Detection and Eradication of *Alternaria radicina* on Carrot Seed

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ABSTRACT

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Alternaria radicina was recovered from carrot seed following a 10-day incubation on a semiselective agar medium, *Alternaria radicina* Selective Agar (ARSA). A significantly higher proportion of *A. radicina*-infested seed was detected in individual seed lots assayed on ARSA than was detected by the standard freezer-blotter seed assay. The identity of *A. radicina* recovered on ARSA was confirmed by inoculation of carrot root tissue. Based on seed assays using ARSA, 24% (seven of 29) of randomly selected commercial carrot seed lots planted in the Cuyama Valley, California, in 1992, contained low levels of *A. radicina* (≤0.1%). Seed treatments for eradicating the pathogen from infested seed were evaluated. Hot water (50 C for 30 min) or hot sodium hypochlorite (0.1% or 1.0% at 50 C for 30 min) eradicated *A. radicina* from infested carrot seed with a minimal reduction in seed germination.

Additional keywords: carrot black rot, *Daucus carota*, seedborne pathogen, seed testing

Alternaria radicina Meier, Drechs., & E.D. Eddy is a seedborne fungal pathogen responsible for black rot disease of carrots (*Daucus carota* L.) (2,4,10,11). The fungus is believed to have originated in northern Europe, but it now occurs in most major carrot production areas of the world due to the planting of infested commercial carrot seed (12). Once introduced into an area, *A. radicina* can persist in the soil for up to eight years (8).

A. radicina infects carrot roots both in the field and postharvest. Generally, the greatest damage occurs during postharvest storage. The fungus produces large black, sunken lesions along the root; and under moist storage conditions it spreads easily from carrot to carrot (10,12). Infection rates of up to 62% have been reported (4).

In the field, disease symptoms include a black decay of the leaf petiole, carrot crown, and less frequently, the carrot root. In the past, black rot disease in the field was a concern only when the disease progressed into root tissue. However, the use of mechanical harvesters now requires healthy, intact petioles that can be mechanically clasped and pulled. Extensive petiole decay results in petiole breakage during harvesting and reduced yields due to unharvested carrots left in the field.

California produces over 65% of the fresh market carrots in the United States, and black rot is generally not a severe problem. Field losses are usually less than 1% (J. Guerrard, *personal communication*). However, in the Cuyama Valley of California, which is a relatively new carrot production area, the incidence of black rot is high. Despite attempts to manage the disease by fungicide applications and 2- or 3-yr crop rotations, black rot is found in most fields. In some fields, nearly 100% of the carrots are infected.

The introduction of *A. radicina* into new carrot production areas such as the Cuyama Valley has likely been via seed. However, most commercial seed is not assayed for *Alternaria* spp. (*A. radicina* and *A. dauci*, causal agent of Alternaria leaf blight), even though there are several methods available to detect and quantify seed infestation (6,13,16,17). The most commonly used method for assaying carrot seed for *A. radicina* infestation involves incubation of seed on moistened blotter paper for 10 days. To facilitate fungal enumeration, seed germination is inhibited by freezing seed at −20 C for 16 hr (freezer-blotter method) (13). The herbicide 2,4-Dichlorophenoxyacetic acid (2,4-D) has also been used to inhibit seed germination during seed assays (13,17). After incubation, the level of infestation is determined by microscopic examination of each seed for the presence of *A. radicina* conidia.

The freezer-blotter method has several drawbacks. Carrot seed is usually infested with various saprophytic fungi, including *Alternaria alternata, Rhizopus* spp., *Fusarium* spp., and *Ulocladium* spp. In seed lots heavily contaminated with saprophytic fungi, *A. radicina* is frequently overgrown on blotter paper and may not be detected (13). If the pathogen grows but does not produce conidia, identification is impossible. When conidia are produced, differentiating *A. radicina* from related fungi such as *A. alternata, Ulocladium* spp., and *Stemphylium* spp. can be difficult due to similarities in conidial morphology, particularly if few conidia are present for examination (10,12,15).

