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Research note

Effect of electrolyzed water on *Pseudomonas syringae* pv. Tomato under *in vitro* conditions

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Abstract

Pseudomonas syringae pv. Tomato causes bacterial speck in tomato crops, causing stunted growth, late fruit ripening, and yield losses. Pesticides are used to control this phytopathogen, which have been reported to be a source of soil contamination. Because the use of pesticides has increased and, with it, the negative impact, it is necessary to look for efficient and environmentally friendly alternatives. Electrolyzed water is an alternative to the use of pesticides as it has been shown to have an antimicrobial effect. It is produced by electrolysis of dilute solutions of NaCl in an electrolysis cell. The present work evaluated the effect of acidic and alkaline electrolyzed water on PstDC3000 cultures. The experiment was conducted in the agrobiotechnology and electrochemistry laboratory of the Technological Institute of Tepic in 2020. Treatments with acidic and alkaline electrolyzed water were applied for 1, 3, and 6 minutes. The results show total inhibition of PstDC3000 with acidic electrolyzed water treatment at minute 01. On the other hand, treatment with acidic electrolyzed water managed to reduce 26.9%, 42.16%, and 58.53% CFU ml^{-1} at minutes 1, 3, and 6, respectively. Finally, it was concluded that electrolyzed water affects the growth of PstDC3000 and that pH is a factor that influences its bactericidal activity.

Keywords:

acidic water, alkaline water, antibacterial.

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Pseudomonas syringae pv. Tomato (PstDC3000) infects tomato plants and *Arabidopsis thaliana* (Zhao *et al*., 2003; Cunnac *et al*., 2009). In tomato crops, it causes the bacterial speck disease. Although it is a weak epiphyte, it is a highly aggressive pathogen once it is found within host tissues (Xin and He, 2013). This bacterium survives epiphytically in leaves, can be found in plant remains in the soil and dried seeds, and becomes activated during germination (Beuzón and Ruiz, 2014). The bacterium enters the plant through stomata or wounds, multiplying endophytically and asymptomatically before developing symptoms (Preston, 2000).

In addition, PstDC3000 can cause stunted growth, late fruit ripening, and yield losses, mainly in infected young plants (Preston, 2000). Because of the damage caused by the bacteria, controlling the disease it causes is of utmost importance. Pesticides are used in agriculture to control diseases caused by phytopathogens (SAGARPA, 2011), which have been reported to be a source of soil contamination and a cause of soil degradation (Sanaullah *et al*., 2020).

Because the use of pesticides has increased and with it, the negative impact on ecosystems (Abhilash and Singh, 2009) and human health (Gomiero, 2018), it is necessary to look for efficient and environmentally friendly alternatives. Electrolyzed water is an alternative to pesticide use that has been shown to have an antimicrobial effect on bacteria and fungi (Rahman *et al*., 2016). It is produced by the electrolysis of dilute NaCl solutions in an electrolysis cell consisting of two electrodes (anode and cathode) and divided by a diaphragmatic membrane (Fujiwara *et al*., 2009; Ovissipour *et al*., 2015).

At the end of the electrolysis process, one obtains acidic electrolyzed water in the anodic part (Pangloli and Hung, 2011) with a pH of 2-3 and a free chlorine concentration of 10-90 ppm (Hao *et al*., 2011) and alkaline electrolyzed water in the cathodic part (Shiroodi and Ovissipour, 2018) with a pH of 10-13 (Huang *et al*., 2008; Hao *et al*., 2011). This work aimed to evaluate the effect of acidic and alkaline electrolyzed water on the reduction of bacterial populations of PstDC3000 under *in vitro* conditions.

The experiment was conducted from August to December 2020 in the Agrobiotechnology and Electrochemistry Laboratory of the Technological Institute of Tepic.

Obtaining electrolyzed water

This study used acidic electrolyzed water and alkaline electrolyzed water, which were generated by LeveLuck SD501 TYH-401 equipment. The pH of the two types of electrolyzed water obtained was determined with a Laqua-PC 1 100 potentiometer from Horiba Advanced Techno Co., Ltd., and the free chlorine concentration (FCC) was determined using a Hanna HI771 colorimeter.

