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CROP PROTECTION

Antibacterial activity of electrolyzed water on *Pseudomonas syringae* and *Clavibacter michiganensis* and its effect on seed germination

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ABSTRACT: Tomato plants are a crop of great economic importance worldwide. Mexico is considered the world's leading exporter of this vegetable, with a 24% market share. However, this crop can be affected by diseases such as bacterial freckle and bacterial cancer that can be introduced to plantations through infected seeds. Pesticides are used in agriculture for disease control and are considered a source of environmental pollution. Alternatives to the use of pesticides must therefore be sought. To this effect, electrolyzed water is a technology that has been shown to have antimicrobial activity. In this study, the antimicrobial activity of electrolyzed water on *Pseudomonas syringae* pv. tomato and *Clavibacter michiganensis subsp. michiganensis* was evaluated on cells suspension and tomato seed germination. Electrolyzed oxidizing water (EOW) and electrolyzed reduced water (ERW) treatments were applied for 1, 3 and 6 minutes. In addition, seeds were infected with each of the bacterial strains separately and electrolyzed water treatments were applied. Results show that oxidizing water is more efficient as an antimicrobial agent, as it achieved 100% growth reduction of the two bacterial strains after one minute of treatment. These treatments did not affect germination percentage, since germination percentages above 90% were obtained in all treatments and the seedlings were not affected in any of the evaluated variables.
Key words: electrolyzed oxidizing water, electrolyzed reduced water, free chlorine, antibacterial.

Atividade antibacteriana da água eletrolisada sobre *Pseudomonas syringae* e *Clavibacter michiganensis* e seu efeito na germinação de sementes

RESUMO: O tomate é uma colheita de grande importância econômica em todo o mundo. O México é considerado o primeiro exportador desta hortaliça no mundo, com 24% de participação. No entanto, esta cultura pode ser afetada por doenças como a mancha bacteriana e o cancro bacteriano que podem ser introduzidos nas colheitas através de sementes infectadas. Os agrotóxicos são utilizados na agricultura para controlar doenças e são considerados uma fonte de contaminação ambiental. Por esse motivo, é necessário buscar alternativas ao uso de agrotóxicos. Nesse sentido, a água eletrolisada é uma tecnologia que tem demonstrado atividade antimicrobiana. No presente estudo, a atividade antimicrobiana da água eletrolisada sobre Pseudomonas syringae pv. tomate e Clavibacter michiganensis subsp. michiganensis em células em suspensão e na germinação de sementes de tomate. Os tratamentos com água eletrolisada oxidante e redutora foram aplicados por 1, 3 e 6 minutos. Por outro lado, as sementes foram infectadas com cada uma das cepas bacterianas de forma independente e foram aplicados tratamentos com água eletrolisada. Os resultados mostram que a água oxidante é mais eficiente como agente antimicrobiano, pois conseguiu uma redução de 100% no crescimento das duas cepas bacterianas após um minuto de tratamento. Os tratamentos não afetaram a porcentagem de germinação, pois em todos os tratamentos obtiveram-se porcentagens de germinação acima de 90% e as plântulas não foram afetadas em nenhuma das variáveis estudadas.
Palavras-chave: Água eletrolisada oxidante, água eletrolisada redutora, cloro livre, antibacteriano.

INTRODUCTION

Tomatoes are a crop of great economic importance and one of the most consumed vegetables in the world. In 2020, 186,821,216 tons were produced worldwide. In the same year, Mexico produced 4,149,241 tons of this vegetable (FAOSTAT, 2021). Moreover, Mexico is considered the world's leading exporter, with a share of 24% (INTERNATIONAL TRADE CENTER, 2020). During cultivation, this crop is affected by various diseases that affect quality and yield. Diseases affecting this crop include bacterial freckling caused by *Pseudomonas syringae* pv. tomato (PEŇÁZOVÁ et al., 2020) and bacterial cancer caused by *Clavibacter michiganensis* subsp. *michiganensis* (NANDI et al., 2018).