The objective of this research was to compare two carrot seed assay methods, the freezer-blotter method and a method based on the use of a semiselective agar medium, for sensitivity in detecting *A. radicina*-infested seed. A pathogenicity test, in which carrot root sections were inoculated, was also evaluated as a method to confirm that isolates recovered on the selective medium were *A. radicina*. By using the semiselective medium in seed assays, the level of *A. radicina* infestation in commercial carrot seed lots used in the Cuyama Valley in 1992 was estimated. In addition, the efficacy of several seed treatments, including hot water, hot sodium hypochlorite, and hot fungicide soaks, were evaluated for the eradication of *A. radicina* from infested seed.

MATERIALS AND METHODS

Seed assay methods. The standard freezer-blotter method for assaying carrot seed for *A. radicina* infestation was compared to plating seeds directly on a semiselective agar medium. Freezer-blotter seed assays were performed in 20 × 100 mm glass petri dishes lined with two Whatman No. 1 filter paper disks and moistened with 2.5 ml of sterile water. Fifty seeds were evenly spaced in each dish, and the dishes were sealed with parafilm. After 24 hr at room temperature, the dishes were placed at −20 C for 16 hr to arrest seed germination. The dishes were placed in a single layer 30 cm below fluorescent lights (cool-white) set to a 12-hr light–dark cycle and incubated at room temperature. After 10 days, each seed was examined using a dissecting microscope at 40× magnification for characteristic *A. radicina* conidia.

Seed assays on semiselective agar were performed in 15 × 100 mm plastic petri dishes that contained 15 ml of a medium to be referred to as *Alternaria radicina* Selective Agar (ARSA). This medium was developed specifically for the isolation and enumeration of *A. radicina* from carrot seed and field soil (14). ARSA was prepared in two parts: part A consisted of 16.0 g of agar, 1.0 g of KH_2PO_4, 1.0 g of KNO_3, 0.5 g of KCl, 0.5 g of $MgSO_4$, and 500 ml of H_2O; and part B consisted of 5.0 g of sodium polypectate (Sigma P-1879) and 500 ml of H_2O. Parts A and B were autoclaved separately, cooled to

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50 C, and combined. Added to the combined parts were 50 mg of chlortetracycline HCl (Sigma C-4881), 50 mg of streptomycin sulfate (Sigma S-6501), 4 mg of dicloran (5 mg of Botran 75WP), 100 mg of triadimefon (200 mg of Bayleton 50WP), 106 mg of thiabendazole (0.25 ml of Mertect 340-F), and 10 mg of 2,4-D (Sigma D-8407). The herbicide 2,4-D was added from a stock solution that consisted of 200 mg of 2,4-D dissolved in 5 ml of hot ethanol and added slowly to 100 ml of H_2O. Fifty seeds were evenly spaced over the agar surface in each dish. Dishes were stacked five deep in plastic trays and incubated at 28 C in darkness. After 10 days, the undersurfaces of the dishes were examined using a dissecting microscope at 7× magnification for characteristic vegetative growth of *A. radicina* emerging from infested seed.

Four experimental carrot seed lots known to be infested with *A. radicina* were assayed by each method. These lots, designated A, B, C, and D, were also used in subsequent studies. One thousand seeds from each lot were assayed by each of the two methods, and the assays were repeated three times. The data were subjected to analysis of variance (ANOVA).

Carrot disk pathogenicity test. A carrot disk pathogenicity test was developed to confirm the identity of *A. radicina* isolates recovered from seed. To prepare carrot disks for inoculation, mature carrot roots purchased from local markets were washed in tap water and sliced into disks approximately 5 mm thick. The disks were surface-disinfested by soaking in 1.0% sodium hypochlorite for 5 min with occasional agitation, then triple-rinsed with water and placed on a paper towel for 1 hr to dry. The carrot disks were then placed in 20 × 100 mm glass petri dishes containing two Whatman No. 1 filter papers moistened with 2 ml of an antibiotic solution (0.05% streptomycin sulfate and 0.05% chlortetracycline HCl).

