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Nahed Msayleb
*Iowa State University*

Rameshwar S. Kanwar
*Iowa State University*, rskanwar@iastate.edu

Huaiqing Wu
*Iowa State University*, isuhwu@iastate.edu

Johannes van Leeuwen
*Iowa State University*, leeuwen@iastate.edu

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**Keywords**
Methyl bromide alternatives, Nematicide alternatives, Nematodes, Ozone, Soil ozonation, Sustainability

**Disciplines**
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# Soil Ozonation for Nematode Disinfestation as an Alternative to Methyl Bromide and Nematicides

***Nahed Msayleb[1], Ramesh Kanwar[1], Huaiqing Wu[2] and J (Hans) van Leeuwen[1,3,4]****

[1]*Department of Agricultural & Biosystems Engineering, Iowa State University, USA*

[2]*Department of Statistics, Iowa State University, USA*

[3]*Department of Civil, Construction & Environmental Engineering, Iowa State University, USA*

[4]*Department of Food Science and Human Nutrition, Iowa State University, USA*

## Abstract

Phytoparasitic nematodes are important pests that cause severe crop yield losses. In the past, methyl bromide and other proprietary nematicides have been used as management practices, but these practices are unsustainable and lead to atmospheric pollution and ozone layer destruction. Ozonation was studied as an alternative management practice since it is highly effective against microorganisms and degenerates quickly to oxygen. Soil samples that were naturally infested with nematodes were treated with different levels of gaseous ozone at 21 °C and 5 °C. Regression analysis results show that a medium level of ozonation (2.1 g $O_3$ $kg^{-1}$ for 15 min at a rate of ozonation 0.14 g $O_3$ $kg^{-1}$ $min^{-1}$) and low temperature (5 °C) resulted in 94% mean nematode inhibition. The data and analysis results imply that ozone may be an efficient and sustainable alternative to other practices.

## Keywords

Methyl bromide alternatives, Nematicide alternatives, Nematodes, Ozone, Soil ozonation, Sustainability

## Introduction

Plant-parasitic nematodes are microscopic, nonsegmented roundworm parasites that live in the soil and attack the plants through their roots. Endoparasitic nematodes infect and colonize the roots of plants (e.g. lance, root-lesion, and root-knot) while ectoparasitic nematodes remain outside of the root tissue (e.g. dagger, needle, spiral, sting, stubby root and stunt). Nematodes feed on the nutrients found in plant roots and vascular tissues, weakening the plant and leading to decreased yields. An international survey determined annual crop losses due to nematodes as follows: cotton, 10.7%; peanut, 12%; wheat, 7%; and soybean, 10.6% [1]. Nematodes can cause up to 75% yield loss in some crops, in addition to vectoring plant viruses and creating root wounds through which other pathogens can enter [2]. In 2000, global production losses to nematodes in all crops were estimated at US $121 billion, $9.1 billion of which in the U.S [3].

Currently, there are only a handful of chemicals registered for pre-plant nematode control [4,5]. The most important remaining nematicide, methyl bromide (MeBr), was the fourth most abundantly used pesticide in the U.S. in 1997 [6], but is now under phaseout due to its degradation of the stratospheric ozone layer. Approximately 25,000 to 27,000 metric tons of MeBr were still applied annually between 1990 and 1994 [7], with more than 75% of its use for pre-plant soil fumigation [8]. In 2013, only 562 metric tons of MeBr were allowed by the EPA as "critical use exemption", in compliance with the MeBr phaseout plan mandated by the Montreal Protocol [9] to protect the stratospheric ozone layer.

Ozone is a potent oxidant and it has been implemented successfully against numerous pathogens including vi-

***Corresponding author:** J (Hans) van Leeuwen, Department of Agricultural & Biosystems Engineering, Civil, Construction & Environmental Engineering, Food Science and Human Nutrition, Iowa State University, 476 Town Engineering, IA 50011-3232, Ames, USA, Tel: +1-515-294-5251, E-mail: leeuwen@iastate.edu

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ruses, bacteria, protozoa and also metazoa [10-12]. Ozone is often used to disinfect drinking water and waste water [13,14] and disinfest ships ballast water [15,16] due to its oxidizing properties.

