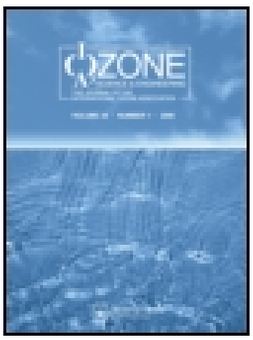

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Tomato Crop Improvement Using Ozone Disinfection of Irrigation Water

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ABSTRACT

Plant–parasitic nematodes diminish the yield of tomato crops worldwide. Irrigation water may be contaminated with such parasites, including *Meloidogyne enterolobii*. In this study, ozone treatment was applied to inactivate *M. enterolobii* eggs. Eggs were rendered inviable in water after exposure to 0.55 $mgO_3\ L^{-1}$ ozone residual for 15 min. Additionally, the effect of temperature, alkalinity, and organic matter of the water were evaluated. The results showed that ozonation is an effective and promising alternative method for nematode control in irrigation water, which could reduce tomato crop infestation caused by plant–parasitic nematodes.

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KEYWORDS
Ozone; disinfection; irrigation water; nematodes inactivation; *Meloidogyne enterolobii*; plant–parasite nematodes

Introduction

Data from the Food and Agriculture Organization of the United Nations (FAO) show that tomato (*Lycopersicon esculentum*) is the second largest crop in the world after potato. Currently, annual global production is about 177 million tons of fresh fruit from 4.7 million ha farmed. Mexico, The Netherlands, and Spain are the world's largest fresh tomato exporters. These three countries account for about half of the world's exports of fresh tomatoes and are responsible for 46% of the 7,845,698 tons shipped in 2016 (FAO 2016). Mexico ships most of its fresh tomatoes to the United States, The Netherlands annually sends around a million tons to Germany, and Spain exports mainly to Germany. In Mexico, the tomato sector occupies fifth position in terms of quantity among all agricultural products, surpassing the production of corn, sugar cane, avocado, and chili peppers (FIRA, 2017).

A number of different factors affect agricultural production, and according to the American Phytopathological Society plant–parasitic nematodes account for 14% of annual losses of different crops across the world, including tomatoes. These are microscopic roundworms that inhabit the soil and infect plants through their roots. Another pathway of dissemination of nematodes is through irrigation systems (Hugo and Malan 2010). Contaminated irrigation water becomes a means of transporting eggs, juvenile nematodes, and even infested roots.

Root-knot nematodes (*Meloidogyne* spp.) head the list of nematodes based on their economic effects (Jones et al. 2013). The most important species are *M. arenaria, M. incognita, M. javanica*, and *M. hapla* (Moens, Pery, and Starr 2009). *Meloidogyne* spp. affect crops that are usually high-cost products on the market, such as in tomato, watermelon, and chili peppers, and therefore any decreases in productivity would lead to significant economic losses (Velásquez Valle 2001).

These nematodes are obligate plant parasites and get their name because their feeding causes galls (root-knots) to develop on the roots of their host plants. Root-knots restrict the passage of water and nutrients to the plants, affecting their development and causing general weakening and dehydration (Roberts, Matthews, and Ehlers 2005). Reduced yield is manifested in terms of changes in quantity and quality.

In Mexico, over the last 10 horticultural cycles, root-knots have occurred in various commercial tomato hybrids. In particular, over the 2012–2013 cycle, damage caused by *M. enterolobii* was observed under shade mesh in the indeterminate hybrid tomato cv Ramses, a crop which has high resistance to *M. incognita, M. javanica*, and *M. arenaria* (Gallardo et al. 2015). Subsequently, it has not been possible to control *M. enterolobii* efficiently.

The use of nematicides is the conventional control method for *Meloidogyne* spp. in soil. However, plant–parasitic nematodes are not only found in the rhizosphere soil around plant roots, they may also be dispersed by irrigation water. All of the economically significant plant–

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parasitic nematode genera have been reported in irrigation water sources (Hugo and Malan 2010).

Ozone (O_3) is a promising alternative to nematicides due to its strong oxidizing properties (Msayleb and Ibrahim 2011; Nahed et al. 2017). Over recent years, it has been shown that ozone may increase the shelf life of fruits and vegetables as a result of its oxidative potential. It destroys vital components of the cells of pathogenic microorganisms (unsaturated fatty acids, amino acids, peptides, and proteins), in addition to damaging their nucleic acids, affecting their metabolic and reproductive capacity (Han et al. 2002).