Isolates of *A. radicina* and four other fungi commonly recovered from carrot seed, *A. alternata, A. dauci, Ulocladium atrum,* and *Stemphylium vesicarium,* were tested for growth and pathogenic response on carrot disks. Fungal isolates were obtained from carrot seed or carrot field soil and maintained on acidified potato-dextrose agar. Carrot disks were inoculated with agar plugs (4 mm diameter) cut from the margins of actively growing cultures; one plug was placed upside down on the center of each disk. After inoculation, each disk was lightly misted with the antibiotic solution to inhibit bacterial growth and to prevent the agar plug from drying.

The dishes containing inoculated disks were incubated on wire racks in clear plastic trays for 10 days at room temperature. A small amount of water was added to each tray to maintain humidity. Five days after inoculation, the disks were misted again. After 10 days, fungal growth and pathogenicity were evaluated on each disk based on the following scale: 0 = no discoloration of the disk, 1 = slight discoloration, 2 = black necrosis, and 3 = black necrosis with the production of characteristic *A. radicina* conidia. A total of 48 isolates was tested, and each test was replicated three times. The data were subjected to ANOVA, and the means were separated by the least significant difference (LSD) test.

The reliability of visual identification of *A. radicina* on ARSA was confirmed using the carrot disk pathogenicity test. From a set of 2,000 seeds (lots A and D mixed) assayed on ARSA, all isolates visually identified as *A. radicina* were tested. An agar plug (4 mm diameter) containing the fungal mycelium was taken from beneath the infested seed and placed on carrot disks as described. A total of 185 isolates was tested.

Seed survey. Samples of carrot seed from selected lots to be planted in the Cuyama Valley in 1992 were provided by growers. Since standardized seed sampling protocols for seedborne carrot diseases are not available, seed samples were taken according to the sampling protocol used for germination testing by the California Department of Food and Agriculture (1). Per lot, 1,000 seeds were assayed for *A. radicina* infestation by plating on ARSA. The assays were repeated twice.

Seed treatments. An initial seed treatment experiment was performed on a single infested seed lot (lot A, 13.2% infestation). Treatments consisted of soaking seed in water (22 C), hot water (50 C), hot sodium hypochlorite (1.0% or 0.1%, 50 C), hot thiram (Thiram 65WP, 0.2% a.i. at 30 C), or hot iprodione (Rovral 50WP, 0.2% a.i. at 30 C), for various time periods. A second experiment was conducted in which the superior treatments identified in the first experiment, hot water (50 C) and hot sodium hypochlorite (1.0% or 0.1%, 50 C) treatments for various time periods, were evaluated for three additional seed lots (lots B, C, and D, with 28.1, 0.1, and 4.3% infestation, respectively).

For each treatment, 4-g samples of seed were placed in loose cheesecloth bags. Each bag was submerged in 500 ml of treatment solution in a 1-L beaker placed in a constant temperature water bath. Seeds were soaked in the treatment solution with occasional agitation for the specified time. Following treatment, each bag was rinsed for 30 sec under running tap water, and the seeds were spread out on a paper towel to air-dry overnight. After 14 days of storage in seed envelopes at room temperature, 1,000 seeds from each treatment were assayed for *A. radicina* infestation by plating on ARSA. Each treatment was replicated three times. The data were subjected to ANOVA, and the means were separated by the LSD test.

Germination tests on treated seed were conducted after 14 days of storage. For each treatment, 250 seeds were placed on moistened filter paper (two Whatman No. 1 filter paper disks with 2.5 ml of water) in glass petri dishes (50 seeds per dish), and the dishes were sealed with parafilm. The dishes were stacked in plastic trays and incubated at room temperature for 10 days. After incubation, seed germination was determined by visual examination of emerging radicals. The data were subjected to ANOVA, and the means were separated by the LSD test.

