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# Response of root-knot nematode *Meloidogyne javanica* to ozone

## Resposta do nematoide das galhas *Meloidogyne javanica* ao uso de ozônio

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**ABSTRACT**

Root-knot nematode, *Meloidogyne javanica* is a major causal agent of plant diseases on summer crops in fields and greenhouses. In order to reduce environmental concerns, compounds having pesticidal capacity with insignificant residue can be a good alternative to synthetic pesticides. This study was undertaken to investigate the nematicidal capability of ozone under Petri dish (*in vitro*) and pot (in the rhizosphere of tomato seedlings cv. Super Chief) conditions. The experiments were conducted based on Completely Randomized Design in four replicates with two antimicrobial materials, Cadusafos (0.5, 1.0, 1.5, 2.0, 2.5 and 3 g $kg^{-1}$ soil) and ozone (0.05, 0.1, 0.2, 0.4, 0.8 and 1.6 g $kg^{-1}$ soil). Results confirmed the significant *in vitro* controlling effect of ozone on nematode egg (85%, in 0.4 g $kg^{-1}$ soil) and second stage juvenile (72%, in 0.4 g $kg^{-1}$ soil) populations. Also a toxic effect of ozone was observed on the nematode pathogenicity, i.e. galling (83%, in 0.4 g $kg^{-1}$ soil), on tomato root in pot assays. Plant morphological traits (i.e. root fresh weight, foliage fresh weight, and stem length) were not significantly affected by ozone. Therefore, taking into account the principles of safety in the application of ozone, this material can be suggested as an alternative nematicide, at least at limited and controlled condition.

**Index terms:** Toxicity; antimicrobial effect; ozone mass transfer; soil fumigation.

**RESUMO**

O nematoide das galhas, *Meloidogyne javanica*, é um dos principais agentes causadores de doenças de plantas em cultivos de verão em campos e estufas. A fim de reduzir as preocupações ambientais, os compostos com capacidade pesticida com resíduos insignificantes podem ser uma boa alternativa aos pesticidas químicos inseguros. Este estudo foi realizado para investigar a capacidade nematicida do ozônio sob condições de placa de Petri (*in vitro*) e em vaso (na rizosfera de mudas de tomateiro cv. Super Chief). Os experimentos foram conduzidos com delineamento inteiramente casualizado em quatro repetições com dois materiais antimicrobianos Cadusafos (0.5, 1.0, 1,5, 2,0, 2,5 e 3,0 g $kg^{-1}$ solo) e ozônio (0,05, 0,1, 0,2, 0,4, 0,8 e 1,6 g $kg^{-1}$ solo). Os resultados confirmaram o efeito significativo do controle *in vitro* do ozônio sobre as populações de ovos (85%, em 0.4 g $kg^{-1}$ solo) de nematoides e juvenis de segundo estádio (72%, in 0,4 g $kg^{-1}$ solo). Também foi observado um efeito tóxico do ozônio na patogenicidade do nematóide, isto é, irritação (83%, em 0.4 g $kg^{-1}$ solo) na raiz do tomateiro em ensaios em vasos. As caracterticas morfolicas das plantas (i.e., peso fresco da raiz, peso fresco da folhagem e comprimento do caule) não foram significativamente afetados pelo ozôno. Portanto, levando em conta os princípios de segurança na aplicação do ozônio, este material pode ser indicado como um nematicida alternativo, pelo menos em condições limitadas e controladas.

**Termos para indexação:** Toxicidade; efeito antimicrobiano; transferência de massa de ozônio; fumigação do solo.

## INTRODUCTION

Root-Knot Nematodes (RKNs), *Meloidogyne* spp. have a wide host range of crops and vegetables in most regions of the world (Jones et al., 2013). Synthetic nematicides are commonly used to reduce RKN, *Meloidogyne javanica* (Treub) Chitwood damage on summer crops, such as tomatoes and cucumbers, in Iran. Soil chemotherapy is one of the most common methods of RKNs inhibition; however, problems such as soil pollution, environmental concerns and heavy costs have often been accompanied with the methods (Ahmad; Siddiqui; Babalola, 2013). Therefore, finding a safe chemical compound, which has at least damage to the environment and non-target organisms, with acceptable performance can be a suitable alternative to traditional nematicides such as methyl bromide.

Ozone ($O_3$) as an oxidative matter has been used to refine or clean up contaminated space and surfaces,

and to destroy the pesticides residues (O'Mahony et al., 2006). Since, ozone as a disinfectant has been successful on the microbial populations, including bacteria, viruses, protozoa and fungi, it has been used to disinfect drinking water and in food industry sanitation (Meunier; Canonica; von Gunten, 2006). The fumigation of grain silos and crop stores, with the aim of controlling pests and post-harvest diseases, had been other uses of ozone (White et al., 2010). Megahed, Aldridge and Lowe (2018) proved the antimicrobial capacity of aqueous and gaseous ozone through manure residues disinfection in livestock farms. Also, ozone has led to a prolonged shelf life of fruits and vegetables by reducing the populations of pathogenic microorganisms in post-harvest diseases (Ferreira et al., 2017; Sarig et al., 1996). Ozone micro-bubbles (aqueous ozone) were used as a disinfecting approach against phytopathogens in hydroponic culture solutions (Kobayashi et al., 2011).