Research on the application of ozone for the management of plant–parasitic nematodes in irrigation water is limited. Moens, Moermans, and Hendrickx (1991) examined the sensitivity of *M. incognita* second-stage juveniles (J2) to ozone treatment for different ozonation times. They found that it was possible to inhibit the potential for infection in tomato plants by 4 min of ozone oxidation, but complete inhibition was only achieved after a treatment time of 12 min. Some other species of nematodes have greater resistance, such as the burrowing nematode *Radopholus similis*, which requires 60 min of ozonation for its complete elimination (Runia and Amsing 1996).

In another study, Sanchez et al. (2007) investigated ozone inactivation in synthetic water and irrigation water contaminated with different species of nematodes (*Caenorhabditis elegans, Aphelenchoides fragariae, Heterodera schachtii*, and *Meloidogyne* spp.). In synthetic water *CT* values [mg-min L^{-1}, the product of ozone residual concentration (*C*) in mgO_3 L^{-1} and contact time (*T*) in minutes] required to inactivate 90% of the nematodes ranged from 2 to 8 mg-min L^{-1}, regardless of the species. Inactivation in irrigation water demanded higher *CT* values (up to 12 mg-min L^{-1}), which is as expected if the increased ozone demand of the components present in the water is taken into account.

In the search for effective nematode control, "gas phase ozone" and direct crop irrigation with ozonized water have been applied directly to soil contaminated with nematodes (Behzadi et al., 2012; Msayleb and Ibrahim 2011; Nahed et al. 2017; Qiu, Westerdahl, and Pryor 2009).

Although many studies have corroborated the oxidative action of ozone and its excellent biocidal effect on some types of nematodes, the scientific literature does not provide sufficient information to evaluate the inactivating effect of ozonation on the root-knot nematode *M. enterolobii*. For this reason, the present study evaluated the application of ozone for the control and inactivation of this specie in irrigation water supplied to tomato crops, with the aim of contributing to the reduction of the economic losses suffered by tomato growers.

Materials and methods

Root-knot derived from tomato crop

In this study, the root-knot nematode *M. enterolobii* was investigated. It was previously isolated directly from tomato roots. Nematode eggs were preserved in a saline solution (0.85%, stock suspension) at 4°C. From this stock suspension, 10 mL of a new suspension was prepared, containing approximately 5 eggs for each 10 µL. The number of eggs in the samples was counted under a Zeiss optical microscope model Axio Lab.A1.

For the tests, eggs were inoculated into water with similar characteristics to irrigation water. Subsequently, the disinfection process was carried out in an ozonation unit. The treatments were evaluated by assessing the eggs' viability in terms of their structure, using optical microscopy. After ozone treatment, nematode eggs were recovered by a flotation concentration technique (NMX-AA-113-SCFI-1999 adapted). To evaluate the effect of ozone on nematode viability, the eggs were incubated in vitro at room temperature (25°C) for 5 days following the treatment.

Irrigation water

Disinfection experiments were carried out in distilled water with key chemical components added to simulate an agricultural irrigation water (Alrousan et al. 2009; Nghiem et al. 2004): calcium carbonate (40 mg L^{-1}, J. T. Baker) for alkalinity, humic acids (7.0 mg L^{-1}, Fulka Chemie) such as natural organic matter, sodium sulfate (24 mg L^{-1}, J. T. Baker), potassium nitrate (1.0 mg L^{-1}, Sigma-Aldrich), and sodium phosphate (2.0 mg $L^{-1,}$ J. T. Baker) as inorganic ions. The pH ranged between 8.0 and 8.5. The chemical analysis of "synthetic irrigation water" is given in Table 1 (APHA-AWWA-WPCF 1992).

Ozone treatment

The experimental equipment (Figure 1) was a batch reaction system. Ozone was produced from pure oxy-

Table 1. Chemical analysis of synthetic irrigation water.