Table 1. Comparison of seed assay methods for the recovery of *Alternaria radicina* from carrot seed

Assay method	Seed infestation (%)[y]			
	Lot A	Lot B	Lot C	Lot D
Direct plating on ARSA[z]	13.2 a	28.1 a	0.1 a	4.3 a
Freezer-blotter	5.8 b	13.4 b	0.2 a	0.6 b

[y] Values represent the percentage of infested seed detected in 1,000 seeds assayed. Assays were replicated three times. For each seed lot, means followed by the same letter are not significantly different according to analysis of variance ($P = 0.05$).
[z] *Alternaria radicina* Selective Agar.

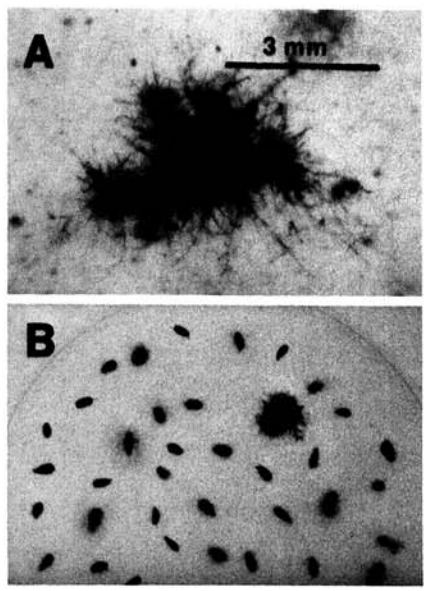

Fig. 1. Characteristic thick, black mycelium of *Alternaria radicina* on semiselective *A. radicina* Selective Agar medium. **(A)** Individual colony and **(B)** growing from an infested carrot seed.

RESULTS

Comparison of seed assay methods. A significantly higher proportion of *A. radicina*-infested seed was detected in individual seed lots assayed on ARSA than by the freezer-blotter method in three of the four seed lots tested (Table 1). There was no difference in recovery from the fourth lot.

Growth of *A. radicina* on ARSA was distinctive and was optimally viewed by examining the undersurfaces of the dishes. Prolific *A. radicina* growth appeared as a dense black mycelial mat (Fig. 1) and was easily distinguished without magnification from the growth of other fungi. Sparse growth of *A. radicina* on ARSA appeared as one to several irregularly branched, thick, olive-black to black hyphae that extended down into the medium from the initial point of seed contact. In these cases, positive identification usually required examination at 7× magnification. The growth of fungal seed saprophytes on ARSA appeared hyaline to olive-brown; hyphae were much thinner than those of *A. radicina*. In contrast to *A. radicina*, the growth of these fungi did not always deeply penetrate the agar medium.

The time required for examination of seed for *A. radicina* contamination was considerably reduced with the ARSA method. Examination of 1,000 seeds on blotter paper at 40× magnification for *A. radicina* conidia generally required at least 1 hr, whereas examination of 1,000 seeds on ARSA at 7× magnification usually required less than 10 min.

Carrot disk pathogenicity tests. Only *A. radicina* isolates induced black necrosis in carrot disks (Table 2). Although slight darkening of carrot tissue was induced by other fungal isolates, especially *A. dauci* and *A. alternata*, black necrosis induced by *A. radicina* was distinct. Isolates of *A. alternata* and *U. atrum* occasionally produced abundant black conidia that covered the surface of the carrot disk. However, when these conidia were scraped off, little discoloration or softening of the carrot disk was observed.

Of the 185 isolates visually identified as *A. radicina* on ARSA, 179 (97%) were confirmed based on the induction of black necrosis in carrot disks and the production of characteristic conidia. The majority of these isolates (85%) were confirmed after 10 days of incubation, whereas the remainder required an additional 10 days of incubation to produce conidia. The six isolates that failed to grow on the carrot disks were assumed to be other species.