Bacterial strain and obtaining the bacterial suspension

The strain of *Pseudomonas syringae* pv. Tomato PstDC3000 used in this study was obtained from the Technological Institute of Tepic. The strain was reactivated in KB solid medium and incubated at 28 °C for 24 h using a Benchmark Incu-Shaker 10 LR incubator. To obtain the bacterial suspension, 50 ml of KB liquid culture medium was inoculated and incubated at 28 °C for 24 h at 180 rpm to obtain a pre-inoculum. From the pre-inoculum, 50 ml of KB medium was inoculated at a DO_{600}= 0.05 and incubated at a temperature of 28 °C at 180 rpm for 24 h.

At the end of the incubation time, 10 ml of the culture medium was taken and centrifuged at 22 °C at 7 000 rpm for 15 min using a Sigma 2-16 KL centrifuge. The supernatant was decanted, and the cell pellet was resuspended in 10 ml of sterile deionized water. It was centrifuged at 22 °C at 7 000 rpm for 15 min. Finally, the cell pellet was resuspended in 10 ml of sterile deionized water.

Treatments with acidic and alkaline electrolyzed water

For the application of the treatments, the protocol suggested by Ovissipour *et al*. (2015) was used, with some modifications. The treatments applied were acidic electrolyzed water and alkaline

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electrolyzed water and sterile distilled water was used as a control. First, 2 ml of the bacterial suspension was placed in a 50 ml Falcon tube, and 38 ml of electrolyzed water was added for 1, 3, and 6 min at room temperature.

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 a 3% sodium thiosulfate solution were added. Regarding the control, only 1 ml of 0.85% NaCl was added and homogenized. One hundred microliters of the treated suspensions were taken, and serial dilutions ($10°$-10^6) were performed. Subsequently, 100 µl aliquots were taken from each dilution, and Petri dishes containing KB solid medium were inoculated and incubated at 28 °C for 48 h (each of the treatment times was performed in triplicate).

Once the incubation time was over, plate counting was carried out, and the colony-forming units per milliliter (CFU ml) were calculated. For data analysis, an Anova was performed with an α= 0.05, followed by a Tukey test to compare means between treatments using the GraphPad Prism 7 software.

Treatments with electrolyzed water were performed using acidic electrolyzed water (AEW) with a pH= 2.4 and a free chlorine concentration (FCC) of 18 ±0.58 ppm and alkaline electrolyzed water (BEW) with a pH= 10.2 and an FCC= 0 ±0 ppm. Sterile distilled water (pH= 8.8 and FCC= 0 ±0) was used as a control. The results of the surviving cells of PstDC3000 after the application of the treatments are shown in (Figure 1).

Figure 1. Surviving cells of PstDC3000 treated with acidic electrolyzed water (AEA= AEW) and alkaline electrolyzed water (BEA= BEW).

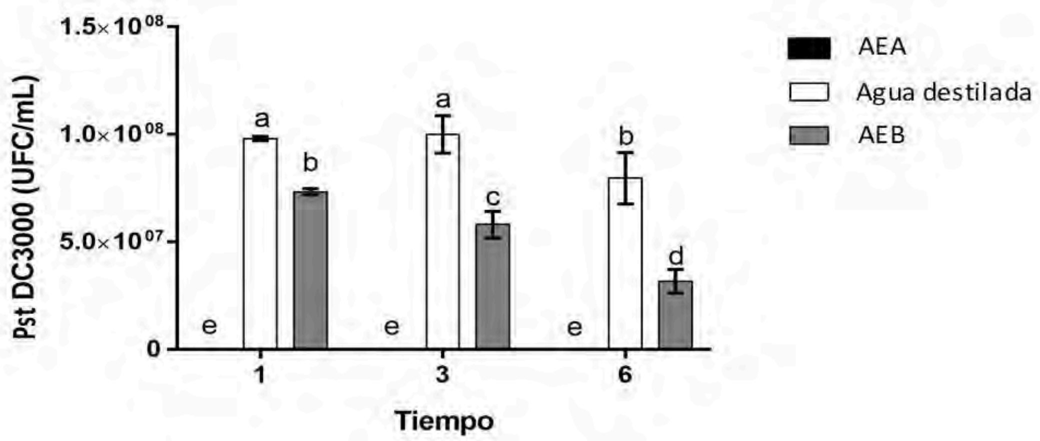

Figure 1 shows that there are statistically significant differences between treatments (p< 0.05). By applying AEW treatment, a 100% reduction of PstDC3000 populations was achieved in a treatment time of 1 min, compared to sterile distilled water control (9.8×10^7 CFU ml^{-1}); in addition, as observed, there were no statistically significant differences between the different AEW treatment times (p> 0.05).