Limited studies have been conducted on the use of ozone in controlling the population of pests and pathogens in soils. Msayleb et al. (2013) investigated elimination effect of ozone on some soil-inhabitant pathogens including plant parasitic nematodes including RKNs, to find a safe alternative compound for methyl bromide. Research has shown that the future of controlling plant parasitic nematodes depends on techniques for integrating cultural practices, genetic resistance and alternative nematicides, so that the populations of these pathogens are kept below the level of economic damage (Zasada et al., 2010). Currently, there are only a limited number of registered chemical pesticides for controlling plant parasitic nematodes (Coyne et al., 2018). Little reports are available on ozone nematicidal capacity, and so there is little information about the use of ozone in the soil and its impact on the population and pathogenicity of RKNs as well as the growth of host plant (Khan; Khan, 1998; Meek; Small, 1996). Also, Veronico et al. (2017) found ozonated water reduced susceptibility in tomato plants to *M. incognita* through the modulation of basal defence mechanisms.

It seems necessary to replace appropriate compounds with minimal environmental damage than conventional chemical nematicides. Ozone does not leave any toxic residues, and its primary substance is atmospheric oxygen (Msayleb; Ibrahim, 2011). Therefore, it was considered to compare the control effect of ozone with a reference synthetic nematicide (Cadusafos) by Petri dish and pot assays. The major scope of the current research includes evaluating nematicidal effect of ozone, on egg hatching and mortality of second stage juveniles ($J_2$s) of *M. javanica* using Petri dish assays; as well as, evaluating the toxic effect of ozone on galling and reproduction of the nematode in a rhizosphere of tomato seedlings in pot assays, in format of three tests of ozone application.

## MATERIAL AND METHODS

### 1. Preparation of materials

#### 1.1. *M. javanica* inoculum

A source of *Meloidogyne*-galled roots was collected from a population maintained in infested fields of tomato cv. Super Chief in Rey region, Iran. Extraction and preparation of *M. javanica* inoculum was carried out using the single egg mass method (Hussey; Barker, 1973). The nematode was identified according to the morphologic and morphometric characteristics of body and perineal pattern of females (Aydinli; Mennan, 2016). The egg mass of *M. javanica* was added to rhizosphere of tomato cv. Super Chief seedlings, and then the nematode was multiplied on the plants grown in steam-sterilized sandy soil in a greenhouse (temperature 27±2 °C and lighting period 10 hours), by the end of the period of plant growth. Eggs of the nematode were extracted by blending the roots in a 1% of NaOCl solution. Available suspension was cleared with distilled water using a sieve 400 mesh. Then the eggs were incubated in distilled water for a week at 25 °C. Achieved $J_2$s were collected and counted as the nematode inoculum, according to Anwar and McKenry (2007).

#### 1.2. Soil, host plant and antimicrobial materials

A reference nematicide was used (as a positive control), Cadusafos (Rugby® Granule 10%), with six concentrations (0.5, 1, 1.5, 2, 2.5 and 3 g $kg^{-1}$ soil). Ozone generator (SH5P, Shamim Sharif Technological Development Company, Iran) was used to prepare ozone. In order to provide oxygen for ozone generation, a pure oxygen cylinder was connected to the generator. The generator produced gaseous ozone at 0.025 $g.min^{-1}$. Thus, the concentrations (0.05, 0.1, 0.2, 0.4, 0.8 and 1.6 g $kg^{-1}$ soil) were considered for gaseous ozone. The four-leaf seedling of tomato cv. Super Chief, previously grown in a sterile sandy substrate, was considered as host plant. Soil was collected from several locations in a field of tomato cv. Super Chief to represent the physical and chemical characteristics of the field soil. The soil (texture: sandy loam, pH: 7.7, EC: 2.6 ds $m^{-1}$, organic mass: 2.2%, clay: 13.4%, silt: 13.8%, sand: 72.8%) was mixed, and then

was sieved through a screen (5 mesh). The soil (≈ 105 kg) was autoclaved, and its moisture content was obtained by 20% ($w.w^{-1}$), according to g of water per g of dried soil × 100, using an oven drying. The autoclaved soil was transferred to 13 plastic bags ((2 antimicrobial materials × 6 concentrations) + 1 negative control (concentration 0)) in quantities of 8 kg (2 kg (a pot) × 4 replicates), according to defined treatments.

## 2. Experimental methods

### 2.1. Evaluation of ozone effect on *M. javanica*

#### 2.1.1. Petri dish assays

##### 2.1.1.1. Egg hatching

The evaluation of egg hatching rate (%) of *M. javanica* was conducted using eggs suspension in a Petri dish assay. In order to prepare an eggs suspension, nematode-infected (galled) roots of tomato were cut into small (2-3 cm) pieces after rinsing by tap water. Using a blender, a suspension was provided by mixing the infected root pieces and some distilled water. The suspension was cleared by sieves of 10, 45, 60, 100 and 400 meshes. The suspension was cleared by sieves of 10, 45, 60, 100 and 400 mesh. The eggs suspension was formed according to centrifuge or sugar flotation method. The final eggs suspension was cleared with distilled water using a sieve 400 mesh. The density of the eggs suspension was estimated by a stereomicroscope (40×). The suspension was transferred to plastic Petri dishes (5 cm diameters) with 200 eggs in 4 ml distilled water per Petri dish. The eggs were exposed to Cadusafos (as a positive control), and gaseous ozone; with six levels of concentration (Cadusafos: 0.5, 1, 1.5, 2, 2.5 and 3 g $l^{-1}$ of distilled water; ozone: 0.05, 0.1, 0.2, 0.4, 0.8 and 1.6 g $l^{-1}$ of distilled water), one by one. Ozone gas was dissolved in a glass tube containing 1 ml of distilled water, and then added to a Petri dish containing eggs suspension, according to the concentrations. Eggs in distilled water (containing no nematicide no ozone) were considered as a negative control. Treated suspension of eggs was incubated at 25 ºC for ten days. Egg hatching rate (%) was determined based on the number of hatched eggs using a stereomicroscope (40×) on the second, fourth, sixth, eighth and tenth days since the start of the assay. Each treatment had four replicates (Petri dishes). In order to check recovery, the eggs were transferred in fresh water and were incubated after ten days of exposure in order to ensure the nematicidal or nematistatic effect of the antimicrobial materials (Habash; Al-Banna, 2011).