Seed survey. *A. radicina* was recovered from seven of the 29 commercial seed lots assayed. Infestation levels for these lots were low (two lots at 0.1%, five lots at 0.05%).

Seed treatments. In the first set of seed treatments, both hot water (50 C for 20 min) and hot sodium hypochlorite (1.0% at 50 C for 20 min) soaks eradicated *A. radicina* from contaminated seed (Table 3). Seed germination was not significantly reduced by these treatments. However, germination was significantly reduced when the hot water treatment was extended to 30 min. The lower concentration of sodium hypochlorite (0.1% at 50 C) also eradicated the pathogen after a 30-min treatment time, but seed germination was significantly reduced. Soaking the seed in cool water (20 C for 10 min) did not eradicate *A. radicina* but substantially reduced the level of infestation without reducing seed germination (Table 3).

In general, sodium hypochlorite treatments appeared to reduce seed quality. These treatments removed the pericarp from many seeds and caused others to adhere in very tight clusters, as though glued. These effects increased with hypochlorite concentration and treatment time. However, in most cases, hypochlorite treatments did not significantly reduce germination; but they made the seed difficult to handle and the overall appearance deteriorated.

Iprodione and thiram treatments (0.2% a.i. at 30 C) failed to eradicate *A. radicina* from seed, although both treatments significantly reduced seed infestation without reducing seed germination (Table 3). In the 4-hr fungicide treatments, less *A. radicina* was detected in seed treated with thiram than with iprodione. There was no difference between the 8-hr fungicide treatments.

In the second set of treatments, *A. radicina* was eradicated from all three lots after a 20-min soak in 1.0% sodium hypochlorite (50 C), a 25-min soak in 0.1% sodium hypochlorite (50 C), or a 30-min soak in H_2O (50 C) (Table 4). However, seed germination was significantly reduced in the 30-min soak in H_2O (50 C) for one of the lots. Germination was also reduced in at least one of the lots tested for the 25-min soak in 1.0% sodium hypochlorite and for the 30-min soak in 0.1% sodium hypochlorite.

Table 2. Comparison of growth and disease expression on carrot disks inoculated with *Alternaria radicina* and other fungi commonly found on carrot seed

Fungus	Isolates tested[y] (no.)	Pathogenicity score[z]
A. radicina	20	2.73 a
A. dauci	7	0.22 b
A. alternata	9	0.13 b
Ulocladium atrum	8	0.07 b
Stemphylium vesicarium	4	0.07 b

[y]Carrot disks were inoculated with a 4-mm-diameter agar plug taken from the margin of an actively growing culture on acidified potato-dextrose agar. Three disks were inoculated per isolate.

[z]Values represent the averaged score for all isolates. Growth and pathogenicity were scored after 10 days according to the following rating system: 0 = no growth or discoloration of disk, 1 = slight discoloration, 2 = black necrosis, 3 = black necrosis and production of characteristic *A. radicina* conidia. Means followed by the same letter were not significantly different according to LSD means separation test ($P = 0.05$).

Table 3. Comparison of seed treatments for eradicating *Alternaria radicina* from carrot seed

Treatment[x]	Temp (C)	Time (min)	Seed infestation[y] (%)	Seed germination[z] (%)
Control	...	...	13.2 a	62.3 b–e
H_2O	20	10	3.90 b	59.0 d–g
H_2O	50	10	0.13 d	64.7 a–c
H_2O	50	20	0.0	58.7 d–g
H_2O	50	30	0.0	55.3 g
H_2O	50	60	0.0	31.6 h
$NaHClO_3$ 0.1%	50	10	0.07 d	60.3 c–g
$NaHClO_3$ 0.1%	50	20	0.07 d	57.7 d–g
$NaHClO_3$ 0.1%	50	30	0.0	56.5 fg
$NaHClO_3$ 0.1%	50	60	0.0	21.7 i
$NaHClO_3$ 1.0%	50	10	0.07 d	66.3 ab
$NaHClO_3$ 1.0%	50	20	0.0	60.3 c–g
$NaHClO_3$ 1.0%	50	30	0.0	57.0 e–g
$NaHClO_3$ 1.0%	50	60	0.0	14.3 j
Thiram 0.2%	30	240	0.20 d	61.3 b–f
Thiram 0.2%	30	480	0.10 d	62.8 b–d
Iprodione 0.2%	30	240	1.27 c	61.0 b–f
Iprodione 0.2%	30	480	0.50 d	69.5 a

