Available online at www.sciencedirect.com

SciVerse ScienceDirect

Physics Procedia 21 (2011) 187 – 192

Physics Procedia

Septième Conférence Internationale sur les Sciences des Matériaux (CSM7)

Treatment of Nematodes with Ozone Gas: A Sustainable Alternative to Nematicides

Nahed Msayleb[a], Saiid Ibrahim[b]

[a]*Iowa State University, 839, 8th Street. Ames, Iowa 50010, U.S.A.*
[b]*Lebanese University, Dekwaneh, Beirut, Lebanon*

Abstract

This study tests Ozone as a Nematicides' alternative.
Nematode-infected soil samples were treated with ascending doses of O_3 by submerging the outlet of an "MB1000 Ozone Generator" in the 40 ml samples; then to test the O_3 nematicidal effect by gas fumigation, Ozone gas was released into a sealed bag containing 80 g of each of the 6 nematode-infected soil samples with ascending doses and a repetition of each.
With water-ozonation, 900 mg O_3 were needed to kill 100% of nematodes, and the O_3–Nematodes LD50 was identified by 420 mg. With the second experiment, O_3 soil fumigation for 50 minutes at a dose of 1,125 mg in an air volume of 5 litres, were needed to control 95% of living nematodes.

Keywords: Ozone, Nematicides Alternatives, Soil Fumigation, Soil Disinfection, Soil Ozonation

1. Introduction

Nematodes are microscopic soil-borne worms that parasite on the plants' roots. Once penetrated, nematodes feed on the nutrients found in the plant's vascular tissues and induce their cells to divide and multiply, which causes root knot galls. These galls interfere with the normal movement of both water and nutrients and increase the plant's susceptibility to diseases. The damage resulting from the infection of the plant's "digestive system" is usually manifested as stunted growth, yellow plants with low productivity. Lyons-Johnson [1] mentioned that *Heterodera glycines* (soybean cyst nematode), is the number one disease pest of soybeans, contributing to annual losses estimated at 64 million bushels in the United States. While Chitwood [2] stated that "Annual production losses at the farm gate (in year 2000, dollars) were $121 billion globally and $9.1 billion in the United States; yield reduction in specific crops can exceed 75% in some locations. More typically, growers are forced to select less profitable crops. In addition to directly causing crop losses, nematodes can vector many plant viruses or create wounds that allow the entry of other root pathogens". Many Nematicides are used to treat this important parasite, including Organophosphates (*i.e.* Fenamiphos, Cadusafos, Terbufos), Carbamates (*i.e* Aldicarb, Aldoxycarb, Carbofuran, Oxzamyl) and Fumigants (*i.e* Dazomet, Chloropicrin, 1,3-Dichloropropene, Metham Sodium and Methyl Bromide). Though, these chemicals even have limited control effect on different nematodes, or are very toxic and environmentally polluting. The toxicity characterizing these chemicals ranges from groundwater contamination (Ethylene Dibromide), Ozone stratospheric layer depletion (Methyl Bromide), to human sterility and carcinogenicity (Ethylene Dibromide, Dibromo Chloroprapane, etc...). Given the economically important yield losses caused by nematodes, and the need for an alternative to control these effectively with a minimal environmental degradation, we sought in this study to test Ozone gas as an alternative to the currently available nematicides. Since Ozone has been characterised by having the strongest oxidising potential amongst all oxidising reagents, it possesses strong disinfecting qualities, and thus could be used in pest control, especially in nematode control, whether through gas Fumigation, or direct soil irrigation with Ozonated Water. Thus, O_3 has a good potential for replacing the highly toxic, environment polluting nematicides, since O_3 degenerates into Oxygen without leaving any toxic residues in nature and its use is economically feasible because its primary material is Oxygen (from water, air or compressed O_2).