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When *Pseudomonas syringae* pv. *tomato* infects tomatoes, it can grow epiphytically and endophytically on plant foliage without causing disease symptoms (SANTAMARÍA-HERNANDO et al., 2019). Although, it is a weak epiphyte, it is a highly aggressive pathogen once inside host tissues. This disease causes necrotic lesions surrounded by chlorotic halos on leaves, stems and fruits. The disease is spread by contaminated tomato seeds and infected weeds in which the bacteria can survive within the root system (PEŇÁZOVÁ et al., 2020).

On the other hand, *Clavibacter michiganensis* subsp *Michiganensis* causes bacterial cancer, one of the most common diseases that affect tomatoes (NANDI et al., 2018). This is considered one of the main bacterial pathogens in tomato production worldwide. It enters the plant through stomata and spreads within the xylem vessels where it colonizes the entire plant and forms extensive biofilm-like structures (PEŇÁZOVÁ et al., 2020). This interferes with water transport thus leading to wilting during the early stages of infection (NANDI et al., 2018). Symptoms of the disease appear on aerial parts and include wilting of leaves and discoloration of vascular tissues (PEŇÁZOVÁ et al., 2020). If an infection occurs at a late stage of the plant's growth, the plants may survive and generate fruits that may show black spots with white halos, that are often referred to as "bird's-eye" spots (PEŇÁZOVÁ et al., 2020).

In agriculture, pesticides are used to control crop diseases as they reduce losses and improve crop yields. Agriculture is reported to be the largest pesticide consuming sector, using approximately 85% of the world's pesticide production (KIM et al., 2017). Excessive use and misuse have generated negative consequences on the environment and on human health (ABHILASH & SINGH, 2009). Because of this, it has become necessary to look for new control alternatives that are more environmentally friendly.

Electrolyzed water has proven to be a feasible technology that has shown antimicrobial activity (RAHMAN et al., 2016). This is produced by electrolysis of dilute sodium chloride (NaCl) solutions in an electrolysis cell, consisting of two electrodes, one positive (anode) and one negative (cathode), divided by a diaphragmatic membrane (RAHMAN et al., 2016; AL-QADIRI et al., 2016). In the anode terminal, the following is generated: hypochlorous acid (HOCl), hypochlorite ion (OCl^-), hydrochloric acid (HCl), oxygen (O_2) and chlorine gas (Cl_2) (RAHMAN et al., 2016; SHIROODI & OVISSIPOUR, 2018). In the cathode terminal is produced sodium hydroxide (NaOH) and hydrogen (H_2) (SHIROODI & OVISSIPOUR, 2018). At the end of the electrolysis process, an acidic solution known as acidic electrolyzed water or electrolyzed oxidizing water is obtained in the anode terminal and an alkaline solution known as alkaline electrolyzed water or electrolyzed reduced water is obtained in the cathode terminal (OVISSIPOUR et al., 2015; SHIROODI & OVISSIPOUR, 2018).

Therefore, this research evaluated the antimicrobial activity of electrolyzed water in decreasing the populations of *Pseudomonas syringae* pv. *tomato* and *Clavibacter michiganensis* subsp. michiganensis on suspension cells and their impact on tomato seed germination.

MATERIALS AND METHODS

Bacterial strains and growth conditions

The experiment was carried out at the Integral Laboratory on Food Science Research of the Technological Institute of Tepic during the year 2020. The strains used were: *Pseudomonas syringae* pv. tomato and *Clavibacter michiganensis* subsp. *michiganensis*. The *Pseudomonas syringae* pv. *tomato* (*PstDC3000*) was obtained from the Technological Institute of Tepic and the *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*) from the National Institute for Research on Forests, Agriculture and Livestock (INIFAP) in Celaya. Strain *PstDC3000* was reactivated on King's B (KB) solid medium and *Cmm* was reactivated on Nutrient Broth Yeast (NBY) solid medium. Strains were incubated for 48 hours at 28 °C in the case of *PstDC3000* and for 96 hours at 27 °C in the case of the *Cmm*.

Production of electrolyzed water

Electrolyzed oxidizing water (EOW) and electrolyzed reduced water (ERW) were obtained using a Leveluk SD501 model No. TYH-401 equipment. Once the water was obtained, the free chlorine concentration and pH for each of the two types of water were determined. The pH was measured using a HORIBA LAQUA PC1100 Benchtop Meter; redox potencial (oxidation / reduction potential, E_{redox}) was determined using a HANNA HI98121 ORP tester, and the free chlorine concentration (FCC) by means of a HANNA HI771 colorimeter checker.