[x]Four grams of seed were placed in a loose cheesecloth bag and submerged in 500 ml of treatment solution for the specified time with occasional agitation. Each treatment was replicated three times.

[y]One thousand seeds per replication were assayed for *A. radicina* on *Alternaria radicina* Selective Agar. Dishes were incubated in darkness at 28 C for 10 days. Means followed by the same letter are not significantly different according to LSD means separation test ($P = 0.05$).

[z]For germination tests, 250 seeds per replication were incubated on moistened blotter paper for 10 days. Means followed by the same letter are not significantly different according to LSD means separation test ($P = 0.05$).

DISCUSSION

The use of ARSA to assay carrot seed for *A. radicina* infestation provides several advantages over the presently used freezer-blotter method. The selective fungicides and antibiotics in ARSA suppress the growth of numerous seed saprophytes that may otherwise compete with and/or obscure the growth of *A. radicina.* Similarly, a 5-min soak in sodium hypochlorite (0.1%) has been reported to increase the recovery of *A. dauci* from carrot seed in a 2,4-D blotter assay by reducing seed saprophytes (17). Although this treatment may have eliminated some of the *A. dauci* from seed, the overall increase in recovery was attributed to the reduction of saprophytic fungi. The incorporation of selective fungicides into ARSA did not inhibit the growth of *A. radicina* and eliminated the additional step of pretreating seeds before assaying.

The identification of *A. radicina* based on its vegetative growth on ARSA is subject to less variation than are methods based on the production and identification of conidia, such as the freezer-blotter method. The production of conidia by many *Alternaria* spp., including *A. radicina,* is subject to the influence of a number of environmental conditions, such as temperature and the quantity and quality of light (3,5,13, 17,18). Variation in either light or temperature will affect the sensitivity and reproducibility of seed assays performed on blotter paper, and special incubation chambers are needed to avoid such variation. ARSA seed assays are carried out in darkness and incubated at 28 C, which is the optimal temperature for *A. radicina* growth in culture (4). Thus, standard laboratory incubators are sufficient for conducting ARSA seed assays.

By using vegetative growth on ARSA, *A. radicina* can be identified at a magnification of 7× or less. This results in a substantial reduction in time required for enumeration and considerably less eyestrain and technician fatigue, which are important considerations when examining large numbers of seeds. The higher magnifications required to differentiate conidia in the freezer-blotter method reduce field of view and depth of field, thus increasing examination time.

The growth of certain fungi associated with carrot seed was not inhibited on ARSA, particularly fungi closely related to *A. radicina,* such as *A. alternata, A. dauci, Ulocladium* spp., and *Stemphylium* spp. However, *A. radicina* was differentiated from these fungi on the basis of its distinctive vegetative growth on ARSA. *A. radicina* produces distinctive dark hyphae that penetrate into the agar, whereas *A. alternata, A. dauci,* and *Ulocladium* spp. produce light brown to dark olive-brown mycelia that frequently only grow on the surface of ARSA. *Stemphylium* spp. produce hyaline mycelia on ARSA and, in some cases, may also produce perithecia. The pronounced difference between the vegetative growth of *A. radicina* and of *Stemphylium* spp. on ARSA is particularly important for differentiating these fungi in seed assays, because the conidia of these fungi are very similar.