2. Materials and Methods

After submerging nematode-infected soil samples with water overnight and collecting the extract and counting the nematodes under the stereoscope, 5 samples were treated with ascending doses of O_3 by submerging the outlet of a "1000BT-12 Ozone Generator" –generating 1g O_3/hr from pure oxygen by Corona Discharge, and the conversion of oxygen to ozone occurs in a reaction cell excited by a high-voltage potential- directly in the 40 ml samples of free-living nematodes. The treated samples are then inspected under the stereoscope for detection of living/active Nematodes and comparison with the control sample's count. One another experiment was carried out to test the O_3 gas fumigation to 5 soil samples with 1 control and a repetition of each, in an ascending-dose scale. After treatment, the samples were viewed under the stereoscope to detect living/active Nematodes and determine their rate of mortality under the effect of O_3 compared with the control samples.

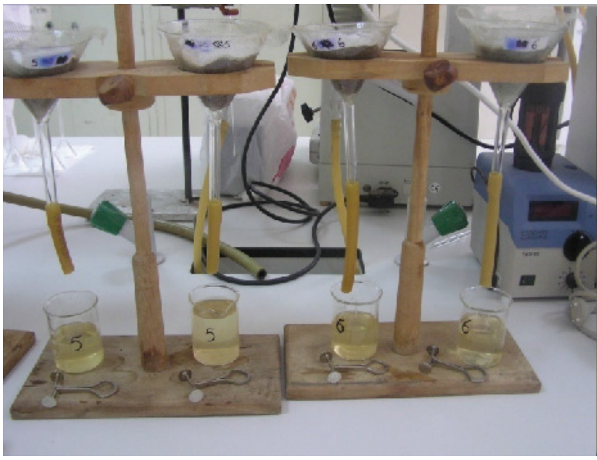

Figure 1: Extraction of nematodes from soil

Figure. 2: Ozone generator & direct ozonation

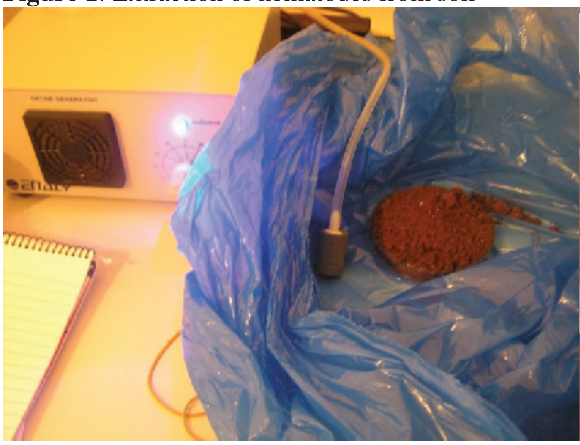

Figure 3: Soil fumigation with O_3 gas

Figure 4: Nematodes' detection & counting

3. Results

Table 1: Nematodes' Mortality Rates Due to Direct Ozonation to Free-Living Nematodes in Solution

Ozone level = 20 in Samples 1, 2 & 3. Ozone level = 50 in Samples 4 & 5 – Inlet O_2 pressure = 10 PSI – Samples' volume = 40 ml

Sample No.	Direct Ozonation time (minutes)	Applied O_3 dose* (mg)	Nematodes mortality rate after treatment
Sample 0 (Control)	0	0	0%
Sample 1	5	0	0%
Sample 2	10	0	0%
Sample 3	15	0	0%
Sample 4	25	300	25%
Sample 5	45	1,350	100%

*O_3 dose is calculated as follows:
{flow rate (ml/min.) x [concentration] (µg/ml) x time (min.)}/1000 = O_3 dose (mg)

The above table shows that Ozone is lethal to nematodes (with a mortality rate of 100%) at a dose of 1,350 mg.