##### 2.1.1.2. $J_2$s mortality

In order to evaluate the effect of ozone on $J_2$s mortality (%) of *M. javanica*, an assay was performed using eggs suspension. An eggs suspension was incubated at 25 °C for a week. Then, the active emerged $J_2$s were collected and counted. $J_2$s population was set to 200 $J_2$s in 4 ml distilled water per plastic Petri dish (with 5 cm diameter). The $J_2$s were exposed to the pesticide or gaseous ozone at defined concentrations in the test hatching eggs for four days at 25 ºC. Ozone gas was dissolved in a glass tube containing 1 ml of distilled water, and then added to a Petri dish containing $J_2$s suspension, according to the concentrations. The number of dead $J_2$s was determined every day (for up to five days) using a stereomicroscope (40×), and then the $J_2$s mortality rate (%) was estimated. The $J_2$s were considered dead due to inactivity, skin damage, and deformity of body, mouth and esophagus. $J_2$s population in distilled water was taken as a negative control. Each treatment had four replicates (Petri dishes). In order to check recovery, the dead $J_2$s were transferred in fresh water and were incubated after one day of exposure in order to ensure the nematicidal or nematistatic effect of the antimicrobial materials (Habash; Al-Banna, 2011).

#### 2.1.2. Pot assays

##### 2.1.2.1. Test I

A suspension (10 ml) of the nematode inoculum (5 $J_2$s $g^{-1}$ soil) was treated with defined concentrations of ozone and Cadusafos, according to the treatments, and then the treated suspension was transferred into the bags containing 8 kg of soil, and mixed with soil thoroughly. Afterwards, the nematode-inoculated soil was transferred to plastic pots (2 kg soil per pot). Four-leaf seedlings of tomato cv. Super Chief, previously grown in a sterile sandy substrate, were cultivated to the pots (one seedling per pot). A pot containing one plant was represented as a replicate. The pots were kept in a greenhouse (temperature 27±2 °C and lighting period 12 hours) for two months. The plants were irrigated sufficiently (Soheili; Saeedizadeh, 2017; Pradhan et al., 2012; Dubey; Trivedi, 2011).

##### 2.1.2.2. Test II

The suspension (10 ml) of the nematode inoculum (5 $J_2$s $g^{-1}$ soil) was added to the sealed bags containing 8 kg of soil, and mixed with it thoroughly. Then, the nematode-inoculated soil was treated with Cadusafos and ozone, according to the treatments. The treated soil was transferred to plastic pots (2 kg soil per pot); and then

four-leaf seedlings of tomato cv. Super Chief, previously grown in a sterile sandy substrate, were cultivated to the pots (one seedling per pot). The pot containing one plant was represented as a replicate. The pots were kept in the greenhouse for two months. The plants were irrigated sufficiently (Soheili; Saeedizadeh, 2017, Pradhan et al., 2012; Dubey; Trivedi, 2011; Saeedizadeh, 2016).

#### 2.1.2.3. Test III

After inoculation of nematode (5 $J_2$s $g^{-1}$ soil) to the sealed bags containing 8 kg of soil, the nematode-inoculated soils were transferred to plastic pots (2 kg soil per pot); and then four-leaf seedlings of tomato cv. Super Chief were cultivated to the pots (one seedling per pot). Afterwards, the pots were treated with defined concentrations of ozone and Cadusafos (at a depth of 3 cm soil around crown), according to defined treatments. The pot containing one plant was represented as a replicate. The pots were kept in the greenhouse for two months. The plants were irrigated sufficiently (Soheili; Saeedizadeh, 2017; Pradhan et al., 2012; Dubey; Trivedi, 2011).

#### 2.1.2.4. Studied traits of *M. javanica* activity

The pathogenicity incidence of *M. javanica* was evaluated according to the number of knots (galls) and egg masses per root, as well as reproduction factor (RF). Two months after cultivation of seedlings to the pots, the plants were taken and the roots were investigated in terms of galling and egg mass, and also soil samples were collected for the nematode population analysis. The roots were washed by running tap water and were drained on blotting paper. After counting galls, in order to specify the number of egg masses, the roots were cut into pieces of 2-3 cm long, and then egg masses were stained with Floxin solution B (0.15 g $L^{-1}$ of water), bleached by lactophenol and counted using a stereomicroscope (40×) (Soheili; Saeedizadeh, 2017). Also, RF was calculated, according to the study conducted by Oostenbrink (1966), as follows: $RF= Pf\ Pi^{-1}$, in which Pf is final population of nematodes, and Pi is primary population of nematodes (the nematode inoculum). To estimate of nematode final population ($J_2$s and males in soil, plus eggs, females, $J_3$s and $J_4$s in roots), a 100g subsample of well mixed soil from each replicate (pot) was processed by extraction method according to the study by Jenkins (1964), centrifuge or sugar flotation method. The nematode suspension was collected, the number of nematodes (males and $J_2$s) was counted using a stereomicroscope (40×), and then it was applied to estimate the population of nematodes per soil of pot. Also, in order to estimate the number of nematodes per root (eggs, females, $J_3$s and $J_4$s), 2g subsample of well mixed chopped root was macerated in a Warring blender and counts were done on the suspension after preparation. Then, the numbers of nematodes present in the suspension was counted using a stereomicroscope (40×). So, the numbers of nematodes were estimated by considering the total weight of the root (Tabatabaei; Saeedizadeh, 2017).