Parameter	Concentration (mg L^{-1})
Chemical oxygen demand (COD)	17 ± 1.0
Alkalinity as $CaCO_3$	40 ± 1.5
Nitrate as N	0.8 ± 0.1
Total phosphate as P	2.07 ± 0.21
Total suspended solid (TSS)	25 ± 2.16

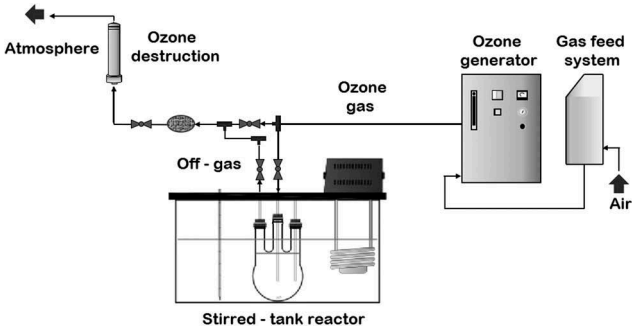

Figure 1. Lab-scale ozonation unit.

gen by an ozone generator (Labo 76, Emery Trailigaz, USA) with a production capacity of 19 gO_3 h^{-1} at a flow rate of 200 mL min^{-1}. Ozone was injected at the bottom of a stirred tank reactor (effective volume of 1.2 L) using a glass diffuser (10–15 µm pore sizes). The ozone was continuously bubbled into the batch reactor filled with 1 L synthetic irrigation water artificially infested with *M. enterolobii* eggs.

Gas-phase ozone concentrations were determined via the iodometric method (Birdsall et al., 1952) and dissolved ozone was measured using the indigo method (Bader and Hoigné, 1982).

The initial aim was to test variations of ozone residual (0.2, 0.4, and 1.6 mgO_3 L^{-1}), contact time (4, 10, and 15 min), and nematode eggs concentration (5, 10, and 20 Ne L^{-1}) on the inactivation rate of eggs. The reaction between nematode eggs and ozone was stopped by adding sodium thiosulfate. Subsequently, other variables were also analyzed: temperature (20°C and 35°C), organic matter (5 and 10 mg L^{-1}), and alkalinity (40 and 100 $mgCaCO_3$ L^{-1}).

The results obtained from the experimental work were subjected to statistical analysis using STATGRAPHICS Centurion software (StatPoint Technologies, Inc.) to assess the significance of variables involved in the inactivation of the nematode eggs.

Results and discussion

Effects of ozone on M. enterolobii

Viable nematode eggs had defined covers and their internal and external structures were intact (Figure 2). Nematode eggs subjected to ozone treatment underwent changes in their structure. Ozone oxidized and modified the eggs' cell walls, their membranes lost their integrity, and hence the potential viability of the nematode was lost. Nonviable eggs showed deformations and damage to the cell wall, fragmentation of the coat protein and disintegration when the damage was severe (Figure 3). The development of the eggs was interrupted by ozonation, and it was no longer possible for them to reach the infectious stage.

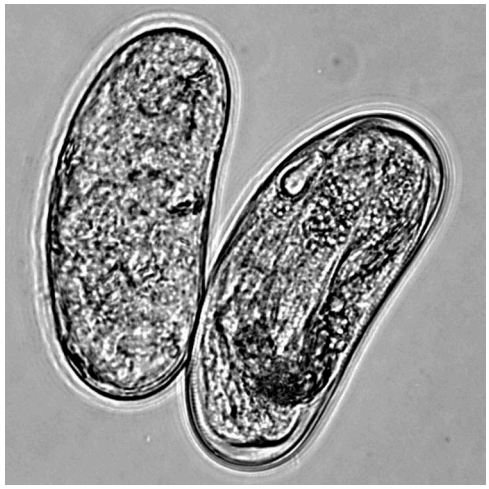

Figure 2. Viable eggs.

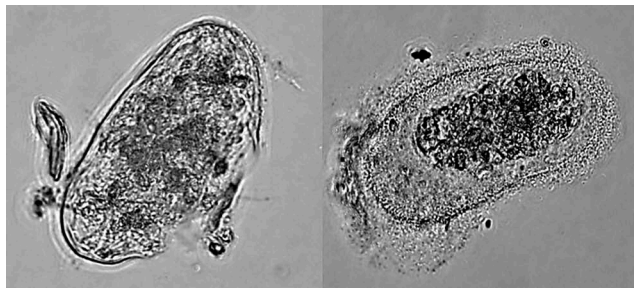

Figure 3. Nonviable eggs.

Disinfection conditions to inactivate the M. enterolobii *nematode in irrigation water*

According to the variance analysis, for F-distribution at a confidence interval of 0.95, ozone residual concentration (*C*), contact time (*T*), and the interaction *CT* seem to be relevant variables in the inactivation of nematode eggs. The concentration of nematode eggs was not statistically significant.