In a study conducted by Al-Qadiri *et al*. (2016), when applying electrolyzed water with an FCC= 60 and 120 ppm, they reported that, by increasing the treatment time from 1 to 5 min and the concentration of free chlorine in the electrolyzed water, the reduction in the number of microorganisms increases. This effect was not observed in the present study since, when AEW with an FCC= 18 ±0.58 ppm was applied, a 100% reduction was achieved.

Nevertheless, this effect was observed when applying BEW (FCC= 0 ppm) since, at minute 1, a bacterial reduction of 26.9% was achieved compared to the control, and this reduction of microorganisms increased with the treatment time to 58.53%, observing statistically significant differences between the different BEW treatment times (p< 0.05).

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Authors such as Hao *et al*. (2016) reported that AEW (FCC= 60.43 ±0.34 ppm) and slightly acidic electrolyzed water (FCC= 25.27 ±0.38 ppm) may have equivalent antibacterial efficiency; however, when diluted, the efficiency of the AEW is lower compared to slightly acidic electrolyzed water, suggesting that the concentration of free chlorine is not the main factor contributing to the antibacterial activity of electrolyzed water.

In this sense, and according to the results obtained, it is suggested that chlorine concentration is not the main factor in the reduction of bacterial populations since it was possible to reduce bacterial populations with the two types of water. On the other hand, it was observed that the treatment time when applying AEW is not a factor that influences bactericidal activity, but it did influence with the BEW treatment, which suggests that pH is the factor that most influenced since, when applying AEW (pH= 2.4), a 100% inhibition of bacterial populations was achieved compared to BEW (pH= 10.2) and control treatment (pH= 8.8).

Effect of pH on the bactericidal efficiency of electrolyzed water

To determine whether the pH of acidic or alkaline electrolyzed water exerts any effect on the ability of electrolyzed water to reduce the number of microorganisms, the Pareto diagram and the response surface were generated by evaluating the variables of time and pH of electrolyzed water. The Figure 2a presents the Pareto diagram showing that both the pH of the electrolyzed water and the treatment time influence the growth of PstDC3000 ($p < 0.05$) and that the pH factor is the factor that has the most effect.

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Figure 2. a) Pareto diagram of the effect of the variables of time, pH, or type of water, and their interactions and b) growth response surface of PstDC3000.