### 2.2. Evaluation of ozone effect on host plant

The probability of soil ozonation destructive effect on tomato seedling growth properties was evaluated through soil treatment with ozone under the same conditions of test II and without the nematode inoculum. After soil treatment with the antimicrobial materials (according to the concentrations) and transferring it to the pots (2 kg soil per pot), three seeds of tomato cv. Super Chief were sowed per pot. After germination, the seedlings were thinned so that remained a seedling per pot. The pot containing one plant was represented a replicate. The pots were kept in the greenhouse; and the plants were irrigated as needed. Two months after sowing seeds, plants were taken, rewetted, fresh root and foliage were weighed, and length of stem was measured.

### 3. Experimental design and statistical analysis

All tests performed in this research were conducted based on Completely Randomized Design in four replicates using two antimicrobial materials including Cadusafos (as a positive control), and ozone; with six levels of concentration by Petri dish and pot assays. The free-antimicrobial materials condition (concentration 0) was considered as a negative control. All experiments, Petri dish and pot assays, were repeated. The normality of data was performed previous statistical analyses. A *Duncan's test* was applied to compare means of treatment data. SAS software version 9.1 was used to analyze all the data.

## RESULTS AND DISSCUSSION

According to the results of the egg hatching test, the highest hatched eggs was obtained in the control (92%) on tenth day of exposure times. There was no significant ($P<0.05$) difference in egg hatching rate in the Petri dishes of the control on sixth, eighth and tenth days of exposure times. In the Cadusafos treatments, the highest egg hatching rate was obtained at concentrations of 0.5, 1, 1.5, 2, 2.5, and 3 g $L^{-1}$ at 8, 6, 6, 4, 2 and 2 days after the beginning of the assay, respectively. It means

the incremental trend of egg hatching has been stopped during these days. The concentrations 2.5 and 3 g $L^{-1}$ of Cadusafos have prevented hatching eggs (0%). In ozone treatments, the highest egg hatching rate (70%) was observed at the concentration of 0.05 g $L^{-1}$ and on tenth day of exposure times. The stopping in incremental trend of the egg hatching is only seen at the concentration of 1.6 g $L^{-1}$ on 8$^{th}$ day after the beginning of the assay, while at other concentrations, the highest hatched eggs was obtained on tenth day of exposure times. However, similar to Cadusafos, an increase in the concentration level has reduced egg hatching rate in ozone treatments (Figure 1).

According to the results of the $J_2$s mortality test, the dead $J_2$s was observed in all concentrations of Cadusafos and ozone. By increasing the concentration level, the $J_2$s mortality rate was increased during the exposure time periods. The fastest mortality 100% was occurred in Cadusafos at the concentration 0.5 g $L^{-1}$ on the first day of exposure times. The mortality rate was 100% at all ozone concentrations in the last days of evaluation. The mortality rate was equal to 100% at the concentration 1.6 g $L^{-1}$ in ozone on the first day of the test, while the mortality rate was equal to 100% in ozone at the concentration 0.4 g $L^{-1}$ on the fifth day (Figure 2).