Obtaining the bacterial suspension and the application of treatments

Obtaining the bacterial suspension and the application of the treatments were carried out according to the protocol described by OVISSIPOUR

et al. (2015) under certain modifications. For which 50 mL of King Broth (KB) culture medium were inoculated with the bacterial strain PstDC3000 and incubated at 28 °C for 24 h to obtain a pre-inoculum. From the pre-inoculum, 50 mL of KB medium were inoculated at $D.O._{600}$= 0.05 and incubated at 28 °C for 24 h at 180 rpm. In the case of Cmm, 50 mL of NBY culture medium were inoculated, incubated at 27 °C for 24 h at 180 rpm to obtain the pre-inoculum. From the pre-inoculum, 50 mL of NBY medium were inoculated at $D.O._{600}$ =0.05 and incubated for 24 h at 180 rpm at a temperature of 27 °C. After the incubation time of the bacterial strains, 10 mL of the culture medium were taken and centrifuged at 22 °C at 7000 rpm for 15 min. The supernatant was decanted and the cell pellet was suspended in 10 mL of sterile deionized water. It was centrifuged at 22 °C and 7000 rpm for 15 min. The supernatant was discarded and the cell pellet was resuspended in 10 mL of sterile deionized water. For the application of the treatments, 2 mL of the bacterial suspension were placed in a 50 mL Falcon tube and 38 mL of electrolyzed water (oxidizing or reducing) were added for 1, 3, and 6 min at room temperature. As a positive control, the bacterial suspension was treated with a 2% sodium hypochlorite (NaOCl) solution and distilled water was used as a negative control. At the end of each of the treatment times, 1 mL of the treated suspension was taken and 1 mL of 0.85% NaCl solution and 1 mL of 3% sodium thiosulfate solution were added to neutralize free chlorine. In the case of the control with distilled water, only 1 mL of the 0.85% NaCl solution was added. After the application of the treatments, 100 µL of the treated suspensions were taken and then serial dilutions were performed. Aliquots of 100 µL of each dilution were taken and inoculated into Petri dishes containing solid KB medium (peptone 2 %, $K_2HPO_4 \cdot 3H_2O$ 0.15 %, $MgSO_4 \cdot 7H_2O$ 0.15 %, agar 1.5 %) for the *PstDC300* and nutrient agar for the *Cmm* (six replicates for each treatment). Inoculated containers with *PstDC3000* were incubated at 28 °C for 48 hours and those with *Cmm* were incubated at 27 °C for 96 hours. At the end of the incubation time, the colonies were counted and the colony forming units per milliliter (CFU/mL) were calculated.

Electrolyzed water treatments on tomato seed

For the application of the treatments, firstly, the seed was obtained from fresh tomato fruits of the saladette variety. Fruits were cut in half and then seeds were recovered. Subsequently, the seeds were placed on a wire mesh and rubbed gently so that the pulp would pass through the mesh and the seed was retained. Seeds were washed with sterile distilled water and placed on sheets of paper in a laminar flow hood for drying. Once seeds were dry, a manual selection was made to remove smaller or damaged seeds.

After selection, the seeds were inoculated with either a bacterial suspension of *PstDC3000* or with one of *Cmm* at a concentration of 10^8 CFU/mL. Subsequently, the seeds were treated by washing with electrolyzed water (oxidizing or reduced) for which 10 mL of electrolyzed water were added for 8 minutes in tubes of 50 mL containing the infected seeds in accordance to the protocol described by RODRIGUEZ et al. (2019). As a positive control, a wash was performed with a 2% solution of sodium hypochlorite, then three washes were performed with sterile distilled water (10 mL for 8 minutes for each wash). Seeds were dried in a laminar flow hood and placed in sterile tubes until sowing.