In contrast to other disinfection methods and conventional pesticides used in the treatment of soil pests, such as soil fumigants MeBr, metam sodium and chloropicrin described above, the use of ozone as a disinfection method has the advantage that it does not produce pollutants, because its rapid decomposition produces oxygen only. The use of other nematicides is prohibited within 100 feet of drinking-water wells to protect groundwater from potential contamination [9], while ozone could be used safely near groundwater bodies.

Sopher, et al. [17,18] reported the successful use of gaseous ozone soil fumigation in increasing plant yield and reducing the detrimental effects of soil pathogens in a range of crops and soils under different climatic conditions. They reported positive effects of preplant ozone application, and theoretically attributed these effects to the decrease in soil pathogens and increased nutrient availability. However, they recommended further studies to confirm this theory and predict specific responses achieved from ozonation under different crops, soils, pathogens and climatic conditions. Nevertheless, to our knowledge, no further studies have been done in this regard.

The high oxidative power of ozone, its effectiveness in inhibiting pathogens without leaving toxic residues in the environment, and the limited research on ozone use in the domain of soil fumigation as alternative to nematicides inspired the current research. Furthermore, the economic importance of phytoparasitic nematodes, and the need for efficient and environmentally safe alternative treatments to the currently adopted fumigant nematicides, made treatment with ozone a realistic aim for further investigation. This study evaluated the effect of ozone on nematode viability in soil samples collected from a field in Iowa. The objectives were to evaluate (i) the effectiveness of different ozone doses and ozonation rates at reducing the viability of nematodes in the soil, and (ii) the efficacy of soil ozonation at low soil temperature (5 ˚C) versus high soil temperature (21˚C).

## Background

Phytoparasitic nematodes survive in the soil or in plant roots, and active nematode stages are more susceptible to nematicides than resting stages [19,20]. Most systemic nematicides are needed in high concentrations (e.g. 1000 ppm of Vydate) to control nematodes within plant roots, which is impractical under field conditions [19]. Hence, it is difficult to deliver a nematicide in efficiently sufficient concentration directly in contact with nematodes within plant roots and root surroundings. Total eradication of nematode populations with a nematicide or fumigant is difficult to achieve due to the heterogeneous nature of soil that offers protection to some individuals or ova [3]. However, management should be aimed at inhibiting or deactivating the number of phytoparasitic nematodes in the soil below their economic threshold. Most nematicides are broad-spectrum, highly volatile fumigants that are able to move through the soil pores. Many of the most efficient volatile nematicides have been deregistered (e.g. ethylene dibromide and dibromochloropropane) [3], because they were associated with environmental and human health risks. Ethylene dibromide was the most abundantly used nematicide in the world, until 1983 when it was prohibited in the U.S. because of groundwater contamination and possible carcinogenicity [21,22]. Similarly, 1,3-dichloropropane was prohibited because it was classified as a probable carcinogen [6] while 1,2-dibromo-3-chloropropane (DBCP) was suspended in the U.S. in the late 1980s because it was found to cause male infertility and was a probable carcinogen [23]. Carbamates used as nematicides (i.e. aldicarb, carbofuran and oxamyl) are highly toxic to humans and animals [6,24] and organophosphates (ethoprop, fenamiphos, cadusafos, fosthiazate and phorate) have been reviewed by the U.S. Environmental Protection Agency (EPA), and several were withdrawn from use [25]. Some nematicides, however, have recently undergone re-registration eligibility decisions (REDs) by the U.S. EPA [26]. These include metam sodium, which has limited efficiency in controlling nematodes in some circumstances [21,27,28], and the fumigants chloropicrin, metam-potassium, and dazomet.