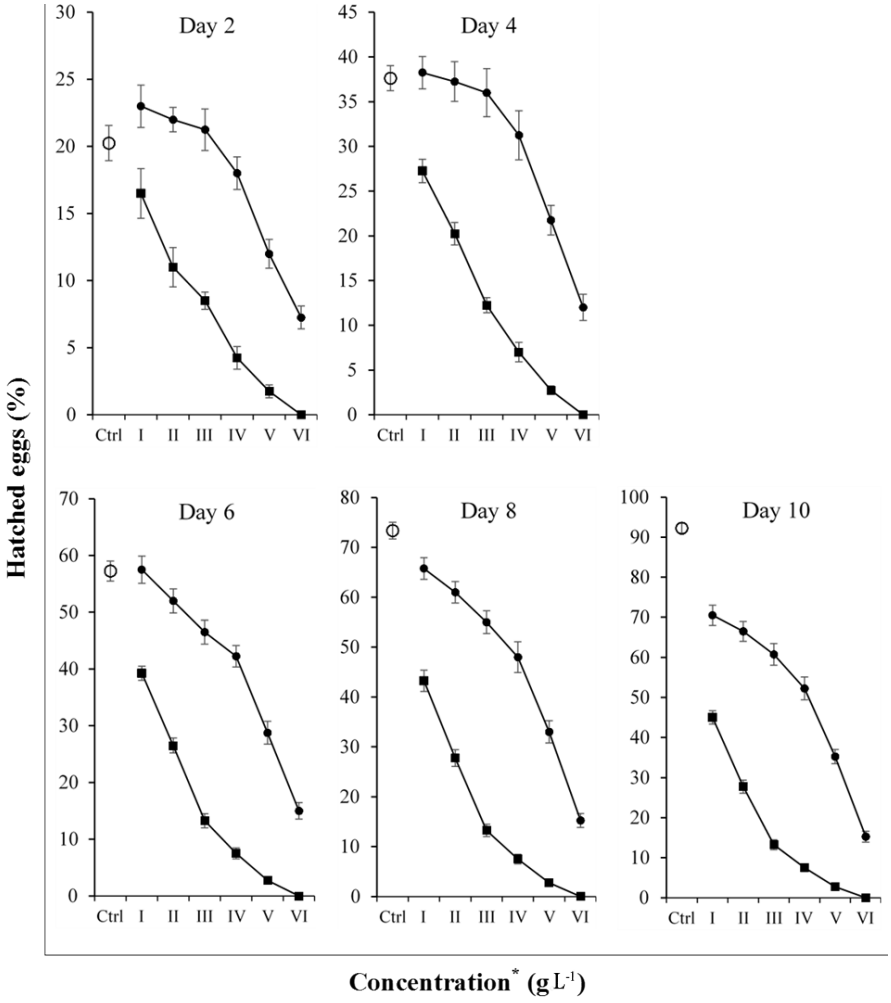


**Figure 1:** Hatched egg mean rate (%) of Meloidogyne janvanica response to concentrations of Cadusafos and ozone at exposure times *in vitro*.

Symbols of I, II, III, IV, V and VI referred to concentrations of Cadusafos 0.5, 1, 1.5, 2, 2.5 and 3 g $kg^{-1}$ soil substrates, and ozone 0.05, 0.1, 0.2, 0.4, 0.8 and 1.6 g $kg^{-1}$ soil substrates.

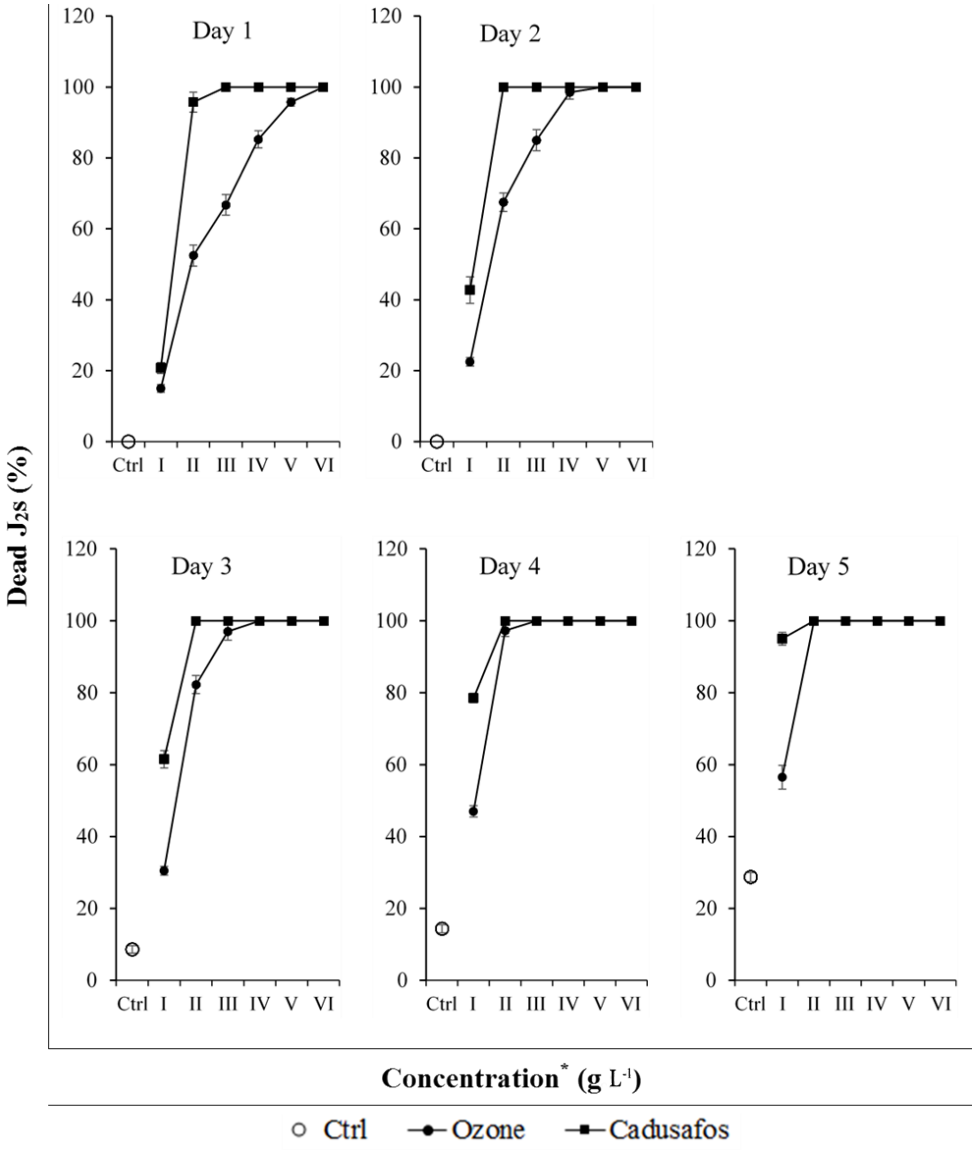


**Figure 2:** J2s mortality mean rate (%) of Meloidogyne janvanica response to concentrations of Cadusafos and ozone at exposure times *in vitro*.

* Symbols of I, II, III, IV, V and VI referred to concentrations of Cadusafos 0.5, 1, 1.5, 2, 2.5 and 3 g $kg^{-1}$ soil substrates, and ozone 0.05, 0.1, 0.2, 0.4, 0.8 and 1.6 g $kg^{-1}$ soil substrates.

