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ORIGINAL ARTICLE

Evaluation of physical and chemical disinfection methods of *Brassica oleracea* seeds naturally contaminated with *Xanthomonas campestris* pv. *campestris*

M. Sanna[1] · G. Gilardi[1] · M. L. Gullino[1] · M. Mezzalama[1,2]

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Abstract
Xanthomonas campestris pv. *campestris* (Xcc), the causal agent of black rot of crucifers, is a seedborne pathogen that causes severe yield losses worldwide. Seed treatments represent the most effective strategies to control the pathogen. In this study several physical and chemical treatments were evaluated for their efficacy in containing the contamination of the seeds with Xcc, without compromising the germination. The experiments were carried out with a naturally contaminated seed batch, selected among four lots for its higher contamination with Xcc (9.5×10^1 cells/g of seed). The evaluation was performed under controlled conditions following a modified protocol from ISTA (2019), highlighting different degrees of effectiveness of the tested disinfection treatments. Seeds disinfected under laboratory conditions with hydrogen peroxide (3%) for 30 min showed the 100% containment of the level of contamination with Xcc with the germination rate at 95.3% after the treatment, if compared with the untreated control. The same treatment was tested at the operation site of the seed company to evaluate the applicability of the method under commercial conditions. Under practical conditions, reducing the length of the treatment to 15 min, the strict control of the temperature of the disinfecting bath, the drying temperature at 37 °C and the use of small quantities of seeds, between 50 and 300 g, were crucial to obtain disinfected and viable seed.

Keywords Black rot · Cabbage · Seedborne pathogen

Introduction

Cabbage (*Brassica oleracea* L.) is a vegetable crop consumed worldwide, with a cultivated area of 2 million ha and a production of 70 million tons. The world's largest producer is China, followed by India and Russia, while in Europe the largest producers are Ukraine and Poland (Faostat 2021). Italy and New Zealand are the two countries in the world where cabbage seed companies multiply primary seed for international distribution, and which is used for producing seedling transplants. Crucial in this activity is the use of healthy seed to prevent the spread of seedborne diseases around the world.

Xanthomonas campestris is a pathogenic, gram-negative bacterium, genetically differentiated into over 140 pathovar (pv.) associated with a specific host. *Xanthomonas campestris* pv. *campestris* (Xcc) is a seedborne pathogen and the causal agent of black rot in the crucifers, responsible of severe yield losses worldwide. It invades and blackens vascular tissues and causes V-shaped marginal chlorotic to necrotic leaf lesions. As the disease progresses, necrosis spreads and the plant withers to rot. The black rot disease is widespread all over the world and is considered one of the most destructive diseases of crucifers (Alvarez et al. 2000).

Primary source of infection is the use of infected seed, where Xcc can survive up to 3 years. The pathogen also survives and overwinters in soil and in crop debris. During the germination of infected seeds, Xcc invades the vascular tissues, infects the foliage and it is released at the edges of the leaves allowing the spread of the inoculum in the field during the cropping cycle through splashes and aerosols coming from nearby infected fields, infected weeds,

✉ M. Mezzalama
monica.mezzalama@unito.it

[1] AGROINNOVA–Centre of Competence for the Innovation in the Agro-environmental Sector, Università Di Torino, Largo Paolo Braccini 2, 10095 Grugliasco, TO, Italy

[2] DISAFA–Dept Agricultural, Forestry and Food Sciences, Università Di Torino, Largo Paolo Braccini 2, 10095 Grugliasco, TO, Italy

insects and machineries (Williams 1980). Severe epidemics of the disease were recorded in areas characterized by high temperatures and humidity, in fact bacterial growth and symptom expression is strongly favored by temperatures of 25–30 °C, that can aggravate the damages (Köhl and van der Wolf 2005).

Seeds are usually produced in temperate regions and the production starts in midsummer to reduce the susceptibility of the seedlings during the late summer, characterized by favorable temperatures for bacterial growth (Köhl and van der Wolf 2005). Spread of the pathogen from infected seedlings occurs also in misted seedbeds production systems (Shigaki et al. 2000).

Xcc poses an important hurdle for cabbage production. The pathogen can be prevented and controlled by adopting various strategies such as using pathogen-free seeds (Bila et al. 2013) or by disinfecting seeds with physical and chemical methods. The physical treatments include the use of hot water, effective against various seedborne bacterial and fungal pathogens of vegetables (Nega et al. 2003). Other studies demonstrated the effectiveness of seed treatment with sodium hypochlorite (Carisse et al. 2000) and with hydrogen peroxide (Duval and Nesmith 2000), well known for their bactericidal action. Recently an electrically charged disinfectant was developed and tested for its effectiveness against Xcc demonstrating its ability to reduce the level of contamination of the seeds (Sakudo et al. 2020).