Many commodities have become dependent on MeBr for nematode control, which necessitates identifying effective alternatives [29]. Zasada, et al. [30] believed that it would be too difficult to manage phytoparasitic nematodes without MeBr. Methyl bromide is an effective preplant soil fumigant used to control soil pests (weed seeds, nematodes, insects, fungi, bacteria and viruses) [31], in many high-input, high-value crops in U.S. agriculture, including vegetables, nursery plants, ornamentals, fruit trees, strawberries and grapes [30]. This broad-spectrum pest control, along with its higher efficacy compared to other fumigants [32], and its volatility that enables it to penetrate treated soil [4], has made some crop production systems highly MeBr-dependent, e.g. strawberries and fresh market tomatoes, and led to reductions in crop rotation and in diversification of production [33].

Ozone has also been applied in mold prevention on stored corn [34,35]. Scanning electron microscopy showed that ozone causes damage to the surface of the ova of *Toxocara canis*, a nematode parasite of dogs and other canides [36]. Ozone is also capable of diffusing across bacterial membranes and reacting with cytoplasmic biomolecules,

such as DNA, which results in cell death [37]. Furthermore, ozone reacts with biomolecules such as proteins, carbohydrates and polyunsaturated fatty acids bound to albumin, dyes, and is involved in lipid peroxidation [38,39].

Ozone has been approved by the U.S. Food and Drug Administration for direct use in human food, drugs, and cosmetics and also as compounds in food contact materials such as cutting boards and other surfaces that come in contact with unprotected food [40]. In addition, ozone is listed by the National Organic Program under the list of *"The National List of Allowed and Prohibited Substances"* with code (§205.605) referring to: *"Nonagricultural (nonorganic) substances allowed as ingredients in or on processed products labeled as organic or made with organic (specified ingredients or food group(s))"* [41].

## Methods and Materials

### Soil samples

Soil for this experiment was collected from the Hinds Farm (Iowa State University research farm, near Ames, Story County, Iowa). This soil belongs to the Clarion-NiCollett-Webster "principal association area", and Zenor soil series (Iowa Soil Properties and Interpretations Database-ISPAID). The soil was analyzed for texture and organic matter content and was found to contain 79% sand, 4.9% coarse silt, 4.7% fine silt and 10.4% clay. The soil had low organic matter content (1.4%) and low total carbon (0.7%). Accordingly, the soil texture is sandy loam, with fast draining rate, and low water retention and cation exchange capacity.

The species composition of nematodes present in the soil was determined by centrifugal flotation and species identification, with the aid of an inverted compound microscope, on four soil samples (100 g each). The soil contained an average of 225 non-plant parasitic nematodes, 2 spiral (*Helicotylenchus sp.*) and 0.5 ring (*Criconemoides sp.*) nematodes per 100 g soil. Non-plant parasitic species lack for feeding stylet, a mouth part necessary in plant parasitism. These nematodes belong to the group of free-living terrestrial nematodes, constituting 25% of all nematode species. Spiral nematode is one of the most common ectoparasites that occur in corn fields and floor of forests. Damage potential of spiral nematode is low, with a threshold of 500-1000 per 100 $cm^3$ soil. Ring nematode is an ectoparasite with a damage threshold of 100 per 100 $cm^3$ soil. Accordingly, both spiral and ring nematodes detected were well below damage thresholds.