According to the results of the pot assays, the lowest to the highest ozone control effect on the nematode galling was observed in the results of tests III, II and I, respectively. So, the test I had the greatest toxic effect on the nematode pathogenicity. Comparison of results of tests related to ozone and Cadusafos indicated the highest control effect was obtained (under concentration 2 g $kg^{-1}$ soil and 0.4 g $kg^{-1}$ soil for Cadusafos and ozone, respectively) in Cadusafos test I (100%), Cadusafos test II (93%), ozone test I (92%), Cadusafos test III (91%), ozone test II (86%) and ozone test III (83%), respectively. Here, the toxic effect has also increased along with the increase in concentrations (Figure 3). The results of the tests regarding the traits of the egg mass and the RF were similar to the gall (Figures 4 and 5).

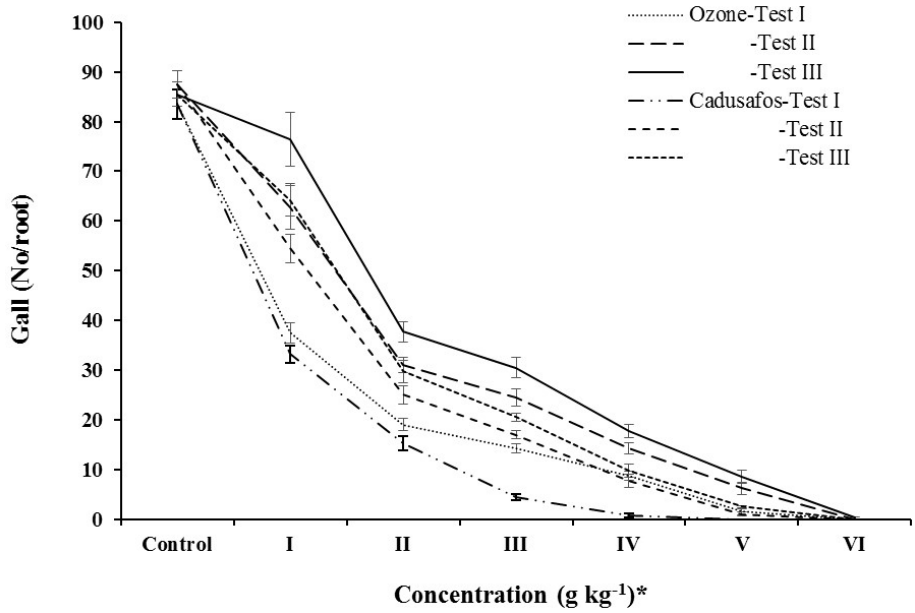


**Figure 3:** The number of galls of Meloidogyne javanica on the roots of tomato plants exposed to different concentrations of ozone and Cadusafos under three tests of pot assay.

* Symbols of I, II, III, IV, V and VI referred to concentrations of Cadusafos 0.5, 1, 1.5, 2, 2.5 and 3 g $kg^{-1}$ soil substrates, and ozone 0.05, 0.1, 0.2, 0.4, 0.8 and 1.6 g $kg^{-1}$ soil substrates.

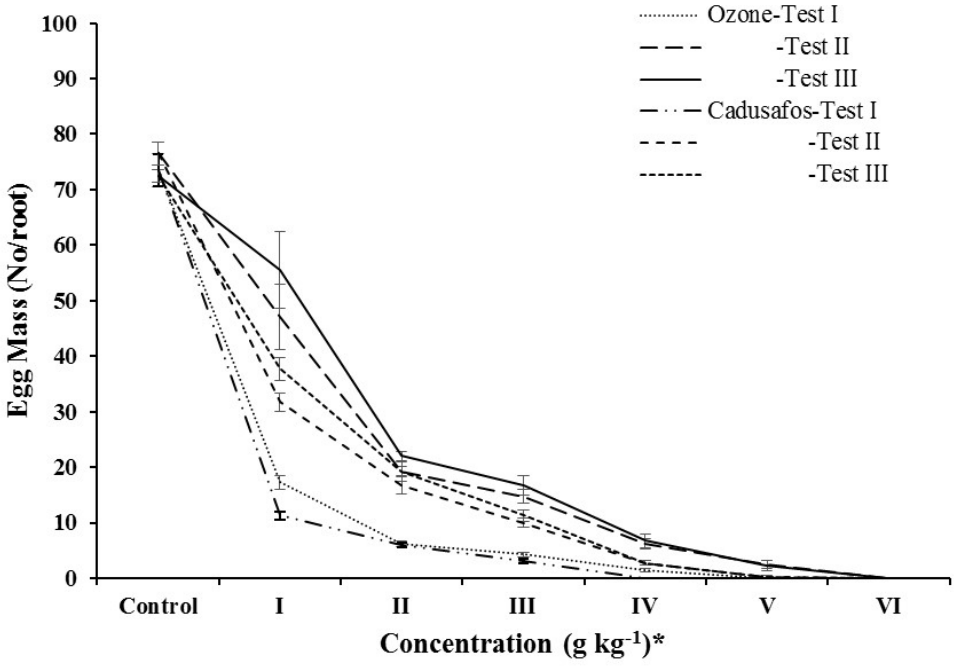


**Figure 4:** The number of egg masses of *Meloidogyne javanica* on the roots of tomato plants treated with different concentrations of ozone and Cadusafos under three tests of pot assay.