The aim of this study was to evaluate the effectiveness of one physical and four chemical disinfection methods to reduce the level of contamination with Xcc of cabbage seed, comparing concentrations and durations of exposure to optimize the benefits for the vegetable without affecting seed germination. The evaluation was performed under controlled laboratory conditions and the most effective treatment was transferred to the company facilities to confirm its applicability under commercial conditions.

Material and methods

Cabbage seed samples

The 4 batches of cabbage seeds (MB1414 batch 7669, MB1414 batch 54, MB1415 batch 717 and MB1415 batch 720) used in this study were provided by the company ANSEME (Cesena, Italy).

Phytosanitary evaluation of seeds

To evaluate the phytosanitary conditions of the seed batches a modified protocol from ISTA (2019) was used. A total of 1000 seeds (4 g) from each batch were suspended in 25 ml of sterile PBS solution (0.05 M phosphate) plus Tween™ 20 (0.02% v/v) (Merck, Readington, New Jersey, USA), preliminary chilled to 2–4 °C, in a 100 ml conical flask. The suspension was shaken for 2.5 h at room temperature on a rotary shaker at 100–125 rpm. Two tenfold serial dilutions were obtained and 100 µl of each solution and of the undiluted seed extract were plated on the semi-selective medium mFS, and distributed over the surface with a sterile bent rod. The plates were incubated at 28–30 °C upside down and examined after 4 days. A negative and a positive control were included in each test. The negative control was obtained preparing dilutions from a sample of the extraction medium (PBS plus Tween™ 20), without seeds, and plating on the media used for the samples. The positive control was obtained preparing a suspension of a strain of *Xcc* in sterile PBS plus Tween™ 20. The suspension concentration was adjusted to 10^2 to 10^4 CFU/ml and 100 µl of it was plated on the selective media and spread over the surface with a sterile bent rod.

To confirm the identification, representative colonies were plated on YDC medium and incubated at 28–30 °C for 24–48 h.

Molecular assay

Three representative bacterial colonies, isolated from lot 720, were tested with specific real-time PCR protocol to confirm the identification as Xcc according to the ISTA protocol (2019). The DNA was extracted from the colonies (Xcc1, Xcc2, Xcc3) obtaining a suspension of each colony in 1 ml of sterile Ringer's solution, then centrifuged at 8000 rpm for 5 min at room temperature. The supernatant was removed, and the pellet suspended again in 500 µl of NaOH (0.5 M) and incubated for 10 min at 100 °C.

The DNA was tested using the specific primers and probe designed by Köhl et al. (2011) and the universal primers and probes designed by Wu et al. (2008) to validate the reaction. The reaction was set up in a final volume of 25 µl containing 0.5 µl of each primer and probe, 5 µl of GoTaq® Probe qPCR Master Mix (Promega, Madison, USA), 11.5 µl of nuclease free water and 5 µl of DNA. The cycle was carried out in a StepOne Plus ™ Real-Time PCR System (Applied Biosystems) thermal cycler with an incubation at 95 °C for 10 min, followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C. The results were visualized through the "StepOne Software" program, connected to the thermal cycler.

Germination test

A total of 400 seeds from each sample were incubated on top of paper, soaked with a 0.05% sodium hypochlorite solution, at 20 °C for 5 days, under a 12 h near-ultraviolet light (NUV) 12 h dark cycle. The same protocol was adopted to test the germinability of the seeds after disinfection treatments.

Disinfection of cabbage seeds under controlled conditions

The seed lot no.720 was used to evaluate the different methods applied because of its high level of contamination with *Xcc*. A total of 1400 seeds, for each treatment tested, were closed in two layers of sterile gauze and submerged in the disinfectant solutions, following the experimental scheme shown in Table 1. At the end of the treatment the seeds were let dry under the flow of a laminar hood overnight. Four hundred seeds were used for the germination test, while the other 1000 seeds were used to evaluate the effectiveness of the treatment in reducing their level of contamination, with the method described above for phytosanitary evaluation of seeds. Hot water (50 °C) was tested for its effectiveness in containing Xcc by immersion of the seeds for 20 and 30 min. Seeds were disinfected with: (1) a solution of hydrogen peroxide, H_2O_2 (3%); (2) with a 0.5% sodium hypochlorite (NaClO) water solution; (3) a commercial product formulated for sanitization of different surfaces containing a mixture of peracetic acid (CH_3CO_3H, 5%), hydrogen peroxide (H_2O_2, 20%) and acetic acid (CH_3CO_2H, 10%) (JetFive, Certis Europe B.V., Italy) from now PAAH, at the final concentration of 0.8%, tested at room temperature and at 50 °C; and at the concentration of 1.6% tested at room temperature; 4) electrolyzed water, obtained by applying an electrical charge to a mixture of tap water and salt causing the release of hypochlorous acid (HClO) which acts as disinfectant (Anolyte—Envirolyte, Italy). Each treatment was performed for 30 min, except treatment with hydrogen peroxide (3%), that was tested also for 15 min.