### Ozone treatment of soil

Prior to ozone treatment, the soil was sieved and mixed well. Samples of 100 g were treated with incrementally larger ozone doses (low, medium and high; Table 1) by increasing the ozone generation time (Figure 1a), at a flow rate of 0.1 L/min. Each experiment consisted of five samples of 100 g each: three ozonated at the same dose, and two non-treated control samples. Doses of ozone applied ranged from 0.35 to 3.9 g $O_3$/kg soil. The effect of temperature on the efficacy of ozone to reduce the viability of nematodes was also tested. Two temperatures (5 ˚C and 21 ˚C) were tested for each ozone dose. For experiments at 5

**Table 1:** Experimental design with the number of samples and replicates at each level of ozone, dose, time of ozonation, rate of ozonation, and temperature.

| Temperature (ºC) | Level of ozonation | Dose (g $O_3$ $kg^{-1}$) | Time (min) | Rate (g $O_3$ $kg^{-1}$ $min^{-1}$) | Samples[a] × replicates |
|---|---|---|---|---|---|
| **5 (ºC)** | Low | 0.6 | 5 | 0.12 | 5 × 2 |
| | | 0.8 | 5 | 0.16 | 5 × 2 |
| | | 1 | 5 | 0.2 | 5 × 2 |
| | Medium | 1.4 | 7.5 | 0.19 | 5 × 2 |
| | | 1.9 | 7.5 | 0.25 | 5 × 2 |
| | | 2.1 | 10 | 0.21 | 5 × 2 |
| | High | 2.5 | 13 | 0.19 | 5 × 2 |
| | | 2.5 | 13 | 0.19 | 5 × 2 |
| | | 3 | 15 | 0.2 | 5 × 2 |
| | | 3.2 | 15 | 0.21 | 5 × 2 |
| **21 (ºC)** | Low | 0.4 | 1 | 0.4 | 5 × 2 |
| | | 0.4 | 1.5 | 0.27 | 5 × 2 |
| | | 0.6 | 2 | 0.3 | 5 × 2 |
| | | 0.7 | 2 | 0.35 | 5 × 2 |
| | Medium | 1.1 | 4 | 0.28 | 5 × 2 |
| | | 1.1 | 5 | 0.22 | 5 × 2 |
| | | 1.5 | 7.5 | 0.2 | 5 × 2 |
| | | 1.5 | 7.5 | 0.2 | 5 × 2 |
| | High | 2.2 | 10 | 0.22 | 5 × 2 |
| | | 3.5 | 15 | 0.23 | 5 × 2 |

[a]Each experiment consisted of five samples (100 g soil each): three ozonated and two controls. The experiment was repeated twice.

