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ORIGINAL RESEARCH ARTICLE

Novel control of house fly *Musca domestica* and bacterial isolates by ozone gas

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Abstract

Ozone is a sturdy oxidant which canrub insects and microorganisms. Thus, it could be useful in eliminate the Housefly in hospitals. The purpose of test here is to determine degree of ozone exposure required to remove houseflies and reduction of pathogenic bacteria. Ozone was effective with housefly, requiring only low minutes of treatment (1 to 14 min.) at minimum concentrations of ozone gas O_3 (0.125 g/m^3 to 1 g/m^3), the insects were eliminated the ozone. Thus, ozone exhibit potential as a fumigant for ousefly nesting materials, but moreover research is needed to evaluate its acceptability and efficacy in that field. The need for a dependable method to decontaminate housefly nesting materials as part of an overall ousefly (*Musca domestica*) system at hospitals sterilization is discussed. In this paper, we are using ozone gas as an alternative housefly pest and to remove house flies at hospitals, and we also illustrate data on the efficacy of ozone against the housefly (*Musca domestica*) and its effect on specific characteristics and properties of the treated zone application is currently attracting attention, particularly since. (a) There are no residues on the product. (b) There is no need for aeration to remove the gas. Green synthesis industrial applications and improvements in ozone technology together with new regulatory actions worldwide have emerged in last years, making it easier to use and applicable in a wide range - as the same with the pathogenic bacteria we are using ozone gas to control it, the numbers of bacterial cells remaining after ozone treatments were less than for untreated cells. The lowest dose of ozone gas for complete reduction of pathogenic bacteria was 0.125 g/m^3 after 24 h of incubation.

Keywords Ozone · Insects · Housefly (*Musca domestica*) · Pathogenic Bacteria

Introduction

Ozone is a kind of oxygen, or trioxygen, which is substantially less stable than O_2 and breaks down with a half-life of 20 to 50 min at room temperature to normal O_2 (O_3), which is a severe oxidant. It is frequently used as a treatment for both drinking water and swimming pools because it may disinfect as well as remove tastes, odors, and color's (Usepa 1999). It has recently been created as an agricultural fumigant; for instance, it is used to protect stored grains from rot and to get rid of insect and fungal pests (Kells et al. 2001; Allen et al. 2003; Mendez et al. 2003; Ballinger et al. 2005; Awad et al. 2022; Boopathy et al. 2022). O_3 has been used successfully as a disinfectant and sanitizer to eliminate pesticides, inorganic, and organic substances as well as to control insects and microbes (Boopathy et al. 2022). So, it is a powerful oxidant and highly harmful to living things at high doses, it is effective for these uses. Thus, it must be used in a sealed fumigation chamber, past from workers, and it is acutely harmful to people as well (Fouda et al. 2022). Ozone, however, quickly breaks down into oxygen (O_3), so it does not linger in the air or on wood or plastic. If ozone is produced directly from air and administered in a fumigation chamber in the hospitals and residences (Bernhardt et al. 2019), the registration procedures are quite straightforward. O_3 Application has the advantage of being more environmentally friendly than conventional fungicides and insecticides. Only air and power are necessary to produce ozone at the treatment facility. O_3 hence offers various safety

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benefits over traditional fungicides and insecticides. There are no harmful chemicals present, no residues on the products, no risk of chemical mixing, and no concerns with the disposal of old insecticides or containers (Légeron 1980; Law and Kiss 1991; Bornholdt et al. 2002; Lu et al. 2009; Rangel et al. 2021). The housefly may complete its life cycle in human and pet environments and is widespread in human activity areas such as hospitals, food markets, slaughterhouses, food centers or restaurants, poultry, and cattle farms (Francke et al. 2003; Fontes et al. 2012). Over 100 distinct bacteria capable of causing human diseases are colonized by houseflies in Central Europe (Fotedar and Roberts 1992; Falkenstein and Coogan 1997). Like *Staphylococcus aureus* (Gad et al. 2021), *Klebsiella* spp., *Escherichia coli* O157:H7 (Falkenstein and Coogan 1997; Hughes and Parkes 2007; Pereira et al. 2008; Rozado et al. 2008), *Salmonella* spp., *Shigella* spp. and *Proteus* (Jian et al. 2013). The goal of this study was to determine whether ozone can kill houseflies (*Musca domestica*) and, if so, to develop response curves for different ozone concentrations as well as pathogenic bacteria. The concentration of ozone required to kill bacteria has been recorded to range between 0.04 and 0.1 ppm.