Table 2: Determining Ozone LD50 on Nematodes with Direct Ozonation to Free-Living Nematode Extracts

Ozone level = 80 – Inlet O_2 Pressure = 5 PSI – Samples' volume = 40 ml

Sample No.	Direct Ozonation time (minutes)	Applied O_3 dose (mg)	Nematodes mortality rate after treatment
Sample 0 (Control)	0	0	0%
Sample 1	3.5	210	5%
Sample 2	4	240	10%
Sample 3	7	420	50%
Sample 4	11	660	75%
Sample 5	15	900	100%

Table 2 shows that the Ozone-nematode LD50 is 7 minutes of direct ozonation at a dose of 420 mg.

Table 3: Nematodes' Mortality Rates Due to Soil Fumigation With Ozone Gas

Ozone level = 100 – Inlet O_2 pressure = 0.1 PSI in samples 3, 3'', 4, 4'', 5, 5'', 6 & 6'', and 5 PSI in samples 2 & 2'' – Soil samples' weight = 80 gr – Bag volume = 5 ltr.

Sample No.	O_3 application time (minutes)	Applied O_3 dose (mg)	Nematodes mortality rate after treatment
Sample 1 (Control)	0	0	0
Sample 1' (Ctl-Repetition)	0	0	0
Sample 2	15	900	55%
Sample 2'	15	900	55%
Sample 3	20	450	55%
Sample 3'	20	450	55%
Sample 4	30	675	85%
Sample 4'	30	675	85%
Sample 5	40	900	90%
Sample 5'	40	900	75%
Sample 6	50	1,125	95%
Sample 6'	50	1,125	95%

Results shown in table 3 demonstrate that soil (sample) fumigation with ozone reached a 95% of nematodes' mortality at 1,125 mg dose. These results could've been enhanced (higher efficiency at a shorter time and may be smaller doses), if soil samples were wetted prior to the ozone fumigation. Though, reaching these results without even irrigating the soil (as it's usually done while using gas fumigants in soil treatment) means expected better results when the soil gets irrigated prior to the application of the ozone gas.

4. Discussion and Conclusion

These results show the high lethal effect of O_3 on nematodes, whether by gas fumigation or through direct samples ozonation; added to its high oxidising potential and wide spectrum of effect on pathogens, O_3 proves to have a high potential for being the ideal alternative for nematicides, especially those that function in the same way as gas fumigants (i.e. Methyl Bromide). Nevertheless, further *in vivo* and *in vitro* experiments should be carried on to fine-tune the practical applications of ozone in soil disinfection and nematode treatment, since this study isn't thorough enough to be considered as base for quantitative data, but only as qualitative indication to the effect of O_3 on nematodes. Experiments should test ozone treatment effect according to the following parameters:

- Soil Texture, water pH (when ozone application is done through water irrigation),
- Temperature (water temperature, ambient temperature, soil temperature), as O_3 is unstable at high temperatures, and degenerates more quickly when temperature rises,
- Soil Organic Matter (OM) content. Since any oxidisable matter will consume O_3 first and increase the need to O_3 for disinfection, thus the necessity to evaluate the effect of OM on the O_3 need for soil disinfection,
- Interaction with soil metals and its effect on the soil fertility in terms of oxidised metals' availability to plants due to ozonation,
- Application type (soil fumigation or irrigation with ozonated water) to determine the optimal measure according to the crop,
- Application date/season, to determine ozone effect on different nematode-growth stages (hibernating cysts and larvae),
- Ozone application Duration for optimal results, especially in the presence of crops, and the needed maintenance time after application,
- Treatment of nematode-infected Plants,
- Necessity of Integration with other agricultural practices, especially those practiced in Organic Farming, for best results,

The appropriate technology is now available for practical and massive agricultural application (e.g. an ozone generator with a capacity of generating 300L/hr of ozonated water at a concentration of 6 g/L for irrigation, is now available), which makes it possible for *in vivo* experiments to be exerted.