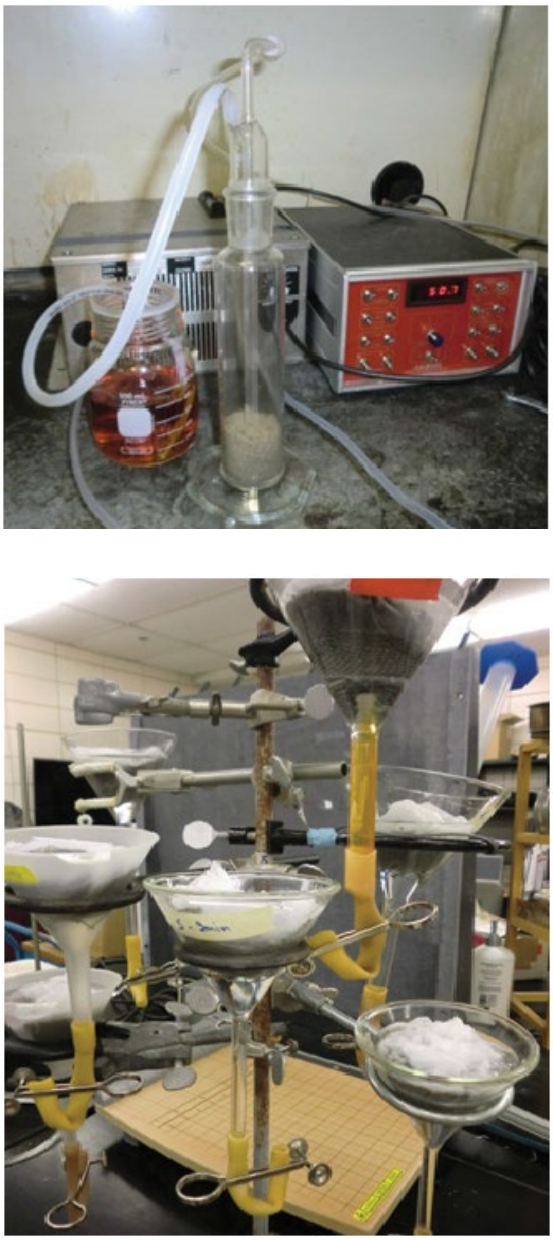


**Figure 1:** a) Ozone generator, reactor, and sample ozonation; b) Soaking of samples after ozonation in Baermann funnels for 24 h and 48 h and draw-off of the filtrate to collect viable nematodes for counting and assessment of treatment.

˚C, soil was kept in a refrigerator at 5 ˚C until the ozonation experiments. Increasing the level of ozonation was obtained by ozone from oxygen, while lower levels were generating ozone from air. After ozonation, the five samples were soaked in Baermann funnels (Figure 1b) [42] at room temperature. Since only viable nematodes migrate down through the soil sample, penetrate the filter and fall down into the distillate, nematode viability was easily determined by comparing nematode counts in the treated and untreated samples in the distillate after 24 h and 48 h. Nematodes were counted with the aid of an inverted compound microscope at x40 magnification. Viability was determined as the total number of nematodes in each treated sample divided by the average number of nematodes in the two control samples as a percentage. The experiment was repeated twice as shown in the experimental design (Table 1). As the experiments following the experimental design were carried out, it was found difficult to use the time of ozonation as a basis to achieve a certain ozone dose level. There were some fluctuations in the generated doses and some earlier experiments were repeated more times than others. Thus, the data from all of the experiments included some other experimental conditions not listed in Table 1, with a total of 106 obsevations (viability values as percentages).

## Ozonation

The ozone generator used was a 1000BT-12 Triogen Model TOG C2B, generating a maximum of 1 g $O_3$/h from pure oxygen by corona discharge. The reactor was made of glass (Figure 1a), and all tubing of silicone material. The operating volume in the reactor was 250 $cm^3$. In each test the ozone flow rate was maintained at 1 L $min^{-1}L^{-1}$ gas-flow/liter volume of soil sample [43]. The excess and unreacted ozone was captured in a solution of 2% potassium iodide (KI). The amount of absorbed ozone by the soil sample was measured by the iodometric wet-chemistry method [44]. Well-established, standardized methods for ozonation and ozone measurement were used [45].

## Data analysis

We analysed the data using multiple linear regression and R software (version 3.1.0, The R Foundation for Statistical Computing). The response variable is the percent nematode viability; the predictors are time (of ozonation), rate (of ozonation), dose, temperature (temp), time*temp, rate*temp, and dose*temp. Since dose = time*rate, only three of the predictors (time, rate, and temp) can vary their values independently; the other four are the interaction terms. We used leaps with Mallows's $C_p$ to select (among the $2^7 = 128$ models) the best model. One observation (1.3 g $O_3$ $kg^{-1}$ for 7.5 min at 5 ˚C) with the highest viability of 90% was removed as an outlier from the final analysis, because it had an unusually large residual of 72% (compared with the next largest residual of 30%). For the remaining 105 observations, leaps with Mallows $C_p$ gave the best model with an R-square = 0.364 and the F statistic = 11.34 (with the P-value = 1.16E-8) and the following estimated coefficients and their P-values:

| | Intercept | Time | Dose | Time* Temp | Rate* Temp | Dose* Temp |
|---|---|---|---|---|---|---|
| Estimate | 19.097 | -3.833 | 12.726 | 0.506 | 2.617 | -2.078 |
| P-value | 4.75E-4 | 0.0020 | 0.024 | 4.80E-6 | 0.0075 | 6.63E-5 |

We then used JMP software (Pro 12, The SAS Institute) to check the adequacy of the above model more

conveniently. The plot of residuals versus predicted values, the normal probability plot, and the lack of fit test (with F = 0.73 and the P-value = 0.82) indicate that the model is adequate.