The seeds used were separated into groups of 30 seeds. The groups consisted of seeds treated with EOW, seeds treated with ERW, seeds treated with NaOCl at 2%, seeds with the pathogen that received no treatment, and finally seeds that were not infected with any of the pathogens and that did not receive any treatment. Seeds were placed in seedbeds containing peat moss as substrate for germination. The germination percentage (GP) of the seeds was determined by using the following equation:

$$GP = \frac{number\ of\ seeds\ germinated}{total\ number\ of\ seeds\ planted} * 100$$

In order to determine whether the treatments applied had an influence on seed germination, the germination speed was evaluated. Tomato seeds were placed in 200-cavity seedbeds and peat moss was used as a substrate. The speed of emergence (SE) was evaluated during a period of 14 days and emerged seedlings were considered to be those that presented both cotyledons above the substrate surface. The speed of emergence was obtained as the square root of the germination percentage for each of the days evaluated.

Statistical analysis

Data analysis with respect to cell suspension treatments, germination percentage and emergence speed was performed by ANOVA with an α=0.05, followed by a Tukey test for comparison of means between treatments using GraphPad Prism 7 software. A completely randomized block design with 3 replications was used for germination percentage and emergence speed. All measured variables were analyzed using SAS software version 9.

RESULTS AND DISCUSSION

In this study, the effect of oxidizing and alkaline electrolyzed water on the viability of the bacteria *Pseudomonas syringae* pv. *tomato* DC3000 and *Clavibacter michiganensis* subp. *michiganensis* was evaluated. The properties of EOW, ERW, distilled water (DW) and NaOCl solution at 2% used during the experimentation showed statistical differences in pH, FCC, and E_{redox} (Table 1).

Results showed that EOW and the NaOCl at 2% solution have completely eliminated *PstDC3000* and *Cmm* in a treatment period of 1 minute (Figure 1a, 1b), with no significant statistical differences between these treatments ($P < 0.05$). Conversely, ERW managed to reduce $2.72X10^7$, $4.30X10^7$ and $4.45X10^7$ CFU/mL in 1, 3 and 6 minutes, respectively ($P < 0.05$), in comparison to the DW control for *PstDC3000* (Figure 1a). In the case of *Cmm* cells treated with reduced water, a decrease of $3.83X10^7$, $3.80X10^7$ and $5.03X10^7$ CFU/ mL was observed in 1, 3 and 6 minutes, respectively ($P < 0.05$), with respect to the distilled water control (Figure 1b).

Some studies have shown a decrease in the number of microorganisms when oxidizing or reduced electrolyzed water has been applied to cells in suspension. OVISSIPOUR et al. (2015) applied electrolyzed reduced water (E_{redox} from -715 to -840 mV; pH = 10.47 - 11.1) and oxidizing (pH 3.1-3.55, FCC 10-20 mg/ L and E_{redox} 950-1150 mV) to cells in suspension of *E. coli O104:H4*, *Listeria monocytogenes*, *Campilobacter jejuni*, *Aeromonas hydrophila* and *Vibrio parahaemolyticus*, reporting a 100% reduction of all strains, other than *E. coli*, with the oxidizing water treatment within a two-minute treatment time, however, this reduction in *E. coli* populations was achieved after 4 minutes of treatment. In the presente research, a 100% reduction of *PstDC3000* and *Cmm* was achieved following a one-minute treatment with oxidizing water (pH=2.4 ± 0.13, E_{redox}= 836 ± 1.73 mV and FCC=18 ± 0.58 ppm). It has been suggested that oxidizing water can steal electrons, making the cell membrane unstable (OVISSIPOUR et al., 2015). Oxidative stress caused by the E_{redox} of oxidizing electrolyzed water causes the formation of disulfide bridges due to the oxidation of cysteine residues present in proteins, thus modifying their structure and function (LIAO et al., 2007). These same authors comment that high potential values can damage the bacterial cell membrane causing the release of cytoplasmic components to the outside of the cell. The oxidation reduction potential value of the oxidizing electrolyzed water used in this research was of 836 ± 1.73 mV, a value slightly above the optimal value (+200 to +800 mV) for bacterial growth (RAHMAN et al., 2016; SHIROODI & OVISSIPOUR, 2018). In addition, it has been reported that E_{redox} values of +800 to +1150 mV can damage the bacterial cell membrane of *E. coli* O157:H7 (LIAO et al., 2007), affecting its growth. In the present study, it has been observed that the application of oxidizing (E_{redox}= 836 ± 1.73 mV) and reduced (E_{redox}= -738.33 ± 0.58 mV) water treatments decreases the number of bacteria, suggesting that E_{redox} is a factor that may influence bacterial growth.