Seed disinfection procedure at the company facilities

The seeds belonging to two batches (717 and 7669) were tested to assess the efficacy of the treatment with hydrogen peroxide (3%). Different amounts of seeds (50 g, 100 g, 200 g, 300 g, 500 g, 1000 g) were closed in cotton bags and submerged in the solution for 30 min. At the end of the treatment the seeds were rinsed under tap water for 3 min, centrifugated at 150 rpm for 2 min at room temperature and then dried in the oven at 37 °C for 1 or 2 h. Seeds were tested to assess the viability and the efficacy of the treatment by following the protocols described above.

Data analyses

The data were subjected to the analysis of variance (ANOVA), following a Log10 transformation of the quantity of Xcc recorded, for data normalization. The Tukey's test was used to explore differences between multiple group means ($P \leq 0.05$). Data were finally back-transformed to the original. Statistical analysis were performed with the Statistical Package for Social Science (SPSS, IBM, Chicago, IL, USA) version 27.0.

Results

Phytosanitary evaluation of seeds

The evaluation of the phytosanitary condition of the naturally contaminated seed lots is shown in Fig. 1. A higher concentration of Xcc was observed in the seed sample MB1415 lot 720 with 9.5×10^1 cells/g of seed, while no cells/g of

Table 1 Different treatments (active ingredients, commercial name, temperature and duration of exposure) evaluated for their effectiveness in containing the level of infection of *Brassica* seeds

Treatment	Active ingredient	Commercial name	Temperature	Time (minutes)	Rinsing
1	Water	–	50 °C	20	–
2	Water	–	50 °C	30	–
3	Hydrogen peroxide (10%)	–	Room temperature (25–27 °C)	30	Yes No
4	Hydrogen peroxide (10%)	–	Room temperature (25–27 °C)	15	Yes No
5a	Peracetic acid 5% + Hydrogen peroxide 20% + Acetic acid 10% (0.8%)	JetFive	Room temperature (25–27 °C)	30	Yes No
5b	Peracetic acid 5% + Hydrogen peroxide 20% + Acetic acid 10% (0.8%)	JetFive	50 °C	30	Yes No
6	Peracetic acid 5% + Hydrogen peroxide 20% + Acetic acid 10% (1.6%)	JetFive	Room temperature (25–27 °C)	30	Yes No
7	Sodium hypochlorite (0.5%)	–	Room temperature (25–27 °C)	30	Yes No
8	Electrolyzed water (HClO)	Anolyte	Room temperature (25–27 °C)	30	Yes No

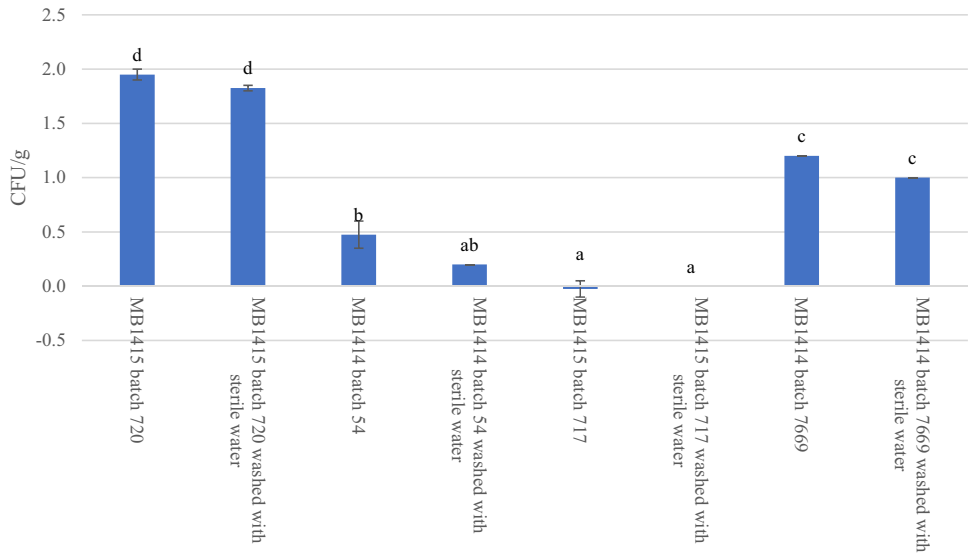

Fig. 1 Quantification of *Xanthomonas campestris* pv. *campestris* concentration expressed in the four tested cabbage seed batches (ANOVA and Tukey $P < 0.05\%$)

seed was found in seed sample MB1415 lot 717. Seed lot 720 was selected for testing the different disinfection methods reported in the experimental scheme (Table 1), as it showed the highest level of seed contamination. Washing seed with sterile water at room temperature did not reduce seed contamination with Xcc that was statistically similar to the level of contamination recorded in the untreated seed samples (Fig. 1).