## Results

The fitted regression model was used to obtain 95% confidence intervals for the mean percent nematode viability values at the experimental conditions listed in Table 1, and some additional selected experimental conditions used in the experiments. The results are given in Table 2. Based on the fitted model, we have the fitted equation for 21 °C as follows: viability = 19.097 - 1.301 time + 13.087 rate + 2.337 dose, and for 5 °C as follows: viability = 19.097 + 6.802 time + 54,967 rate - 30.910 dose.

As Table 2 shows, the estimated mean Nematode viability was between 6% to 7% at the ozone dose levels of 2.1 to 2.4 g $O_3$ $kg^{-1}$ for 15 min and at 5 ˚C, with the 95% upper confidence limits less than 16%, indicating that the viability of nematodes is reduced by 84% or more. A higher dose of ozone did not result in additional reduction in nematode viability. However, the reduction of nematode viability at low ozone dose level at 5 ˚C was somehow acceptable (more than 75% viability reduction as the upper confidence limits are less than 25%), and would be recommended in the case of plant parasitic nematode species with low virulence.

The table also shows that ozonation at 21 ˚C was less effective than at 5 ˚C. Nevertheless, the upper confidence limits for all the cases in Table 2 are still less than 45%, indicating that the viability of nematodes is reduced by 55% or more. At 21 ˚C, the dose levels of 2.2 to 2.4 g $O_3$ $kg^{-1}$ for 7.5 min gave the upper confidence limits less than 25%, indicating that the viability of nematodes is reduced by 75% or more.

It was noticed that the collected filtrate from treated samples was yellow in color (Figure 2), unlike that from untreated samples that was colorless.

Ozonated soil samples were analyzed for pH and the main oxidizable elements: P (Mehlich-3 extraction, showing P in its bioavailable form), Zn, Fe & Mn (analyses of the bioavailable forms by DTPA extraction method). Results did not show any correlation between ozonation dose (expressed in time of ozonation in min. and in dose in g.$kg^{-1}$ $O_3$ in the soil) and any of the analyzed parameters (Table 3).

## Discussion

The overall results of this study clearly indicate that

**Table 2:** Estimated mean percent nematode vaiability values and 95% confidence intervals for the experimental conditions in Table 1 and some additional selected experimental conditions in the experiments

| Temperature (ºC) | Level of ozonation | Dose (g $O_3kg^{-1}$) | Time (min) | Estimated viability | 95% confidence interval |
|---|---|---|---|---|---|
| 5 (°C) | Low | 0.6 | 5 | 15.6 | (9.3, 21.8) |
| | | 0.8 | 5 | 16.6 | (11.1, 22.0) |
| | | 1.0 | 5 | 17.5 | (12.5, 22.6) |
| | Medium | 1.4 | 7.5 | 15.1 | (11.1, 19.0) |
| | | 1.9 | 7.5 | 17.1 | (12.1, 22.1) |
| | | 2.1 | 10 | 13.7 | (9.7, 17.8) |
| | Medium to High | 1.9 | 15 | 5.7 | (0, 16.5) |
| | | 2.1 | 15 | 6.3 | (0, 15.9) |
| | | 2.2 | 15 | 6.6 | (0, 15.7) |
| | | 2.3 | 15 | 7.0 | (0, 15.6) |
| | | 2.4 | 15 | 7.3 | (0, 15.5) |
| | High | 2.5 | 13 | 10.5 | (5.1, 16.0) |
| | | 3.0 | 15 | 9.2 | (2.3, 16.2) |
| | | 3.2 | 15 | 9.9 | (2.7, 17.1) |
| 21 (°C) | Low | 0.4 | 1 | 35.5 | (28.6, 42.4) |
| | | 0.4 | 1.5 | 31.6 | (27.3, 35.9) |
| | | 0.6 | 2 | 30.6 | (26.5, 34.8) |
| | | 0.7 | 2 | 30.3 | (25.4, 35.2) |
| | Medium | 1.1 | 4 | 27.4 | (24.1, 30.7) |
| | | 1.1 | 5 | 31.2 | (27.8, 34.6) |
| | | 1.5 | 7.5 | 34.7 | (29.9, 39.6) |
| | High | 2.2 | 10 | 31.2 | (26.3, 36.1) |
| | | 2.2 | 7.5 | 18.2 | (12.2, 24.2) |
| | | 2.3 | 7.5 | 15.9 | (8.9, 22.9) |
| | | 2.4 | 7.5 | 13.5 | (5.5, 21.6) |
| | | 3.5 | 15 | 25.8 | (18.1, 33.5) |