Another factor influencing the bactericidal effect is also the pH. Bacteria growth has been reported to occur in a pH range between 4-9 (SHIROODI & OVISSIPOUR, 2018). Therefore, when its value is low (pH < 4) it can affect bacterial growth, making bacterial cells more sensitive to active chlorine (LYU et al., 2018). The oxidizing electrolyzed water used in the present study had pH=2.4, suggesting that pH had an influence on reducing the number of bacteria in *PstDC3000* and *Cmm* in comparison with the reduced electrolyzed water treatment of pH=10.2, treatment with oxidizing water was more effective on both bacterial strains.

As for the free chlorine species present in oxidizing water, such as hypochlorous acid (HOCl) and hypochlorite ion (OCl^-), they can react with biological molecules such as proteins, amino acids, peptides, lipids and nucleic acids (FUKUZAKI, 2006). OCl^- carries out an oxidizing action causing the rupture and disintegration of the microbial cell membrane causing the release of cytoplasmic components (FUKUZAKI, 2006; ZENG et al., 2010). OCl^- inactivates functional proteins located in the membrane (RAHMAN et al., 2016) by interacting with thiol groups and peptide bonds causing modifications in the structure that affect the protein functionality; in addition, changes in the redox state

Table 1 - Properties (pH, $E_{redox}$and FCC) of the applied treatment solutions.

Solution	pH	*FCC (ppm)	E_{redox} (mV)
**DW	8.8 ± 0.15	0 ± 0.00	311.67 ± 0.58
**ERW	10.2 ± 0.26	0 ± 0.00	-738.33 ± 0.58
**EOW	2.4 ± 0.13	18 ± 0.58	836.00 ± 1.73
**NaOCl (2%)	12.8 + 0.05	334 ± 2.52	479.33 ± 2.52

*FCC = free chlorine concentration.
**DW = distilled water, ERW = electrolyzed reduced water, EOW = electrolyzed oxidizing water, NaOCl = sodium hypochlorite.