* Symbols of I, II, III, IV, V and VI referred to concentrations of Cadusafos 0.5, 1, 1.5, 2, 2.5 and 3 g $kg^{-1}$ soil substrates, and ozone 0.05, 0.1, 0.2, 0.4, 0.8 and 1.6 g $kg^{-1}$ soil substrates.

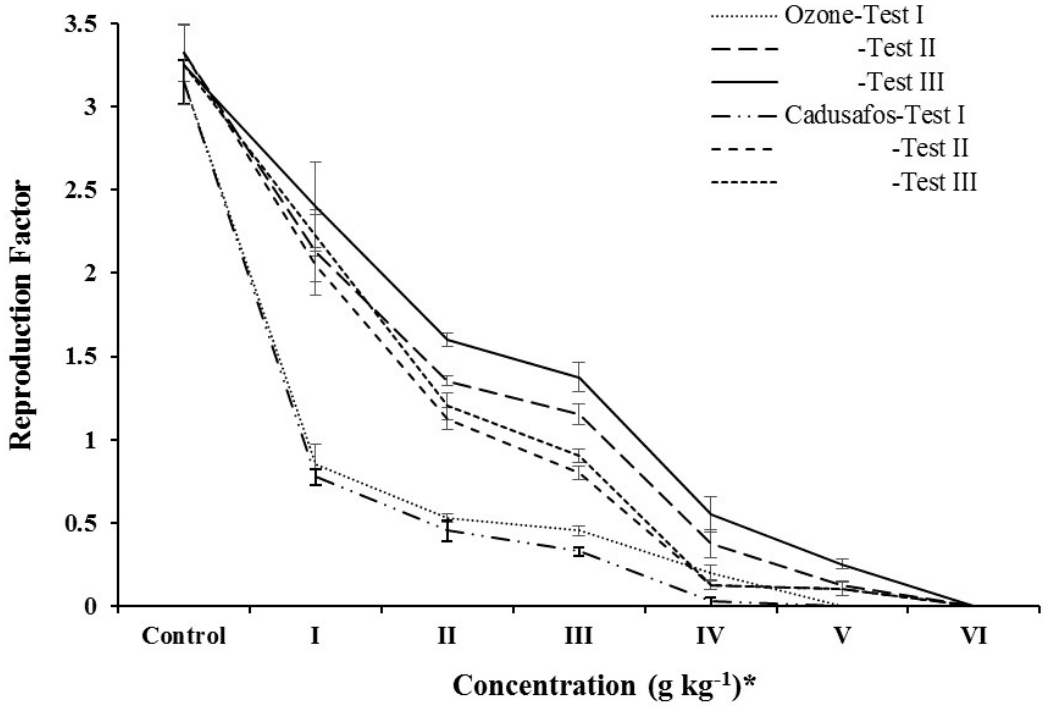


**Figure 5:** Reproduction factor of Meloidogyne javanica on roots of tomato plants treated with different concentrations of ozone and Cadusafos under three tests of pot assay.

* Symbols of I, II, III, IV, V and VI referred to concentrations of Cadusafos 0.5, 1, 1.5, 2, 2.5 and 3 g $kg^{-1}$ soil substrates, and ozone 0.05, 0.1, 0.2, 0.4, 0.8 and 1.6 g $kg^{-1}$ soil substrates.

Overall, studied traits of *M. javanica* pathogenicity (gall, egg mass and RF) has shown a significant ($P<0.05$) reduction in the antimicrobial materials treatments compared to control. The pathogenicity incidence of the nematode was decreased gradually along with the increase in the concentration of the antimicrobial materials. The toxic level of ozone on the nematode population was less than Cadusafos; although, the nematode galling level was zero in ozone, at the concentration of 1.6 g $kg^{-1}$ in soil. Also, the number of egg masses was zero at this concentration. The RF was less than one in ozone at the concentration of 0.4 g $kg^{-1}$ in soil; i.e. the final population of the nematodes was less than primary population at this concentration (Figures 3, 4 and 5). In second test, in non-nematode condition, on treated soils with ozone or Cadusafos, growth properties of plant (fresh weight of foliage and root, and stem length) did not show a significant ($P<0.05$) difference in all treatments compared to control. Based on the treatments, fresh weight of root and foliage as well as stem length did not vary significantly, as a result of increasing the concentration of the antimicrobial materilas (Table 1).

The results of current research overlap with the findings of studies about the toxic effects of ozone on soil-inhabitant nematodes (free-living and plant parasitic) (Msayleb; Ibrahim, 2011; Qiu; Westerdahl; Pryor, 2009; Bao et al., 2014; Meek; Small, 1996).

Msayleb and Ibrahim (2011) used ozone as a pesticide in nematode-infested soil, and found that ozone (900 mg) was sufficient to eliminate (100% control) the soil-inhabitant nematodes populations. Also, Qiu, Westerdahl and Pryor (2009) found ozone mass conduction to the soil reduced population of plant parasitic nematodes to 68%, meanwhile the value of free-living nematodes was about 52%. The results of the current research indicated that nematicidal effect of ozone on $J_2$s population and egg hatching was significant compared to the reference nematicide. Also, soil and nematode inoculum treatment by gaseous ozone decreased the development of the nematode's galling and reproduction on the host plant roots. Ozone had been able to reduce the nematode activity compared to the control according to the greenhouse assay,

tests I, II and III, at low concentrations (0.05 and 0.1 g $kg^{-1}$ soil), so that, at greater concentrations (0.8 and 1.6 g $kg^{-1}$ soil), the number of galls were close to zero. The results indicated the toxic effect of ozone was less than Cadusafos on the nematode populations, however, ozone, at concentrations of 0.8 and 1.6 g $kg^{-1}$ in soil, has stopped galling on the roots.