**Table 3:** Effect of ozone in ascending doses on the soil pH and the release of bioavailable forms of P, Zn, Fe and Mn.

| Time of ozonation | Dose (g.kg$^{-1}$ $O_3$) | pH[a] | M-3 P[b] (ppm) | DTPA[c]-Zn (ppm) | DTPA[c]-Fe (ppm) | DTPA[c]-Mn (ppm) |
|---|---|---|---|---|---|---|
| Control | 0 | 7.9 | 34 | 0.5 | 16 | 116 |
| 10 min | 0.5 | 7.75 | 38 | 0.9 | 24 | 24[d] |
| 13 min | 0.7[e] | 7.7 | 37 | 0.8 | 21 | 141 |
| 15 min | 0.8[e] | 7.8 | 33 | 0.7 | 17 | 115 |
| 17 min | 1.1[e] | 7.8 | 38 | 0.7 | 20 | 123 |
| 20 min | 1.2[e] | 7.8 | 37 | 0.7 | 22 | 24[d] |
| 25 min | 1.4[e] | 7.8 | 36 | 0.7 | 21 | 42[d] |

[a]The soil ozonation did not show a correlation between ozone doses and variation in soil pH.

[b]Bioavailable form of phosphorous in response to ozonation was measured with the Mehlich-3 method, and showed no correlation between ozone dose and M-3 P.

[c]Bioavailable forms of zinc, iron, and manganese in response to ozonation were measured using the DTPA extraction method. No correlation was detected between ozone dose and the variations in DTPA forms of Zn, Fe or Mn.

[d]Differences between DTPA-Mn numbers are of an order of ppm. This is a normal and non-significant difference between soil samples from the same soil.

[e]Difference in dosage increase in response to the same increase in ozonation duration (2 min) is due to the difference in ozone absorption by the soil samples. This fluctuation depended on how tightly submerged the ozone diffuser was in the soil sample. Doses presented are averages of dosage measurements of 12 samples of the same ozonation duration.

**Figure 2:** Filtrates from ozonated (yellow) and control (colorless) soil samples collected after 24 h soaking in Baermann funnels.

ozonating soil infected with nematodes at a dose of 1-2 g.kg$^{-1}$ $O_3$ at 5 ˚C is sufficient to kill 80% of the nematodes. Ozonation at low temperature (5 ˚C) was more efficient at killing soil nematodes than at high temperature (21 ˚C), which favors the application of this treatment at the beginning of the growing season. More than 50% of nematodes were inhibited at ozonation doses below 0.5 g.kg$^{-1}$ $O_3$ executed at all temperatures expected to be encountered. Accordingly, this level of disinfection might be enough to reduce the nematodes viability below damaging thresholds, without harming the soil biotic balance. Biotic balance is a crucial factor in maintaining the soil health and productivity, and non-plant parasitic nematodes and other beneficial microorganisms play an essential role in maintaining that through organic and non-organic nutrients recycling, and by competing with and suppressing, plant parasitic microorganisms. Hence, it is not recommended to use unnecessary higher ozone doses in the control of soil nematodes.