Molecular assay

The real-time PCR assay highlighted the amplification of the DNA of two of the three isolated colonies confirming the identification and presence of Xcc in the seeds (Fig. 2).

Positive colonies were small, pale green, mucoid and surrounded by a zone of starch hydrolysis on mFS, and pale yellow and mucoid on YDC.

Germination test

The effect of the disinfection treatments on seed germination is shown in Table 2. The treatment with hydrogen peroxide (3%) did not affect the germinability of the sample (95.3%) which was statistically similar to the percentage recorded in the untreated control (97%), and no rinsing effect emerged. The treatment of the seeds with electrolyzed water produced a lower but statistically similar percentage of seed germination (94.0%) if compared to the untreated control, while

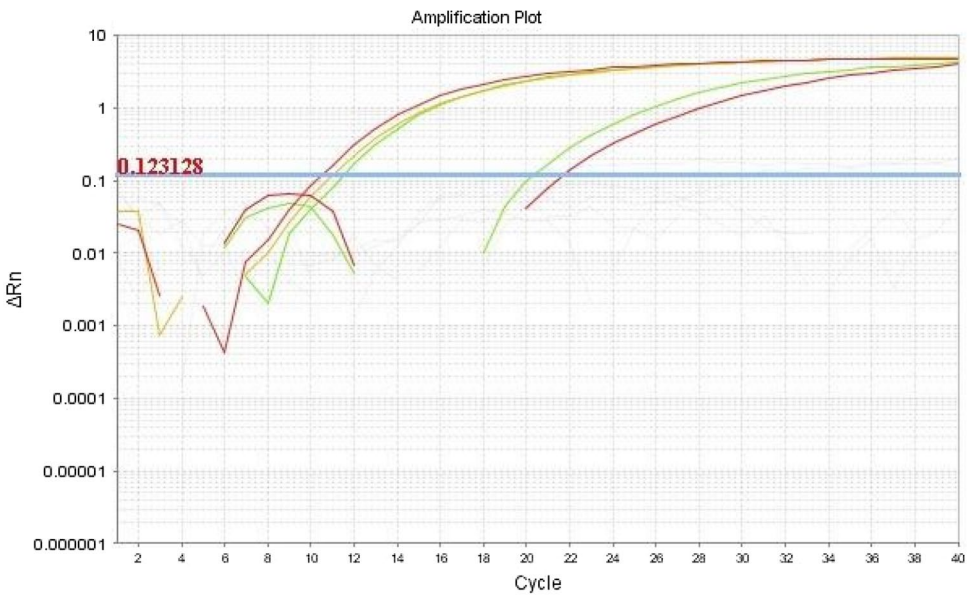

Fig. 2 Amplification with real-time PCR protocol of DNA extracted from Xcc1 (Ct 24.45) and Xcc3 (Ct 13.51) colonies isolated from cabbage seeds

Table 2 Effect of different treatments on cabbage seed germination

Treatment	Rinsing	Percentage of seeds					
		Germinated		Dead		Abnormal	
–	–	97.0	a*	0.0	a	3.0	ab
Hot water (50 °C) for 20 min	–	95.0	a	2.5	b	2.5	ab
Hot water (50 °C) for 30 min	–	94.3	ab	2.5	b	3.3	ab
Hydrogen peroxide (3%) for 30 min	No	95.3	a	2.0	b	2.8	ab
Hydrogen peroxide (3%) for 30 min	Yes	95.3	a	2.3	b	2.5	ab
Hydrogen peroxide (3%) for 15 min	No	95.5	a	1.0	a	3.5	ab
Hydrogen peroxide (3%) for 15 min	Yes	97.5	a	1.0	a	1.5	a
Peracetic acid 5% + Hydrogen peroxide 20% + Acetic acid 10% (0.8%)	No	94.8	a	2.5	b	2.8	ab
Peracetic acid 5% + Hydrogen peroxide 20% + Acetic acid 10% (0.8%)	Yes	96.0	a	2.5	b	1.5	a
Peracetic acid 5% + Hydrogen peroxide 20% + Acetic acid 10% (0.8%) at 50 °C	No	88.0	cd	4.8	cd	7.3	a
Peracetic acid 5% + Hydrogen peroxide 20% + Acetic acid 10% (0.8%) at 50 °C	Yes	90.5	bc	3.5	bc	6.0	cd
Peracetic acid 5% + Hydrogen peroxide 20% + Acetic acid 10% (1.6%)	No	86.3	d	8.3	e	5.5	bc
Peracetic acid 5% + Hydrogen peroxide 20% + Acetic acid 10% (1.6%)	Yes	90.3	bc	5.0	d	4.8	b
Sodium hypochlorite (0.5%)	No	81.5	e	12.3	e	6.3	bc
Sodium hypochlorite (0.5%)	Yes	87.8	cd	9.8	e	2.5	ab
Electrolyzed water (HClO)	No	94.0	ab	5.3	d	0.8	a
Electrolyzed water (HClO)	Yes	87.8	cd	10.3	e	2.0	ab