Materials and methods

Ozone system

Ozone gas was obtained from a generator developed by the Center of Plasma Technology. In the gas generation process, the oxygen was used as input, passing through a dielectric barrier discharge (DBD) reactor. This type of discharge is produced by applying a discharge voltage between two coaxial electrodes, having a glass dielectric between them and a free space where the oxygen flows through.

In this free space a filament discharge is produced, where electrons are generated with enough energy to breakdown the oxygen molecules forming ozone.

The diagram of ozone generation is shown in Fig. 1. The ozone was generated using coaxial dielectric barrier discharge (DBD) technique at the Center of Plasma Technology, Faculty of Science, Al-Azhar University, Nasr City, Cairo, Egypt.

The Dielectric barrier discharge (DBD) cell was fed by oxygen gas. The concentration of the generated ozone was controlled by the discharge current, and the oxygen gas flow rate was adjusted to 0.1 L/min.

Effect of Ozone on house fly *Musca domestica*

Ozone was applied directly into the jars containing the adult housefly (*Musca domestica*). The concentration of ozone inside the jars was measured using ozone analyzer (Model H1-AFX-Instrumentation, USA) Fig. 1.

The input voltage of the AC test set (more specifically a variable high voltage transformer) was 220 V at 50 Hz. A voltage transformer was connected to two outer electrode and inner electrode, which were separated by gap. Voltages used to generate ozone were controlled through a transformer control box (Variac Variable AC Power Transformer Regulator).

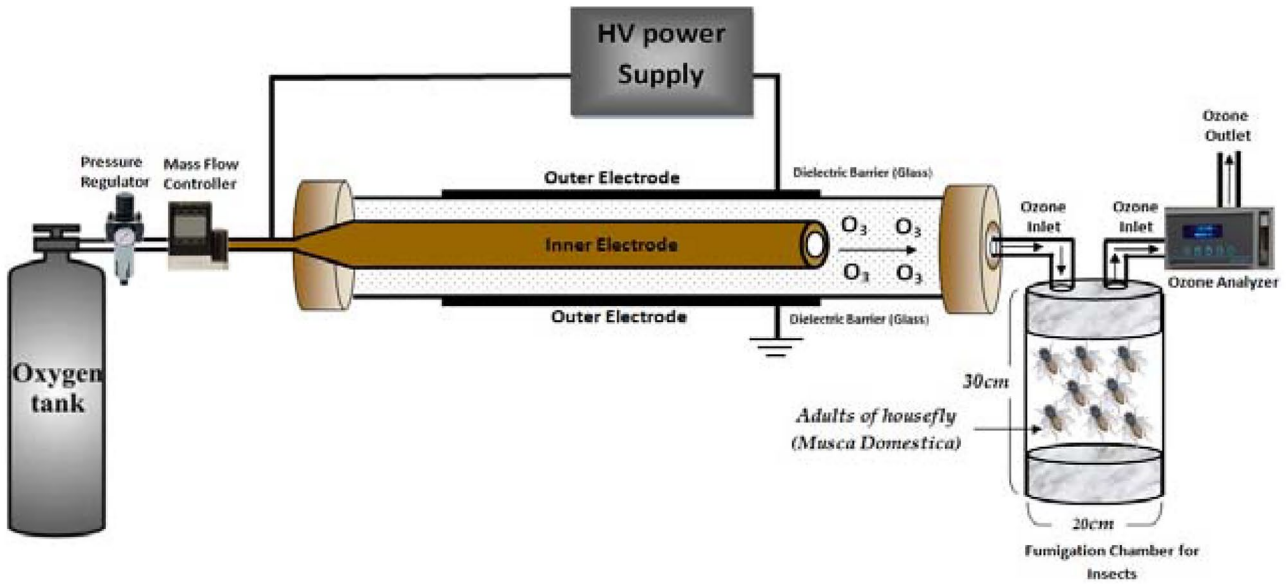

Fig. 1 Conversion of oxygen to O_3 and control of house fly (*Musca domestica*)

