seeds per rep per treatment of each lot were plated onto YDC or PF medium. Plates were sealed with parafilm and rated for the presence of target bacteria. For squash (Table 2b), 50 seeds per rep per treatment of each lot were washed in KPB (pH7.4, 10mM) for 30 minutes, the bacteria were pelleted (10 000 rpm, 15 min) and the pellets were re-suspended in 1 ml sterile water. Samples were serially diluted and plated onto PF media. Colonies were counted after 48 hr and CFU/ml were determined. Data were analyzed by ANOVA and means separated using Fisher's least significance difference test.

Seed lots tested:

1. Tomato: Peto 696-1
2. Tomtao: Peto 696-2
3. Mustard: Green Wave
4. Mustard: Southern Giant
5. Mustard: Red Giant
6. Squash: Spaghetti
7. Squash: Buttercup
8. Squash: Waltham Butternut

Table 2a. Effectiveness of treatments in reducing populations of bacterial pathogens on seed of tomato (*X. campestris* pv. *vesicatoria*) and mustard greens (*P. syringae* pv. *maculicola*).

Treatment	Proportion (%) of Seeds Contaminated				
	Tomato lot 1	Tomato lot 2	Mustard lot 3	Mustard lot 4	Mustard lot 5
Untreated	93.3 a*	90.0 a	-	1.3 a	18.0 a
Hot water (37C 10 min, then 50C 25 min)	0 d	0 c	5.3 bc	0.0 a	0.7 a
Hot water (37C for 10 min, 50C for 15 min)	-	-	6.7 bc	0.0 a	0.7 a
Chlorox	4.0 d	8.0 bc	6.7 bc	0.7 a	4.0 a
Acid	2.7 d	12.0 bc	0.0 c	0.0 a	0.0 a
Sonication	63.3 c	28.0 b	12.0 b	0.0 a	-
Microwave	78.0 b	13.3 bc	34.3 a	1.7 a	8.0 a

*Values are the means of three replicates; means followed by the same letter within a column are not significantly different at $p \leq 0.05$.

Table 2b. Effectiveness of treatments in reducing populations of bacterial pathogens on seed of squash (*P. syringae* pv. *lachrymans*).

Treatment	Bacteria washed from seed (CFU/ml)		
	Squash lot 6	Squash lot 7	Squash lot 8
Untreated	1.7 x 10 ³ a*	-	7.3 x 10 ⁵ a
Hot water (37C 10 min, then 50C 25 min)	0 a	1.5 x 10 ⁵ b	1.6 x 10 ³ b
Chlorox	0 a	1.6 x 10 ⁴ b	0 b
Acid	0 a	0 b	0 b
Sonication	1.1 x 10 ⁴ a	1.3 x 10 ⁷ a	3.3 x 10 ⁴ b
Microwave	0 a	1.3 x 10 ⁷ a	0 b

*Values are the means of three replicates; means followed by the same letter within a column are not significantly different at p<0.05.

Xanthomonas was completely eliminated from both lots of tomato seed by the hot water treatment and relatively low proportions of seed remained infested with the Chlorox and hydrochloric acid treatments. Sonication and microwave treatments were ineffective. For mustard greens, relatively low rates of infestation were achieved, as indicated in the untreated control. Only the hydrochloric acid treatment eliminated all *Pseudomonas* from all three lots of seed. Infestation of squash seed was more effective, with populations of 10³-10⁵ CFU/ml recovered from untreated seed. As observed for mustard seed, only the hydrochloric acid treatment consistently eliminated *Pseudomonas* from seed.

Effect of seed treatments on seedling vigor

Fifty non-infested seeds per repetition from each seed lot were treated according to the procedures described above. For squash seeds, hydrostatic pressure treatments were also included (Table 3b). After treatment, seeds were removed from the cheesecloth, placed on labeled filter papers and dried overnight. Seeds were germinated for 2-4 days and seedlings were scanned using the OSU Seed Biology Seedling Vigor Imaging System (SVIS). Data were analyzed by ANOVA and means separated using Fisher's least significance difference test.

Table 3a. Effect of seed treatments on tomato and mustard greens seedling vigor.

Treatment	Vigor Index				
	Tomato lot 1	Tomato lot 2	Mustard lot 3	Mustard lot 4	Mustard lot 5
Untreated	458 a*	641 a	563 a**	562 ab	515 a
Hot water	462 a	685 a	523 bc	576 a	562 a
Chlorox		666 a	496 c	544 b	567 a
Acid	474 a	662 a	0 d	0 c	130 b
Sonication	446 a	658 a	566 a	554 ab	505 a
Microwave	450 a	608 a	543 ab	541 b	501 a

*Values are the means of three replicates; means followed by the same letter within a column are not significantly different at $p \leq 0.05$.

Table 3b. Effect of seed treatments on seedling vigor.

Treatment	Vigor index		
	Squash 6	Squash 7	Squash 8
Hot water	698 ab*	598 b	551 b
Chlorox	657 bc	575 bc	531 bc
Acid	715 a	706 a	638 a
Sonication	693 ab	583 bc	510 c
Microwave	618 c	561 cd	537 bc
Hydrostatic Pressure, 33mpa, water	544 d	0	479 d
Hydrostatic Pressure, 400mpa, water	456 e	0	462 d
Hydrostatic Pressure, 33mpa	0**	528 d	0
Hydrostatic Pressure, 400mpa	0	486 e	0

*Values are the means of three replicates; means followed by the same letter within a column are not significantly different at $p \leq 0.05$.

**Seeds were destroyed by the hydrostatic pressure and could not be evaluated.

None of the seed treatments significantly reduced tomato seedling vigor. Mustard greens seedling vigor was negatively affected by hydrochloric acid treatment, which was most effective in eliminating *Pseudomonas* from seed. Hot water and Chlorox treatment significantly reduced vigor in one of three mustard greens lots, while sonication and microwaving had no negative effects on vigor. For squash, the hydrostatic pressure treatments destroyed seed, although effects varied depending on squash type/lot. Seedling vigor was highest for seeds treated with hydrochloric acid.

On-farm assessment of seed treatment effectiveness in reducing bacterial disease incidence

Part of a commercial lot of mustard seeds was divided in two, and one-half was treated with hot water according to OSU Extension Bulletin 672, Ohio Vegetable Production Guide. The other half remained untreated. Seed were planted on the Burma farm, Willard Ohio and the crop was managed according to standard practices. Plots were evaluated in the summer; peppery spot disease incidence was low in all plots. There were no significant differences between treated and untreated plots in the amount of disease observed.