Data expressed as percentage of germinated seeds, percentage of dead seeds and percentage of abnormal seeds

*numbers followed by equal letters are not statistically significant (ANOVA and Tukey $P < 0.05\%$)

the treatment with PAAH at a final concentration of 1.6% produced a phytotoxic effect, showing a reduced percentage of germination of 86.3%. The seed disinfection with a 0.5% NaClO water solution significantly reduced the germination of the seed (81–88%), with an additional negative effect on the seed vigor, in terms of quantity of abnormal seeds recorded (12.3%). Rinsing with sterile water, in this case, showed a lower percentage of abnormal seed (9.8%). No negative effects on germination were observed (94–96% of germinated seeds) in the seeds treated with PAAH at the concentration of 0.8% at room temperature, while the disinfection with the solution heated at 50 °C reduced the germination rate of the seeds to 88%. Seeds disinfected with hydrogen peroxide (3%) for 15 min showed a better germinability, statistically similar to that recorded in the untreated control (95.5–97.5%).

Disinfection of cabbage seed under controlled conditions

All treatments provided a significant effect in reducing seed contamination with Xcc of lot n.720 (Fig. 3). Among the various chemical and physical treatments tested, hydrogen peroxide (3%) (30 min), electrolyzed water and PAAH at a concentration of 1.6% showed the best efficacy in containing the levels of contamination of the seed. No rinsing effect on the effectiveness of the treatment emerged. Seed treatment with a 0.8% water solution of PAAH at room temperature produced the same effectiveness as the disinfection with a 0.5% sodium hypochlorite water solution at room temperature, while the disinfection with the 0.8% water solution of PAAH heated at 50 °C significantly reduced the seed levels of contamination. The treatment of the seeds with hydrogen peroxide (3%) for 15 min was less effective than the treatment of the seeds for 30 min with the same disinfecting solution.

Seed disinfection procedure at the company facilities

Immersion of the seed bags in hydrogen peroxide (3%) for 30 min caused an increase of the temperature of the disinfecting bath and breaks of the seed coat. Therefore, the time of immersion was adjusted to 15 min. The optimal number of seeds necessary to obtain a complete drying of the samples is between 50 and 300 g. Larger quantities do not allow the correct drying of the seeds during the 2 h of permanence in the oven and involve the use of excessive quantities of hydrogen peroxide to disinfect the samples.

The treatment with hydrogen peroxide (3%) for 30 min produced a 100% reduction of the seed contamination in