5. Recommendations

Given these promising results, and the availability of the appropriate technology, new horizons could be opened for the use of ozone in soil-disinfection and pathogens' treatment, especially that ozone has empirically (*in vitro*) proved to have high effect on different pathogens (bacteria, fungi and viruses). The potential applications to be tested are mainly recommended to deal with the following fields and aspects:

- Organic cultures, to overcome some of its limitations in the control of pathogens and pests, which contributes to the higher production of better quality, thus leading to the increase in the farmers' profit, and the availability of healthy produce to more people at more convenient prices due to the higher production quantities.

- In nurseries, to produce healthy and certified transplants, which would be considered a good preventive measure against the spread of pests and pathogens.

- Seeds disinfection. The threshold of efficiency on seed-borne pathogens without harming the germplasm should be identified.

- The possibility to overcome the pests and pathogens' control limitation during cold and rainy weathers, as ozone is best working at cold temperatures and under the rain, while it degenerates quickly at high temperatures. As for the rain, it contributes to the wetting of the soil, which enhances the ozone percolation in it, reaching pests and pathogens deeper and more efficiently.

- The ability to control soil pathogens in presence as well as in the absence of crops, since ozone doesn't harm the crops when applied in irrigation or in gas fumigation, unless its application was prolonged for consecutive days in the presence of crops; in practice, O_3 application duration shouldn't exceed a few hours.

- The ability of O_3 soil fumigation to be a sound and good alternative to the highly toxic and polluting Methyl Bromide gas. For this specific goal, O_3 should be tested for its spectrum of effect on soil pathogens, and for the optimum parameters for its efficacy. If proved active in controlling merely the same spectrum of effect and with naturally occurring ambient conditions (temperature, soil pH, variable soil textures, etc...), then this would be an important breakthrough, with consequences having many significances and benefits in the following:

- Agriculture: to control the pathogens and pests effectively and feasibly,

- Environment: ozone doesn't pollute the environment as it should be generated and used on-site from water or compressed oxygen, and it degenerates into oxygen in relatively no time, thus leaving no pollutants like MeBr and other pesticides; if only it replaces MeBr, saving the stratosphere from hundreds of tons of this gas worldwide, will have its huge positive environmental impact; in addition, O_3 doesn't harm the stratospheric ozone layer, but has rather a positive effect on it,

- Public Health: ozone use doesn't leave any residues in the soil or in the produce, necessitating no pre-harvesting caution measures normally taken with other pesticides, and the produce is residues-free and completely healthy for direct consumption,

- Economy: ozone generation isn't expensive as its primary materials are water, air or oxygen; thus its use doesn't increase the agricultural inputs' facture, which leads to the increase in the farmers' profit. The massive scale of use of ozone when replacing the relatively costly MeBr (and potentially other pesticides), and the cumulative increased farmers' profit, will have its positive impact on the national economies.

- In addition to the above-stated aspects and applications, ozone qualifications overcome all Methyl Bromide's alternatives. For instance, it is as environmentally safe as soil solarisation, but it doesn't need 70 days of application and crop removal to give results as this latter. On the other hand, ozone is the most efficient "pesticide", since its primary materials could be water or oxygen, and it's very powerful as a disinfectant at the same time.

For the promising results got in this research, and the theoretic potentials that Ozonation has, the main author of this paper is working on testing many of the parameters mentioned above. The ultimate goal of the further research is to evaluate ozone ability as a powerful tool in sustainable agriculture: first to test its *in vivo* effectiveness and efficiency (economic feasibility), second to evaluate its optimal conditions for best outcomes, and finally to demonstrate its diffusion amongst societies as a viable innovation (social adoption). Worth to mention that the third Sustainability dimension (environmental safety) isn't needed to be proved for ozone, since it is known for its environmental benefits as explained previously.

References

1. D. Lyons-Johnson, 1997, 'Soybean cyst nematodes, look out!', *Agricultural Research*, 45, 9, p. 7, Academic Search Elite, EBSCO *host*, viewed 26 March 2011.
2. D. J. Chitwood, (USDA-A. (2001). Agricultural impact of nematodes. *Pest Control*, *17*(1981), 1104-1115.