Effect of ozone on pathogenic Bacteria

The initial tests with the ***seudomonas aeruginosa, Escherichia coli, lebsiella pneumoniae, Staphylococcus aureus***, **and *Bacillus cereus*** strains demonstrated that doses greater than or equal to 0.125 g/m^3, 0.25 g/m^3, 0.5 g/m^3 and 1 g/m^{3}for 1 ,2, 5 and 10 min.

We used the growth measurement according to (Fontes et al. 2012). The pellets of four broth cultures (control and treated) were serially diluted with distilled water and the dilutions were made up to 10 − 7 from 10 − 5 and 10 dilution 1 ml of each suspension was spreader on nutrient agar plates Fig. 2. The plates were incubated for 37ºC at 24 h. After incubation the bacterial colonies were counted, and result was recorded. No. of cells = Organisms per millimeter / Gram of the sample = No. of colonies (Average of 3 replicates) / Amount plated X dilution.

Results and discussion

Electrical characteristic of DBD

Figure 3 shows waveforms of the applied voltage on the reactor *(coaxial dielectric barrier discharge)* and the associated discharge current measured in Oxygen plasma at gas flow rate of 0.1 (L/min). When the AC applied voltage on the DBD reactor reaches the onset value, the streamer discharge starts in the gap inside the reactor in the form of discrete current spikes. These spikes are related to the formation of micro discharges (filaments) of tens of nanosecond (ns) duration in the gap space (Gherardi et al. 2000). The filaments are randomly distributed over entire electrode surface. The streamers cross the discharge gap and spread on the surface of the dielectric barrier, building up surface charges, which produce electric field opposite to that of the applied voltage. After a short time (several ns), the streamer activity in that spot is extinguished, followed by streamer initiation in another location.

The peak of each individual spike is related to the number of instantaneous microfilaments that were formed at this instant, and hence a high current spike indicates that a high number of micro discharges initiated almost simultaneously.

A simple method for obtaining the consumed power is using the discharge Lissajous figures, obtained when plotting transported electric charge Q through the discharge as a function of the applied periodical voltage (Francke et al. 2003; Nersisyan and Graham 2004). The charge Q is delivered from the voltage drop across a measuring capacitor of 3.35µF. The average electric energy dissipated in a discharge cycle, is simply the area of the characteristic Lissajous figure, which in most cases is nearly a parallelogram (Falkenstein and Coogan 1997). Lissajous diagrams have been plotted at KVs applied voltages where the voltage is difference between the two electrodes has been measured as a function of the charge on the electrodes. Figure 4 shows Lissajous diagrams at 3.6, 3.8, 4, 4.4 KVs measured for Oxygen plasma at gas flow rate of 0.1 (L/min), the area of parallelogram increases with the increase in applied voltage because; the energy dissipated in a discharge cycle is proportional to the area of the parallelogram. The dissipated power has been calculated by multiplying the area of the parallelogram by the frequency of the used AC power supply (50 HZ). The change of the average dissipated power, calculated from the Lissajous figures with the applied voltage is shown in (Table 1). As can be seen from this table, the average dissipated power increases with the increase in applied voltage but remains relatively low even