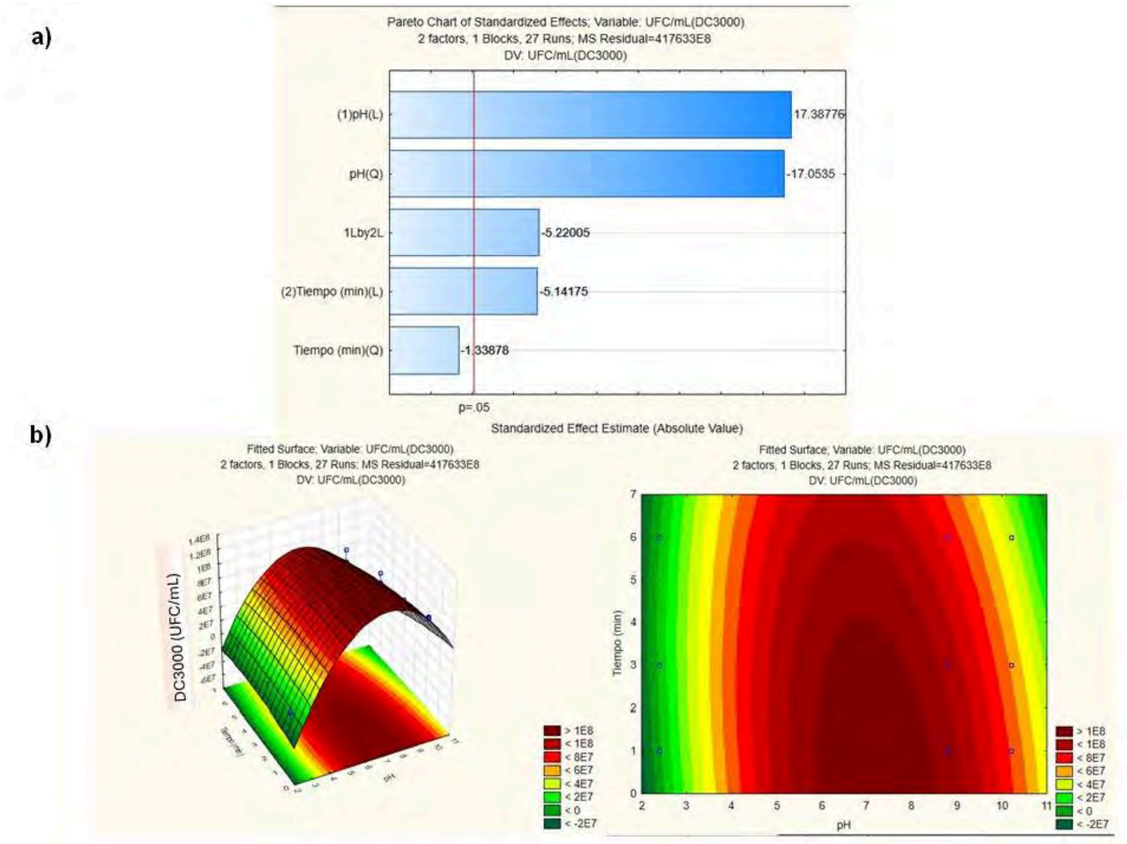

Figure 2b presents the response surface, which shows that at a pH value of 2.4, corresponding to AEW, it is possible to eliminate the PstDC3000 populations completely in one minute of treatment and, at a pH value of 10.2, a reduction of 26.9%, 42.16%, and 58.53% CFU ml^{-1} is achieved in a treatment time of 1, 3, and 6 min, respectively.

Research by Sun *et al*. (2012) reported a 95% reduction in *S. aureus* populations in a treatment time of 2 min when AEW with a pH of 2.5 was applied. A similar effect was observed in the present study, where a 100% reduction was observed when AEW with a pH= 2.4 was applied in a treatment time of 1 min. Nonetheless, the authors report that BEW is less efficient at reducing the number of bacteria.

In the present study, BEW was observed to be less efficient than AEW; however, with a treatment time of 5 min, it was reduced by up to 58.53%. On the other hand, in a study conducted by Issa-Zacharia *et al*. (2010), they reported a greater reduction in *E. coli*, *Salmonella* spp., and *S. aureus* when applying AEW treatments (pH= 2.6) compared to slightly acidic water with a pH of 5.6 and a NaClO solution with a pH of 9.1.

Rahman *et al*. (2016) results report that bacteria grow in a pH range of 4-9. They also comment that the antimicrobial activity of electrolyzed water depends on its pH, that this value determines the chlorine species available in it, and that a low pH reduces microbial growth because it makes bacterial cells more sensitive to active chlorine.

In the present study, an AEW treatment with a pH of 2.4 was applied, and a greater reduction was obtained compared to the BEW treatment (pH= 10.2) and the control (pH= 8.8), suggesting that low pH (below 4) affects bacterial growth. In addition, the pH, together with the concentrations of free chlorine present in AEW, could have exerted a synergistic effect in the reduction of bacterial populations.

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Conclusions

Acidic and alkaline electrolyzed water possess antibacterial activity as the two types of water affected the growth of PstDC3000. Nevertheless, although BEW was shown to have an antimicrobial effect, it was lower than that observed with AEW, so it is necessary to increase the treatment time to reduce the number of microorganisms. On the other hand, pH and treatment time factors contributed to bacterial reduction, with pH being the factor that most influenced its antimicrobial activity.

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