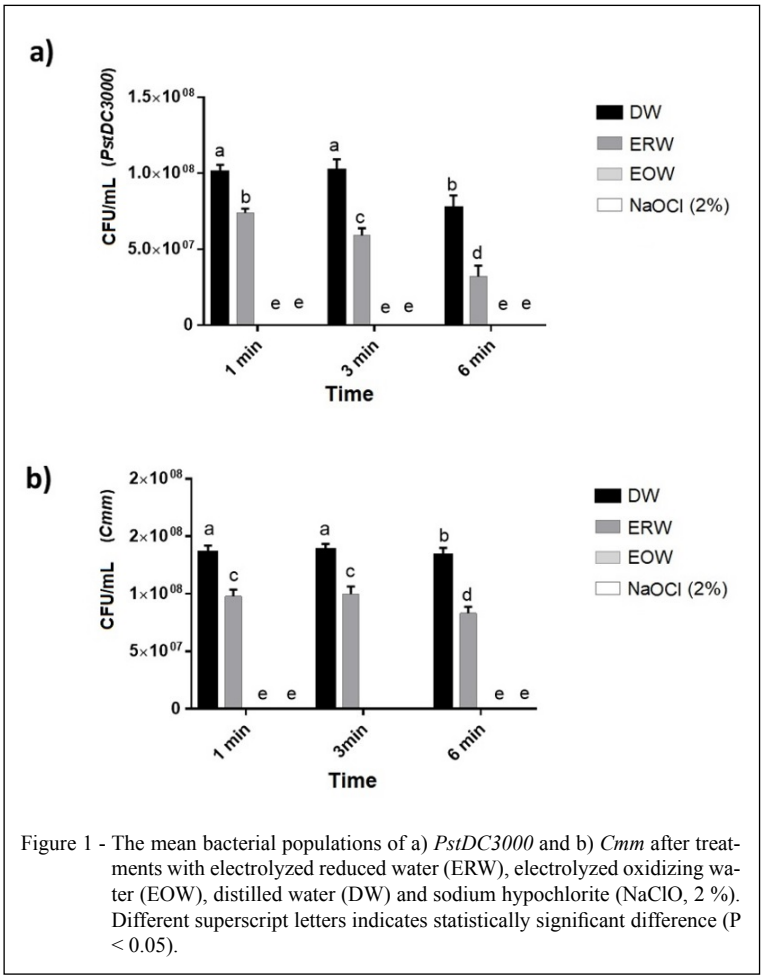

Figure 1 - The mean bacterial populations of a) *PstDC3000* and b) *Cmm* after treatments with electrolyzed reduced water (ERW), electrolyzed oxidizing water (EOW), distilled water (DW) and sodium hypochlorite (NaClO, 2 %). Different superscript letters indicates statistically significant difference ($P < 0.05$).

in thiol groups can be generated, thus affecting the transport capacity across the membrane (FUKUZAKI, 2006). Moreover, HOCl can migrate into the interior of the cell by passive diffusion (SHIROODI & OVISSIPOUR, 2018) across the membrane causing inhibition of enzyme activity by the oxidation of the -SH groups present in the active site. (FUKUZAKI, 2006). This oxidative modification of amino acid residues not only affects enzyme activation but also affects the signal transduction cascade. HOCl can also cause damage to DNA by interacting with nucleic acids, mainly by the chlorination of nitrogenous bases, causing the dissociation of the double helix due to the loss of hydrogen bonds (SHIROODI & OVISSIPOUR, 2018).

In the case of cells that are treated with reduced water, it has been suggested that the type of bacteria affects the action of the reduced water to decrease the number of bacterial populations (OVISSIPOUR et al., 2015). In the present investigation, it has been observed that in the case of *PstDC3000*, the treatment time with reduced electrolyzed water affected the reduction of microorganisms, obtaining the maximum reduction at 6 minutes ($4.45X10^7$ CFU/ mL). No significant statistical differences were observed between the 1- and 3-minute treatment times when using *Cmm* cells. However, significant statistical differences were observed during the 6-minute treatment with respect to the cells treated during 1 and 3 minutes, achieving a decrease of $5.03X10^7$ CFU/ mL. Some studies have reported that the bactericidal effect of reduced water is due to its E_{redox}, which allows it to reduce bacterial free radicals and alter the metabolic flux and the production of adenosine triphosphate (ATP) (RAMÍREZ-OREJEL & CANO-BUENDÍA, 2020). In addition, the NaOH present in the reduced water causes the saponification of fats and can also react with proteins, which destabilizes or dissolves the extracellular polymeric substances surrounding the bacterial cells (OVISSIPOUR et al., 2015).

Moreover, QUAN et al. (2010) have reported a reduction of *Vibrio vulnificus* and *Vibrio parahaemolyticus* of 2.2 $\log_{10}$ CFU/ mL during 60 s when the cells were treated with NaOCl and a complete inactivation of 7 $\log_{10}$ CFU/ mL was obtained with the electrolyzed water treatment during 30 s, showing that electrolyzed water is more efficient than NaOCl at equivalent chlorine concentrations since it is able to reduce bacterial populations completely and in a shorter period of time. In the present study, a 100% reduction of the *PstDC3000* and *Cmm* strains was obtained with treatments of NaOCl at 2% and EOW. The FCC of EOW and NaOCl at 2% were of 18 ppm and 334 ppm, respectively, suggesting that the antibacterial efficiency of the NaOCl solution is attributed to the FCC present in the solution. Therefore, it can be concluded that oxidizing electrolyzed water is a good alternative to the use of NaOCl because the same effect can be obtained with a lower FCC.

The germination percentage of treated seeds is shown in figure 2 (a and b). None of the treatments applied to infected seeds with the *Cmm* strain affected tomato seed germination, since the percentages obtained were above 90% (Figure 2a) and no significant statistical differences were found among the different treatments ($P < 0.05$). The highest germination percentage (100%) was obtained in untreated seeds and the lowest germination percentage (94.44%) was in the seeds treated with NaOCl at 2%. However, in treatments applied to *PstDC3000*-infected seeds (Figure 2b), significant statistical differences were obtained between the EOW treatments (100%) and untreated seeds (100%) compared to the ERW treatment (93.33%) ($P < 0.05$).