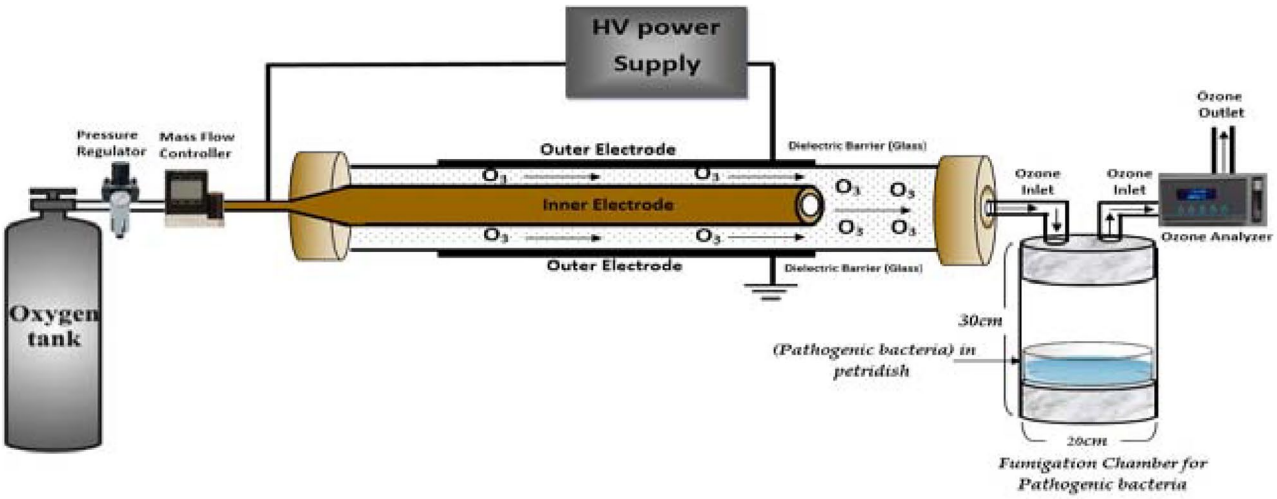

Fig. 2 Conversion of oxygen to O3 and control of Pathogenic Bacteria

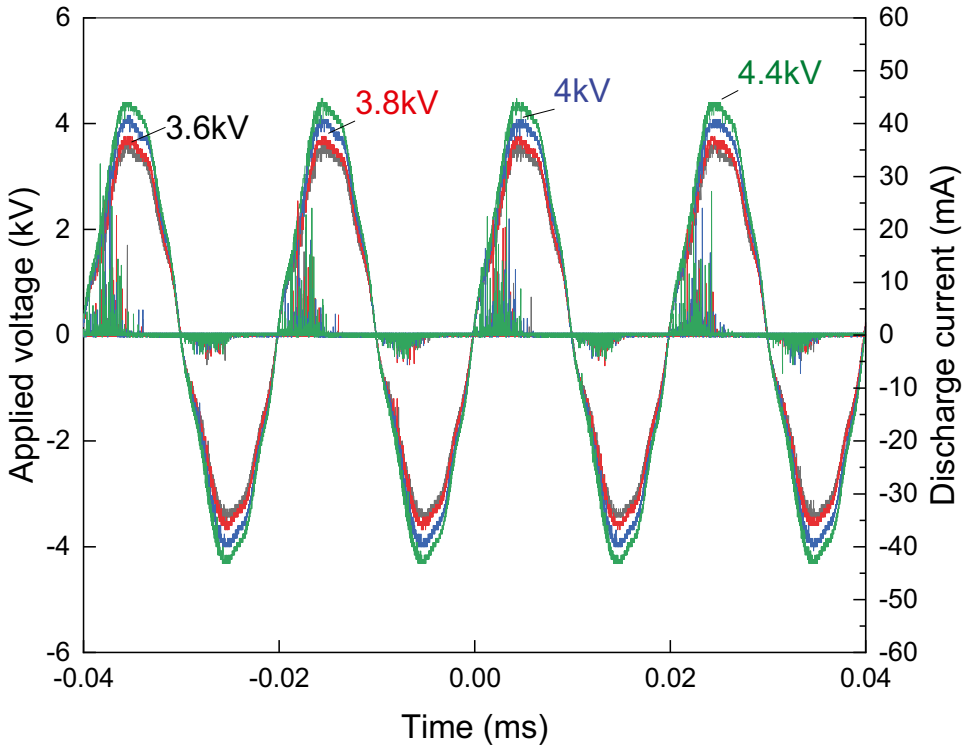

Fig. 3 Wave forms of the applied voltage to reactor and associated current measured for **a** Oxygen plasma at gas flow rate of 0.1 (L/min)

at a higher applied voltage. This result may be referred to the characterized filamentary discharge behavior where, the time of the filament is very short (few tens of nanoseconds).