Ozone was more efficient at reducing nematode viability at lower temperature, similar to the observations of Patil, et al. [46]. This is attributed to the increasing ozone solubility ratio with decreasing temperature [47], and the slower ozone decomposition at lower temperature [48]. Hence, ozone is more stable at 5 ˚C, which prolongs its activity at oxidizing and inhibiting nematodes in the soil. Consistent with these physico-chemical ozone properties, the current study confirms a higher efficacy at a lower temperature. This effect of temperature efficacy does not occur with many nematicides (e.g. EDB and 1,3-D) [49] and fumigants (MeBr) [50]. This is an advantage for ozone use, because nematicides are usually applied at the beginning of the growing season, when temperatures are usually below optimal soil temperature range for nematode development and multiplication (21 ˚C to 27 ˚C). This qualification is an advantage over nematicides and other gas fumigants, because these latter are less efficient at low temperatures.

The results in Table 3 do not show any correlation between ozonation dose and the analyzed soil parameters (pH, Me-3 P, and DTPA- Zn, Fe, & Mn) in response to ozonation, which does not confirm the theory of Sopher, et al. [17,18] of increased nutrient availability by soil ozonation. A plausible explanation of the yellow coloration of ozonated soil filtrate might be the oxidation of soil organic matter. By oxidizing soil organic matter, the organic carbon content transforms from humine to humic acid and then to fulvic acid, which might explain the yellowish coloration of the filtrate. Fulvic acid is the

most soluble and mobile form of organic carbon, and the most active form in chelating nutrients and rendering them available to plants. Hence, this could partially confirm Sopher, et al. [17] theory, since fulvic acid ameliorates the soil physical-chemical properties and increases plant productivity consequently. Moreover, these simple organic acids are effective in killing plant parasitic nematodes, while having little or no effect on free-living nematodes [51]. Accordingly, this suggests that the indirect effect of soil ozonation on plant parasitic nematodes is expected to be greater than the inactivation of free-living nematodes acquired in this experiment, through organic acids mobilization. The soil used in this experiment was sandy loam with low organic matter content. Ozone is known to be able to selectively oxidize colored matter and cause color changes [52,53].

Fumigants diffusion is faster in coarse-textured soil with high moisture [54], and these become less efficient in soils with high organic matter content [18]. Organic matter and metals increase the ozone demand because they are oxidizable. Hence, higher ozone doses will be required than in this research to reach similar nematode inhibition rates in heavier soils with higher organic matter and metal contents.

This study was not species-specific, since the observations were assessing the aggregate number of nematodes inhibited by the treatment unselectively amongst species. Therefore, further experimentation with species specificity is recommended, taking into consideration the significance of nematode inactivation by species. In addition, although the soil that was used in this research did not include significant numbers of plant parasitic nematodes, the high efficiency of ozone in inactivating non-parasitic nematodes could be an indicator for comparable effect on plant-parasitic nematodes as well. Hence, this could be a plausible confirmation of the Sopher, et al. [17,18] assumption that the increased crop yield after soil ozonation was attributed in part to a decrease in soil pathogens by ozone.

Since ozone does not leave toxic residues, and given that low doses are required to inactivate nematodes by half, which would control the nematodes without harming the soil biotic balance, ozonation could be used as a sustainable alternative to the conventional treatments that have been used to manage nematodes and other soil pathogens [55]. Thus, it could play an important role in organic agriculture. Furthermore, due to the complexity of ozone generation systems required in field application and the difficulty of bringing big ozone generators on site, the application of this technique is limited to small crop-lands. Lands that are suitable for soil ozonation are those usually treated with gas fumigants (e.g. MeBr), namely high-value crops and greenhouse crops. Finally, additional research is required to evaluate the economic feasibility of ozonation to control soil nematodes, the species-specific response to ozonation, and the application of soil ozonation at the field level.

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