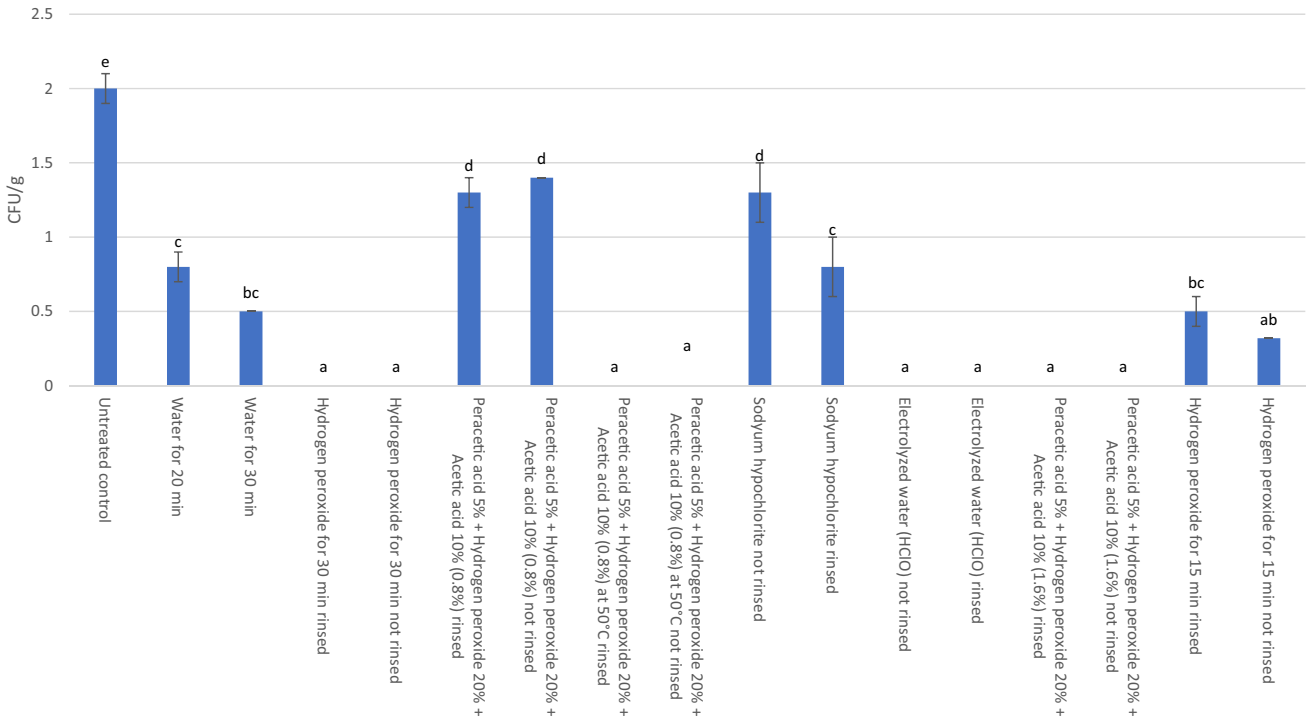

Fig. 3 Effect of different treatments on the presence of *Xanthomonas campestris* pv *campestris* in cabbage seeds. Data expressed as CFU / g (ANOVA and Tukey $P < 0.05\%$)

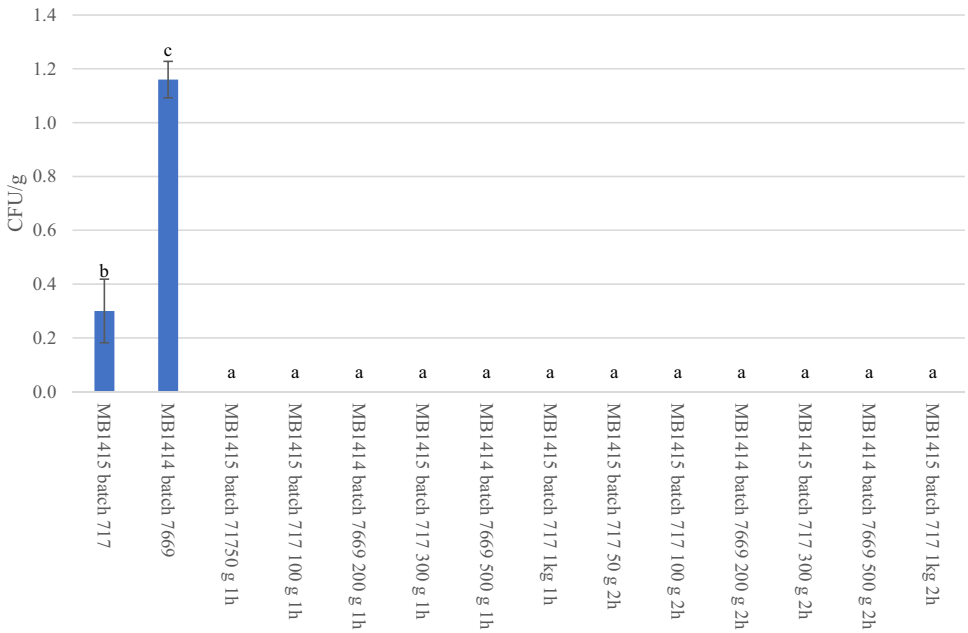

Fig. 4 Effect of the treatment with hydrogen peroxide for 30 min on different quantities of cabbage seeds (50 g, 100 g, 200 g, 300 g, 500 g, 1000 g) and dried at 37 °C for 1 or 2 h. Data expressed as CFU/g (ANOVA and Tukey $P < 0.05\%$)

each sample tested (Fig. 4). Significant differences in the percentage of germination of the seeds of the two batches were observed; a 90% decrease in the viability was recorded in lot 7669 (Table 3).

Discussion

The evaluation of different disinfection treatments was performed under controlled conditions with the application to seeds of physical and different commercial agents.

Table 3 Effect of the treatment with hydrogen peroxide on the germination of different quantities of cabbage seeds (50 g, 100 g, 200 g, 300 g, 500 g, 1000 g) dried at 37 °C for 1 or 2 h