It is noticed that the area of parallelogram increases with the increase in applied voltage because; the energy dissipated in a discharge cycle is proportional to the area of the parallelogram. The dissipated power has been calculated by multiplying the area of the parallelogram by the frequency of the used AC power supply (50 Hz). The change of the average dissipated power calculated from the Lissajous figures with the applied voltage is shown in Table 2.

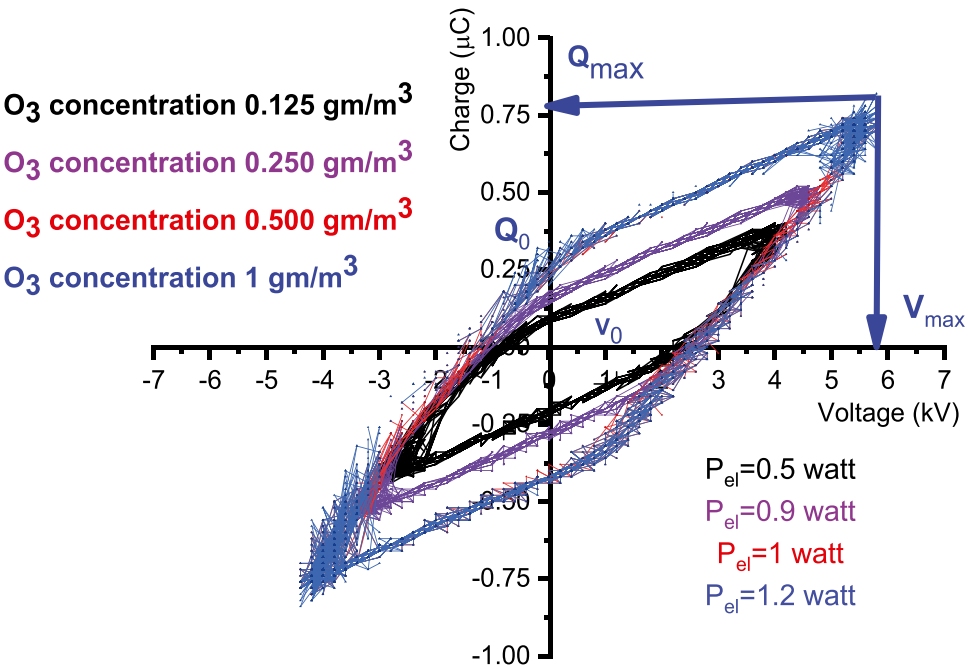

Fig. 4 Lissajous diagrams at 3.6, 3.8, 4, 4.4 KVs measured for Oxygen plasma at gas flow rate of 0.1 (L/min)

Table 1 Values of applied voltage and corresponding power values

Ozone concentration	Applied voltage (kV)	Electric power P_{el}(Watts)
0.125 g/m^3	**3.6 kV**	**0.5 W**
0.250 g/m^3	**3.8 kV**	**0.9 W**
0.5 g/m^3	**4.0 kV**	**1 W**
1.0 g/m^3	**4.4 kV**	**1.2 W**

Power measurement method

The power was analyzed following the original work of (Manley 1943), who has utilized voltage–charge Lissajous figures to characterize the average consumed power through the discharge (Fotedar and Roberts 1992; Gherardi et al. 2000; Francke et al. 2003; Gad et al. 2021). The charge–voltage characteristic plot was revealed in Fig. 2. The two values of effective discharge capacitance are indicated by obtaining of two distinct slopes of the Q–V plot. The reactor (coaxial dielectric barrier discharge) power formula was shown in Eq. (1), where is the total power P_{el} related to the operating frequency (f), the peak voltage V_{max} and the minimum discharge voltage V_{min} at which micro-discharges are monitored in the discharge gap with the capacitances of the dielectric C_D and the gas gap C_g. From this so-called Lissajous Figure the minimum external voltage V_{min} at which the ignition occurs, the electric energy consumed per voltage cycle E_{el} and the electric power P_{el} can be estimated by the following relations (Ballinger et al. 2005):