The carrot disk pathogenicity test confirmed the reliability of the ARSA carrot seed assay. Almost all fungal isolates visually identified as *A. radicina* on ARSA were confirmed by inoculating carrot disks, demonstrating that visual identification of *A. radicina* on ARSA is accurate. However, when the identity of a particular seed isolate cannot be resolved by visual examination, its identity should be confirmed on carrot disks.

Commercial seed lots used in the Cuyama Valley had only low levels of *A. radicina* contamination. However, this amount of inoculum could be substantial if considered on a per acre basis. Planting density for fresh market carrots in the Cuyama Valley is approximately 800,000 seeds per acre. Based on this rate, planting a seed lot with 0.1% *A. radicina* infestation would result in the introduction of 800 infested seeds per acre. The contribution of this low level of seed-borne inoculum on the incidence of black rot in the Cuyama Valley is currently being investigated.

Most commercial carrot seed is treated with the fungicide thiram, applied as a dust or as a slurry. The results of the seed survey indicated this seed dressing does not always eradicate *A. radicina* from seed. Hot fungicide soaks of 24 hr (0.2% a.i. iprodione or thiram at 30 C) have been reported to successfully eradicate *A. radicina* from infested seed (6,7, 9). However, the adverse effects of long treatment time on seed germination after redrying and storage have not been fully addressed, and the treatment is not commonly used commercially. Shorter treatment times, such as those used in our trials, were not sufficient for eradication.

The results of the seed treatment experiments indicate that either hot water or hot bleach can eradicate *A. radicina* from carrot seed. These treatments may be particularly useful, considering the increased restrictions being placed on the use of many fungicides. Although a slight reduction in seed germination occurred with these treatments, the benefit of eradicating *A. radicina* from seed may outweigh this reduction in germination, particularly if the seed is to be planted in new production areas. Further evaluation of these treatments on larger quantities of seed is necessary to determine their commercial applicability.

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Table 4. Comparison of hot water and hot sodium hypochlorite treatments for eradicating *Alternaria radicina* from carrot seed

Treatment[x]	Time (min)	Seed infestation (%)[y]			Seed germination (%)[z]		
		Lot B	Lot C	Lot D	Lot B	Lot C	Lot D
Control	...	28.10 a	0.10 a	4.20 a	61.6 a	53.2 a	70.3 ab
H_2O	20	0.03 b	0.0	0.03 b	64.2 a	51.7 ab	69.3 ab
H_2O	25	0.10 b	0.0	0.0	58.5 b–d	49.7 ab	69.3 ab
H_2O	30	0.0	0.0	0.0	56.8 cd	49.4 ab	67.3 bc
$NaHClO_3$ 0.1%	20	0.0	0.07 a	0.0	58.5 b–d	50.0 ab	73.5 a
$NaHClO_3$ 0.1%	25	0.0	0.0	0.0	57.7 b–d	52.3 ab	67.7 bc
$NaHClO_3$ 0.1%	30	0.0	0.0	0.0	56.5 d	48.3 bc	64.7 c
$NaHClO_3$ 1.0%	20	0.0	0.0	0.0	60.7 a–c	51.5 ab	68.7 bc
$NaHClO_3$ 1.0%	25	0.0	0.0	0.0	57.3 cd	50.0 ab	68.0 bc
$NaHClO_3$ 1.0%	30	0.0	0.0	0.0	55.2 d	45.2 c	66.7 bc

[x]Four grams of seed were placed in a loose cheesecloth bag and submerged in 500 ml of treatment solution for the specified time with occasional agitation. Each treatment was replicated three times.

[y]One thousand seeds per replication were assayed for *A. radicina* infestation on petri dishes containing *Alternaria radicina* Selective Agar. Dishes were incubated in darkness at 28 C for 10 days. Means followed by the same letter are not significantly different according to LSD means separation test ($P = 0.05$).

[z]For germination tests, 250 seeds per replication were incubated on moistened blotter paper for 10 days. Means followed by the same letter are not significantly different according to LSD means separation test ($P = 0.05$).

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