It has been reported that seeds infested with *Clavibacter* and *Pseudomonas* can germinate successfully without affecting the germination percentage (DUTTA et al., 2014). In the present study, it was observed that infection by pathogenic bacteria does not affect the germination of tomato seeds ($P < 0.05$), since a percentage of 100% was obtained in seeds infected with *Cmm* or *PstDC3000*. A similar effect was observed in a study by BIEMOND et al. (2013) on seeds infected with *Pseudomonas syringae*, the authors reported a higher number of germinated cowpea seeds compared to seeds infected with other pathogens such as *M. Phaseolicola* and *F. oxysporum*. In addition, studies show that bacteria on the seed surface can infect emerging seedlings during germination (STÜWE & TIEDEMANN, 2013) and show no visible symptoms of the disease. XU et al. (2010) They concluded that *Cmm* can be reported on the surface of tomato seeds and not affect seed germination, however, it can enter the seedling at the time of radicle emergence and invade the hypocotyl, cotyledons and radicle itself. According to the above, it is suggested that, although *PstDC3000* and *Cmm* did not affect seed germination, the bacteria may be present in the seedlings and then exhibit symptoms at a subsequent growth stage.

Related studies showed that the application of different seed treatments such as essential oils, ozone in scarified seeds or citric acid do not affect the germination percentage of seeds; on the contrary, they promote and may even accelerate germination (MONROY-VÁZQUEZ et al., 2016). These results are in accordance with the results obtained on seeds treated with ERW, EOW or NaOCl at 2%, since no negative effect on germination was observed.

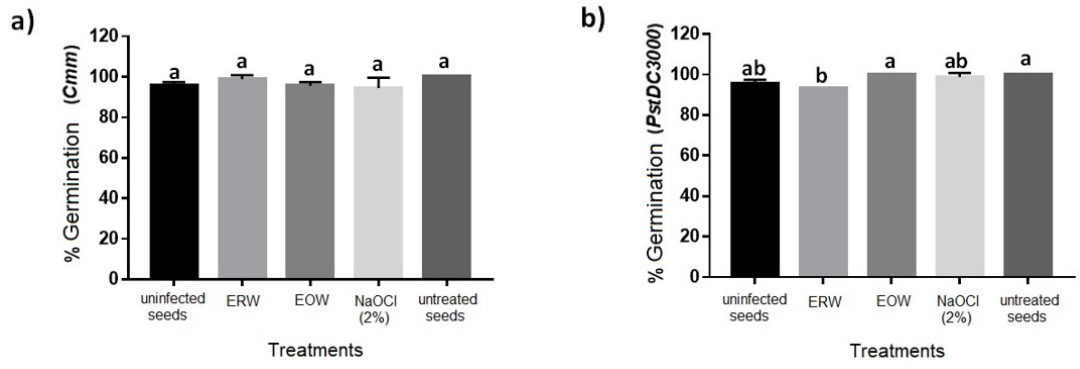

Figure 2 - Germination percentage of seeds treated with electrolyzed reduced water (ERW), electrolyzed oxidizing water (EOW), sodium hypochlorite (NaClO, 2 %), untreated seeds and uninfected seeds. Different superscript letters indicates statistically significant difference ($P < 0.05$).

In addition to the above, ERW, EOW, NaOCl at 2% and pathogenic bacteria had a positive effect on the germination rate of tomato seeds (Figure 3). Seedling emergence speed in both *Cmm*-infected (Figure 3a) and *PstDC3000*-infected (Figure 3b) seeds was obtained during day 6 in seeds treated with ERW, EOW, NaOCl at 2% and untreated seeds, finding no significant statistical differences between these treatments ($P < 0.05$). In addition, significant statistical differences ($P > 0.05$) were observed between the above treatments compared to uninfected seeds starting at day 6.