$$E_{el} = \oint \mathrm{V(t)dQ} = \mathrm{C_{means}} \oint V(t)dV_{\mathrm{means}} = 4C_d \frac{1}{1 + C_g/C_d} V_{\min}(V_{\max} - V_{\min})$$

$$E_{el} = 2(V_{\max}Q_0 - Q_{\max}V_0) \equiv \text{area of } (Q - V) \text{ diagram}$$

$$P_{el} = \frac{E_{el}}{T} = fE_{el} \quad (1)$$

In this study, the consumed power was determined to be 26 W at applied voltage of 11.2 kV treatments was applied in case of Oxygen gas.

As can be seen from this table, the average dissipated power decreases with the increase in applied voltage but remains relatively low even at higher applied voltage. This result may be referred to the characterized filamentary discharge behavior where, the time of the filament is very short (few tens of nanoseconds).

Flies provide opportunity to study the effects of ozone, "They have a high metabolic rate, a short life span (about 30 days), and an extensive tracheal-tubular respiratory system that directly distributes inhaled substances to all tissues in the body. Their entire body is respiratory, Ozone-caused tissue damage even at low concentrations (Falkenstein and Coogan 1997; Kogelschatz et al. 1997; Nersisyan and Graham 2004). If the insect respiratory system was the major entry route of O_3 into the insect body (Kogelschatz et al. 1997), increased respiration rate with an increase of temperature might result in more mortality because the increased gas exchange would increase the amount of O_3 inside insect bodies (Janeco et al. 2011; Biganzoli et al. 2014).

The effects of ozone treatments with concentrations of 0.125, 0.25, 0.5, and 1.0 g/m^3 on the mortality of housefly (*Musca domestica*) adults are presented in Table 2. The results revealed that the mortality of housefly was increased

Table 2 Mean mortality (%) of housefly exposed to different concentrations of ozone at different time

Time exposure (minutes)	Control	Group (1) Mortality (%)	Group (2) Mortality (%)	Group3 Mortality (%)	Group4 Mortality (%)
0	**0%**	**Treatment Concentration (g/m^3) 0.125 g/m^3**	**Treatment Concentration (g/m^3) 0.25 g/m^3**	**Treatment Concentration (g/m^3) 0.5 g/m^3**	**Treatment Concentration (g/m^3) 1 g/m^3**
1	**0%**	**10%**	**30%**	**50%**	**80%**
3	**0%**	**20%**	**50%**	**70%**	**100%**
5	**0%**	**30%**	**70%**	**90%**	
7	**0%**	**50%**	**90%**	**100%**	
9	**0%**	**70%**	**100%**		
11	**0%**	**90%**			
14	**0%**	**100%**			