The results showed that viability was not affected at an ozone residual of 0.2 mgO_3 L^{-1} maintained for 4 min. Under the same ozone concentration but with contact time increased to 15 min, inactivation of almost 90% of nematode eggs was achieved (Figure 4). When the values of these variables were 0.55 mgO_3 L^{-1} and 10 min, more than 95% of the nematode population was inactivated (Figure 5). On the other hand, when the contact time was reduced, the ozone residual had to be 1.6 mgO_3 L^{-1} to reach a considerable level of inactivation. However, some nematode eggs still remained viable (Figure 6).

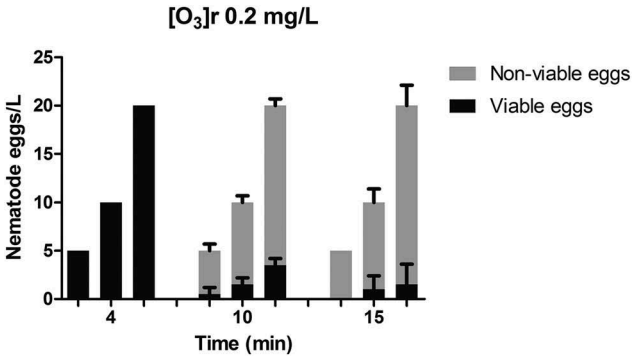

Figure 4. Nematode egg inactivation (ozone residual 0.2 $mgO_3\ L^{-1}$).

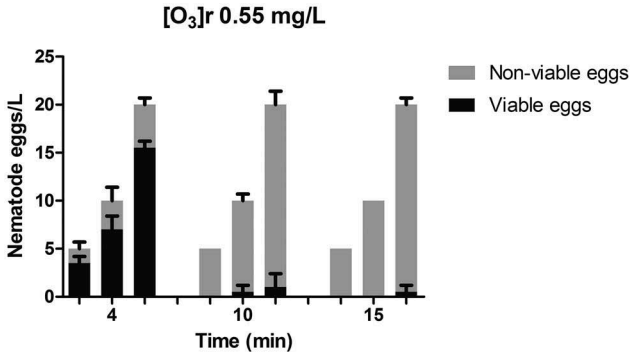

Figure 5. Nematode egg inactivation (ozone residual 0.55 $mgO_3\ L^{-1}$).

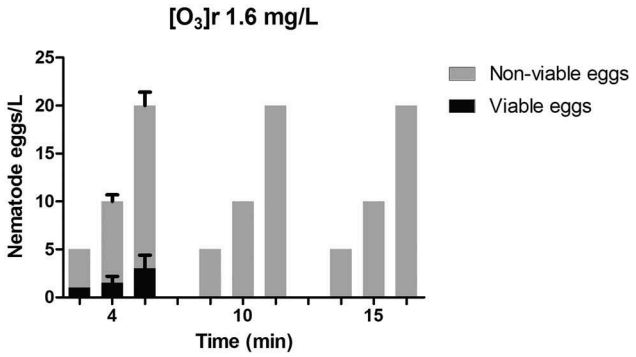

Figure 6. Nematode egg inactivation (ozone residual 1.6 $mgO_3\ L^{-1}$).

It can be concluded that the most relevant factor is the contact time. The optimal conditions to inactivate the total nematode population were with an ozone residual of 0.55 $mgO_3\ L^{-1}$ for 15 min of contact time. The resulting *CT* value is 8.25 mg-min L^{-1} for *M. enterolobii*. This requirement is similar to that required to achieve 99% inactivation of *Cryptosporidium* oocysts. The available data suggest that this microorganism is one of the most resistant to ozone (U.S. EPA, 1999). According to Langlais, Reckhow, and Brink (1991), the efficiency of the disinfection process depends on the specific microorganism as well as the contact time.

Effect of water composition and temperature on inactivation rate

In some areas of tomato cultivation in Mexico, surface water is the most important source for crop irrigation, so the quality and the presence of organic matter and other contaminants can affect the process of disinfection where ozone treatment is used. Similarly, the temperature of water is another factor that must be considered.

In this study, according to the variance analysis, under the conditions evaluated, the organic matter was not a factor that affected the inactivation rate. At high temperatures (35°C), the inactivation rate was reduced by 22.5%, while with an alkalinity content of 100 $mgCaCO_3\ L^{-1}$ a decrease in inactivation rate of 17.5% (in comparison with 40 $mgCaCO_3\ L^{-1}$) was recorded (Figure 7).