Comparing three tests performed in greenhouse conditions (pot assays), it seems that the direct treatment of the initial nematode (inoculum) using gaseous ozone in the test I caused the most toxic effect on the nematode, while in the test II, the establishment of the nematode population in the soil has decreased the toxic effect of ozone on the nematode population. This situation is more pronounced in the test III, so that in this test, the soil ozonation after seedling cultivation has caused the greatest reduction on the nematicidal effect of ozone. Therefore, it seems that soil treatment with ozone before planting and also exposing nematode populations directly to ozone will have more positive effects on nematode management. Ozone is a substantial oxidizing agent and an important disinfectant that acts on microorganisms through the oxidation of biological materials (Mehlman; Borek, 1987). Nematodes are hydrophilic animals that breathe through receiving water-soluble oxygen, so aqueous ozone can damage the cuticle inner layers and internal organs through penetration into the cuticle. Exposing the $J_2$s of *M. javanica* directly to aqueous ozone performed in the test I, was resulted in the highest toxic effect of ozone on the nematode pathogenicity compared to the tests II and III.

Vice versa, results of some studies are not in agreement with the results of current study. In a study, treated-ozone bulbs of Easter lily (*Lilium longiflorum* Thunb.) did not reduce the number of nematode-associated with the bulbs and roots of the plant. Although, it had positive effect on the growth of the plants grown from the bulbs (Giraud et al., 2001). Also, Pryor (2001) has found that ozone had not an important role in reducing nematode populations in soil of tomato fields, but the injection of ozone gas in the soil may lead to an increase in the yield eventually.

According to the obtained results in the present study, ozone can be identified as a suitable nematicide. Our experiments showed that ozone at concentrations of 0.2, 0.4, 0.8 and 1.6 g $kg^{-1}$ in soil has had a nematicidal role against populations of *M. javanica*. Also, growth properties of the host plant; fresh weight of root and foliage, and stem length; did not ($P<0.05$) vary significantly in the nematicides treatments, compared to control. The incidence of plant growth parameters was not influenced by gaseous ozone fumigation to the rhizosphere of tomato seedlings.

**Table 1:** Growth properties of tomato plants in response to different concentrations of Cadusafos and ozone in a free-nematode soil.

| Treatment | Concentration (g.$kg^{-1}$) | Root fresh weight (g) | Foliage fresh weight (g) | Length of stem (cm) |
|---|---|---|---|---|
| Control | 0 | 4.63±0.19 | 33.75±0.95 | 23.25±0.85 |
| Cadusafos | 0.5 | 4.6±0.26 | 33.25±1.03 | 24.5±0.65 |
| | 1.0 | 4.4±0.21 | 32.75±1.03 | 22.5±0.65 |
| | 1.5 | 4.43±0.23 | 33.25±0.48 | 24±0.71 |
| | 2.0 | 4.75±0.18 | 34.25±0.85 | 22.75±0.63 |
| | 2.5 | 4.33±0.17 | 32.75±1.38 | 22.75±0.85 |
| | 3.0 | 4.2±0.11 | 31.75±0.85 | 21.75±0.75 |
| Ozone | 0.05 | 4.55±0.3 | 33.5±0.96 | 24±0.91 |
| | 0.1 | 4.7±0.13 | 34±0.82 | 24±0.41 |
| | 0.2 | 4.4±0.21 | 32.5±1.04 | 23±0.82 |
| | 0.4 | 4.83±0.18 | 34.25±0.85 | 23.25±0.85 |
| | 0.8 | 4.65±0.2 | 34.5±0.65 | 23.25±1.11 |
| | 1.6 | 4.63±0.17 | 32.75±0.75 | 23.25±0.48 |
| | | Non- significant | Non- significant | Non- significant |

Lack of toxic residue in exposed environment is considerable benefit of ozone as a chemical nematicide. However, ozone has a lethal trait for all soil-inhabitant macro and microorganisms. Although using of ozone, may be appropriate for soil sterilization, but this feature puts ozone in list of the undesirable chemical compounds relative to beneficial soil's organisms. The direct effects of ozone on plant organs have not been found yet, it means that ozone may have harmful effects on product quality. However, ozone has been approved by the American 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 (Kobayashi et al., 2011).

Nevertheless, it is necessary to conduct further studies on the effects of ozone on soil fauna and flora, and useful saprophytic microorganisms. So, due to the characteristics of ozone and available knowledge, ozone can be used merely for soil sterilization in a small scale, and observing safety principles. In controlled environments such as greenhouses, it is possible to provide good conditions for plant growth by removing PPNs through soil ozonation, and then transferring beneficial microorganisms to the soil. However, health and safety are very critical topics regarding the use of ozone, and especially its use is hazardous in closed locations. Further studies are needed to evaluate effects of ozone on population of beneficial microorganisms, pH and soluble nutrients in soil as well as effect of temperature and moisture on performance of ozone in soil.

## CONCLUSIONS

The treatment of *M. javanica* inoculum and the nematode-inoculated soil with gaseous ozone caused a significant ($P<0.05$) reduction of pathogenicity of the nematode in the rhizosphere and roots of tomato cv. Super Chief seedlings. The highest toxic effect on the nematode was obtained in the treatments at concentrations of 0.8 and 1.6 g $kg^{-1}$ soil of ozone. Overall, according to the results obtained in this study, control of plant parasitic nematodes can be offered through soil treatment by gaseous ozone injection to a substrate soil before planting.

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