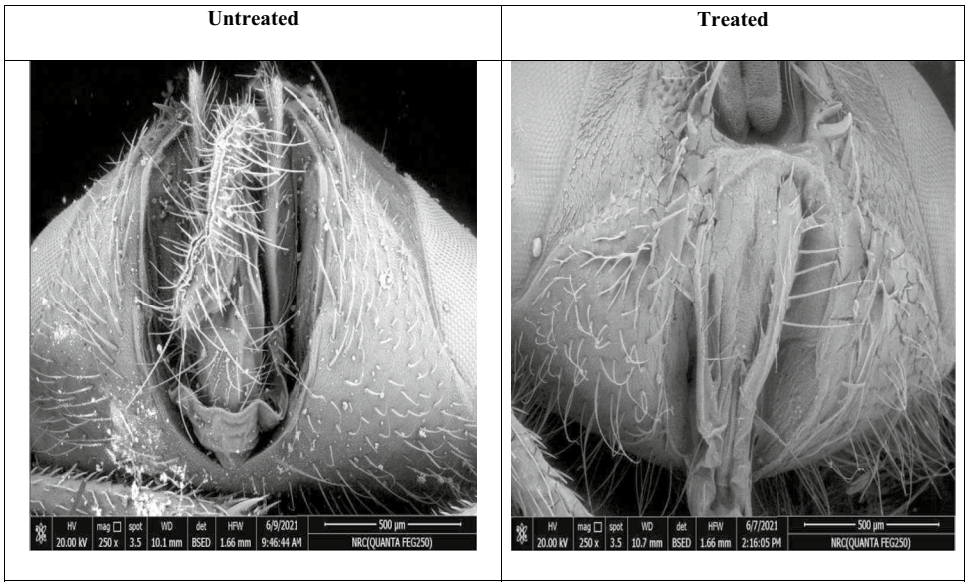

Fig. 5 Scanning electron micrographs of the head of housefly (*Musca domestica*) showing different between treated and untreated house fly and showing damage of tissues of the insect head, antenna and organs due to the exposed to ozone, which caused an explosion in the insects' organs in the treated house flies

with increasing the concentration and the time of exposure of insects to ozone from 10 to 100%. The highest adult mortality percent (100%) was induced with the concentration 0.125 g/m^3 at 14 min, this percent decreased to 90.0% after 11 min and gradually decreased till reached the lowest value 10.0% at the lowest time 1.0 min. Meanwhile, at the concentration 0.25 g/m^3 the highest mortality percent (100%) was recorded after 9 min and the lowest mortality percent was (30.0%) after one minute. Also, complete adult mortality was caused after 7 min at the concentration 0.5 g/m^3, this mortality percent decreased to 50.0% after one minute. At the highest concentration 1.0 g/m^3, the mortality percent was 80.0 and 100.0% after 1.0 and 3.0 min respectively Fig. 5. The effects of ozone treatments at concentrations of 0.125, 0.25, 0.5, and 1.0 g/m^3 on the reduction of pathogenic bacteria after 24 h of incubation were recorded in Table 3. The results revealed that the reduction of pathogenic bacteria was increased with increasing the concentration and time of ozone from 10 to 100%. Complete reduction of ***pseudomonas aeruginosa*** growth was caused at dose 0.25 g/m^3 after 10 min of ozonation. Also, complete reduction of ***Escherichia coli*** growth was caused at 0.125 g/m^3 after 10 min of ozonation. ***Klebsiella pneumoniae***, the

Table 3 Mean reduction (%) of pathogenic bacteria exposed to different concentrations of ozone and different time

Ozone dose / bacteria species		*seudomonas aeruginosa*	*Escherichia coli*	*lebsiella pneumoniae*	*Staphylococcus aureus*	*Bacillus cereus*
		Reduction (%)				
Treatment Concentration (g/m^3) 0.125 g/m^3 = 58.375 PPMV	T = 1 min	10%	30%	25%	35%	40%
	T = 2 min	25%	45%	50%	55%	60%
	T = 5 min	70%	70%	75%	80%	70%
	T = 10 min	95%	100%	100%	100%	100%
Treatment Concentration (g/m^3) 0.25 g/m^3 = 116.75 PPMV	T = 1 min	40%	40%	45%	50%	60%
	T = 2 min	60%	65%	70%	80%	95%
	T = 5 min	80%	95%	100%	100%	100%
	T = 10 min	100%	100%	-	-	-
Treatment Concentration (g/m^3) 0.5 g/m^3 = 233.5PPMV	T = 1 min	45%	60%	80%	75%	70%
	T = 2 min	65%	80%	95%	90%	95%
	T = 5 min	90%	100%	100%	100%	100%
	T = 10 min	100%	-	-	-	-
Treatment Concentration (g/m^3) 1 g/m^3 = 467 PPMV	T = 1 min	60%	80%	90%	85%	90%
	T = 2 min	80%	100%	100%	100%	100%
	T = 5 min	95%	-	-	-	-
	T = 10 min	100%	-	-	-	-

complete reduction proceeds after 10, 5 and 2 min with ozone dose 0.125, and 1.0 g/m^3 respectively. The optimum ozone dose for complete reduction of ***Staphylococcus aureus*** and ***Bacillus cereus*** was caused at 0.25, 0.5 and 1.0 g/m^3 after 10, 5 and 2 min respectively.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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