Sample	Quantity (g)	Drying time (hours)	Percentage of seeds					
			Germinated		Abnormal		Dead	
MB1415–717	–	–	88.5	ab*	7.5	bcd	4.0	a
MB1415–717	50	1	87.5	abc	6.0	b	6.5	bc
MB1415–717	100	1	87.5	abc	8.5	cd	4.0	a
MB1415–717	300	1	87.0	abc	8.0	bcd	5.0	ab
MB1415–717	1000	1	86.5	abc	7.5	bcd	6.0	abc
MB1415–717	50	2	85.0	cd	7.0	bc	8.0	cd
MB1415–717	100	2	89.0	a	6.0	b	5.0	ab
MB1415–717	300	2	82.5	def	7.5	bcd	10.0	de
MB1415–717	1000	2	81.5	e	7.0	bc	11.5	e
MB 1414–7669	–	–	85.5	bcd	7.5	bcd	7.0	bc
MB 1414–7669	200	1	10.5	f	14.0	e	75.5	f
MB 1414–7669	500	1	9.0	f	9.5	d	81.5	g
MB 1414–7669	200	2	0.0	g	1.0	a	99.0	h
MB 1414–7669	500	2	1.0	g	0.0	a	99.0	h

Data expressed as a percentage of germinated seeds, a percentage of non-germinated seeds and a percentage of abnormal seeds

*numbers followed by equal letters are not statistically significant (ANOVA and Tukey $P < 0.05\%$)

The results clearly showed different degrees of effectiveness of the treatments in containing the level of surface contamination of the seed. The use of physical seed treatment methods, like hot water, are efficient in containing bacteria, such as several species belonging to the genera *Xanthomonas* and *Pseudomonas* on vegetable crops like cucumber, pumpkin, and tomato (Grondeau et al. 1994). However, in this study, treating the seeds in hot water for 20 and 30 min did not significantly reduce Xcc, as in the experiment by Carisse et al. (2000) in which lettuce seeds were treated for the containment of *X. campestris* pv. *vitians*. The effect of hot water treatment on germination is variable with the type of seed. In this case, thermotherapy did not produce negative effects on seed germination and vigour, like in the experiments carried out by Singh et al. (2016) where no adverse effects on brassica seeds were observed when treated with hot water (50 °C) for 25 min. The disadvantages of physical methods are underperformance compared with chemicals, failure to eradicate inoculum deep in seeds, and difficulties in the procedure needed to carry out the treatment (Aysan and Horuz 2016).

Treatment with a water solution of sodium hypochlorite has been reported as the best solution to contain the incidence of bacterial leaf spot on lettuce, caused by *X. campestris* pv. *vitians* (Carisse et al. 2000), but has not shown a significant reduction in the level of CFU / g of Xcc and also affect the germination rate of the seeds in our experiment. Hydrogen peroxide was used in seed coatings to promote seed germination and seedling health (Duval and Nesmith 2000). The treatment with this agent for 30 min completely reduce the contamination of the seeds, not affecting the percentage of germinated seeds, as seen for lettuce seeds where no bacteria were found after the treatment with hydrogen peroxide at 5% for 15 min (Pernezny et al. 2002).

Mixtures of acetic acid and hydrogen peroxide were previously tested for disinfection of mung bean from bacterial agents like *Escherichia coli*, *Listeria monocytogenes* and *Salmonella enterica* (Trząskowska et al. 2018). The use of these agents provided a 100% containment of the contamination with Xcc, especially when a 0.8% solution at 50 °C or a 1.6% solution at room temperature were used. However, contrary to what was observed in the experiment of Trząskowska et al. (2018), a significant reduction in germination rate was recorded in each treatment.

Currently there are no chemical seed dressing against bacteria, including Xcc, authorized in Italy. The use of an electrically charged disinfectant may be a solution. The experiment conducted by Sakudo et al. (2020) showed an inactivation of Xcc and a disinfection of the seeds, reducing the level of viable bacterial cells and decreasing the incidence of the disease, as well as in this study the treatment of the seeds with electrolyzed water produced a significant reduction of the contaminated seeds. Seed disinfection with CAC-717 did not seem to affect the germinability of the seeds (Sakudo et al. 2020), while the treatment with electrolyzed water caused a reduction of the viable seeds.

All these treatments were evaluated considering the effect of the rinsing of the seeds after the disinfection and in the majority of the cases no significant effects were observed when seeds were rinsed with sterile water or not.

The treatment with hydrogen peroxide for 30 min was the most effective, both in terms of disinfection rate and of

seed viability, but the side effect on the seed coat observed when the procedure was carried out at the company facilities brought to the adoption of 15 min as maximum time of immersion without losing disinfection efficiency. The reasons for this effect are not very clear, because it was not observed under laboratory conditions. The best method to contain the contamination with Xcc during the disinfection procedure at the company facilities, without compromising the germination of the seeds was the immersion of small amounts of seeds, closed in cotton bags, in a solution of hydrogen peroxide for 15 min at room temperature (25–27 °C), then dried for 2 h, confirming the results obtained under laboratory conditions.

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Availability of data and materials All data are included in the manuscript.

Declarations

Conflict of interest The authors declare no conflict of interest.

Consent of publication All authors declare consent of publication.