Maximum emergence speed was achieved during the seventh day in seeds infected with *Cmm* and *PstDC3000* that were treated with ERW, EOW, NaOCl at 2% and in untreated seeds however, the maximum germination rate in uninfected seeds occurred during the 10th day. The seeds contain a layer of mucilage in their structure, which has a protective function on them and can have an effect as a negative regulator of germination by preventing the transfer of oxygen to the interior of the seeds (VIUDES et al., 2020). In addition to the mucilage layer, seeds have a protective coat called testa that restricts germination by being impermeable to water and/or oxygen, therefore, these layers present on the seeds could have influenced the non-infected seeds to germinate three days later than the treated seeds. As for the treated seeds, it is inferred that they could have germinated early due to the damage or the removal of the mucilage layer during the application of the treatments, since it has been reported that the mucilage layer can be eliminated under acidic conditions by hydrolysis or under alkaline conditions by deesterification of polysaccharides (LIU et al., 2021), thus indicating that the oxidizing water, reduced water and the NaOCl at 2% solution, due to its acidity or alkalinity, could have damaged or eliminated the mucilage layer in the tomato seeds, allowing the entry of water and oxygen inside the seed. In addition, it has been reported that washing with water alone can remove the outer layer of mucilage (LIU et al., 2021), suggesting that in infected seeds that did not receive any treatment, the mucilage may have been affected because the seeds went through a washing process prior to infection and a period of immersion in bacterial suspension.

Conversely, EOW and NaOCl treatments could exert an effect not only on the mucilage layer, but also on the testa or coat whose function is to protect the seed from the attack of pathogens and herbivores (SHALIMU et al., 2016). In this sense, it has been reported that seeds with testa alteration have a shorter dormancy and that oxidative stress may play a crucial role in breaking the dormancy, since oxidative species can cause protein modification through carbonylation, thus contributing to the mobilization of energy reserves and to the synthesis of proteins required for germination. In addition, oxidizing species cause lipid peroxidation (AHAMMED et al., 2018) allowing them to increase the permeability of the testa, which can activate cell signaling pathways that improve seed germination (SUDHAKAR et al., 2011). According to the previously stated, the treatments could have altered the testa of the seeds, making it more permeable, thus allowing the entry of water and oxygen into the seed, causing dormancy breakdown and seed germination.

CONCLUSION

This study showed that oxidizing electrolyzed water and electrolyzed reduced water have an antibacterial effect, which makes electrolyzed water a good alternative instead of using NaOCl, since the same effect (100% reduction) was

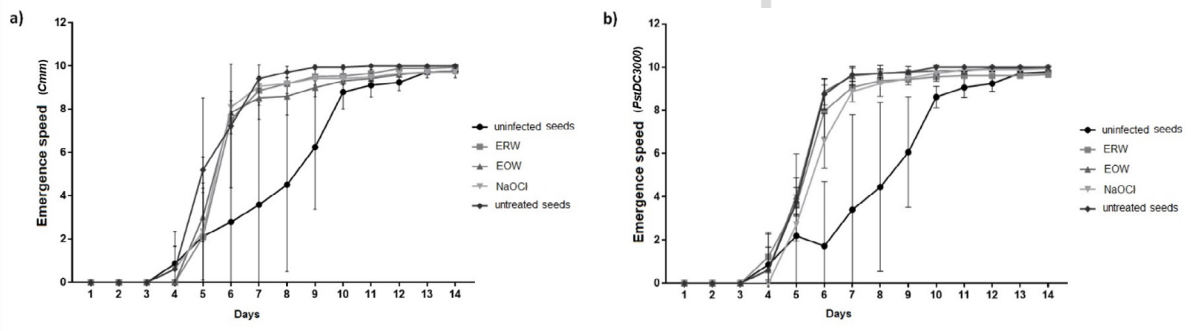

Figure 3 - Behavior of emergence speed in tomato seeds infected with a) *Cmm* and b) *PstDC3000* and treated with electrolyzed reduced water (ERW), electrolyzed oxidizing water (EOW) and sodium hypochlorite (NaClO, 2 %).

obtained with lower concentrations of free chlorine. Conversely, the electrolyzed reduced water showed a lower antibacterial effect than that achieved with the oxidizing water, being necessary to extend the treatment time to achieve a higher reduction of microorganisms. Electrolyzed water treatments do not affect the germination process of tomato seeds; it helps to breakdown seed dormancy and improves seed emergence speed.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest in this work. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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