Ozone is only partially soluble in water, and, as is true for the majority of gases, its solubility increases as temperature decreases or the mixture is pressurized (Henry's Law). For this reason, a greater ozone dosage is required to maintain an ozone residual in the value of 0.55 $mgO_3\ L^{-1}$ as temperature increases in order to maintain the desired rate of inactivation.

However, although the *CT* value of 8.25 mg-min L^{-1} was maintained at 35°C, the disinfection percentages were seen to be slightly lower (Figure 8). This could be because at high temperatures the ozone desorption rate is greater than the oxidation rate of ozone on microorganisms. For this particular nematode, *M. enterolobii*, the disinfection rate was found to be temperature-dependent.

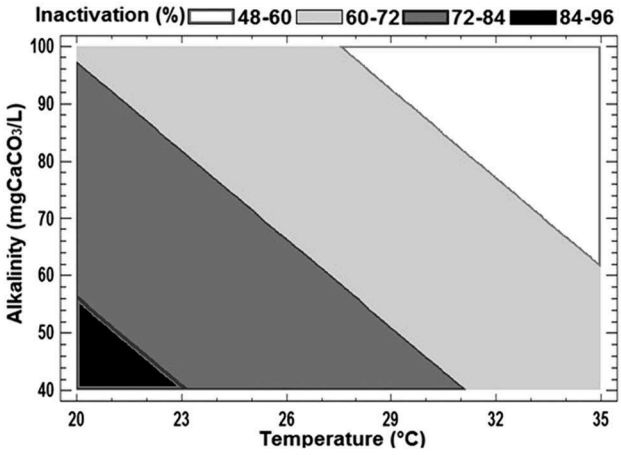

Figure 7. Contour plot of inactivation vs. temperature, alkalinity.

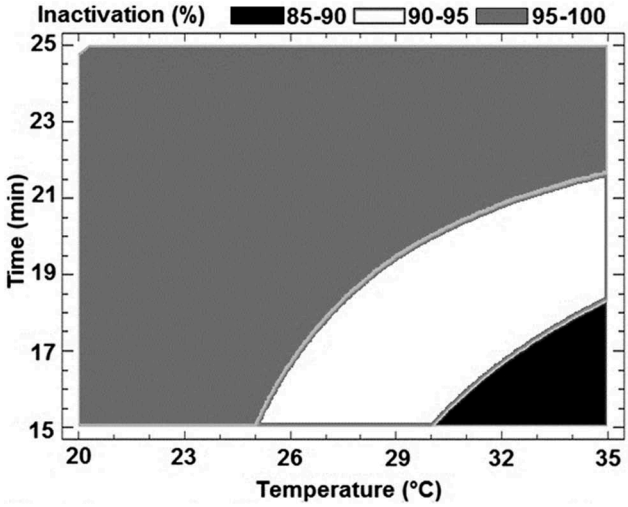

Figure 8. Contour plot of inactivation vs. temperature, time.

The current importance of *M. enterolobii* is due to its highly aggressive nature, increasing geographical distribution, and host range. The application of nematicides is not sufficiently effective when a clean water source is unavailable or nematodes cannot be prevented from entering the irrigation system. For this reason, water treatments should also be considered. A successful management strategy to counter the nematode problem should apply not only to tomato crop productivity, but also to that of other crops contaminated by *M. enterolobii*. This may be achieved by the simultaneous application of nematicides to soil and ozone to irrigation water as a method of disinfection.

Conclusion

Ozone is a promising alternative to increase the productivity of tomato crops affected by *M. enterolobii*. This oxidant is capable to damage the cell wall structure of the nematode eggs, affecting their integrity and their potential viability. Using the ozonation process, it is possible to inactivate *M. enterolobii* eggs in irrigation water with a *CT* value 8.25 mg-min L^{-1}, which is reflective of the high level of resistance of this microorganism. Increasing the ozone residual concentration does not allow a reduction in the contact time necessary to reach the same egg-inactivation rate since this latter depends on the ozone contact time. Temperature affects the inactivation rate when it reaches high values even if the *CT* value applied at 20°C is maintained. Ozonation could contribute to improving tomato crop productivity and reducing the agricultural losses caused by plant–parasitic nematodes.

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