References

Alvarez AM (2000) Black rot of crucifers. In: Slusarenko AJ et al (eds) Mechanisms of resistance to plant diseases. Kluwer Academic Publications, Dordrecht, Netherlands, pp 21–52

Aysan Y, Horuz S (2016) Plant pathogenic bacteria control through seed application. In: Kannan VR, Bastas KK (eds) Sustainable approaches to controlling plant pathogenic bacteria. CRC Press, Boca Raton, pp 323–330

Bila J, Mortensen CN, Andresen M, Vicente JG, Wulff EG (2013) Xanthomonas campestris pv. campestris race 1 is the main causal agent of black rot of Brassicas in Southern Mozambique. Afr J Biotechnol. https://doi.org/10.5897/AJB12.2455

Carisse O, Ouimet A, Toussaint V, Philion V (2000) Evaluation of the effect of seed treatments, bactericides, and cultivars on bacterial leaf spot of lettuce caused by *Xanthomonas campestris* pv. *vitians*. Plant Dis 84:295–299. https://doi.org/10.1094/PDIS.2000.84.3.295

Duval JR, NeSmith DS (2000) Treatment with hydrogen peroxide and seedcoat removal or clipping improve germination of genesis triploid watermelon. HortScience 35:85–86. https://doi.org/10.21273/HORTSCI.35.1.85

FAOSTAT (2021) Food & agriculture organization of the United Nations statistic division. http://www.fao.org/faostat/en/#data/QC/visualize. Accessed 5 January 2022.

Grondeau C, Samson R, Sands DC (1994) A review of thermotherapy to free plant materials from pathogens, especially seeds from bacteria. Crit Rev Plant Sci 13:57–75. https://doi.org/10.1080/07352689409701908

ISTA (2019) 7–019A2 Detection of *Xanthomonas campestris* pv. *campestris* and pv. *raphani* on Brassica seed. Version 6.1. International Rules for Seed Testing. Chapter 7. Validated Seed Health Testing. Effective January 2021. 1–20.

Köhl J, van der Wolf JM (2005) Alternaria brassicicola and Xanthomonas campestris pv. campestris in organic seed production of Brassicae: epidemiology and seed infection (No. 363). Plant Research International, Wageningen, The Netherlands, pp 19–28

Köhl J, Vlaswinkel M, Groenenboom-de Haas BH, Kastelein P, van Hoof RA, van der Wolf JM, Krijger M (2011) Survival of pathogens of Brussels sprouts (*Brassica oleracea* Gemmifera Group) in crop residues. Plant Pathol 60:661–670. https://doi.org/10.1111/j.1365-3059.2010.02422.x

Nega E, Ulrich R, Werner S, Jahn M (2003) Hot water treatment of vegetable seed-an alternative seed treatment method to control seedborne pathogens in organic farming. J Plant Dis Prot 110:220–234

Pernezny K, Nagata R, Raid RN, Collins J, Carroll A (2002) Investigation of seed treatments for management of bacterial leaf spot of lettuce. Plant Dis 86:151–155. https://doi.org/10.1094/PDIS.2002.86.2.151

Sakudo A, Haritani M, Furusaki K, Onishi R, Onodera T (2020) Electrically charged disinfectant containing calcium hydrogen carbonate mesoscopic crystals as a potential measure to control Xanthomonas campestris pv. campestris on cabbage seeds. Microorganisms 8:1606. https://doi.org/10.3390/microorganisms8101606

Shigaki T, Nelson SC, Alvarez AM (2000) Symptomless spread of blight-inducing strains of *Xanthomonas campestris* pv. *campestris* on cabbage seedlings in misted seedbeds. Eur J Plant Pathol 106:339–346. https://doi.org/10.1023/A:1008771217477

Singh D, Rathaur PS, Vicente JG (2016) Characterization, genetic diversity and distribution of *Xanthomonas campestris* pv. *campestris* races causing black rot disease in cruciferous crops of India. Plant Pathol 65:1411–1418. https://doi.org/10.1111/ppa.12508

Trząskowska M, Dai Y, Delaquis P, Wang S (2018) Pathogen reduction on mung bean reduction of *Escherichia coli* O157: H7, *Salmonella enterica* and *Listeria monocytogenes* on mung bean using combined thermal and chemical treatments with acetic acid and hydrogen peroxide. Food Microbiol 76:62–68. https://doi.org/10.1016/j.fm.2018.04.008

Williams PH (1980) Black rot: a continuing threat to world crucifers. Plant Dis 64:736–742. https://doi.org/10.1094/PD-64-736

Wu YD, Chen LH, Wu XJ, Shang SQ, Lou JT, Du LZ, Zhao ZY (2008) Gram stain-specific-probe-based real-time PCR for diagnosis and discrimination of bacterial neonatal sepsis. J Clin Microbiol 46:2613–2619. https://doi.org/10.1128/JCM.02237-07