Short Communication

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In vitro evaluation of acaricidal activity of aqueous ozone against *Dermanyssus gallinae*

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Keywords

Acaricide, aqueous ozone, Dermanyssus gallinae, laboratory conditions

Abstract

The aim of this study was to investigate the in vitro acaricidal effect of aqueous ozone against *Dermanyssus gallinae* (Acari: Dermanyssidae) under laboratory conditions. For this purpose, aqueous ozone at concentrations of 1, 2, 3, 4, 5 and 10 ppm were prepared, and five replicate experiments were carried out using 400 µl of each concentration sprayed on every treatment batch of mites (plus a distilled water control). The mortality rate of mites in treatment and control groups were assessed 24 h post exposure. The mortality rate obtained by concentrations of 4, 5, and 10 ppm were significantly different from the control group ($p < 0.05$). The highest mortality rate (63.99%) was observed at concentration of 10 ppm. In this study, aqueous ozone showed a dose-dependent acaricidal potency against *D. gallinae*.

Abbreviations

D. gallinae: *Dermanyssus gallinae*
ppm: parts per million

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Introduction

Dermanyssus gallinae negatively affects chickens' health via biting and sucking blood which consequently can cause economic losses due to a decrease in egg production, down-grade eggs, increase in mortality of laying hens and transmitting some pathogenic agents [1].

Extensive and repeated usage of conventional chemical compounds in commercial poultry farms has led to the development of acaricide resistance in *D. gallinae* [2, 3]. Acaracide resistance and increased demand for organic foods have motivated researchers to investigate novel ways of controlling this ectoparasite, especially in commercial poultry houses [4].

Ozone is a strong oxidant. It is an allotrope of oxygen and consists of three oxygen atoms. It is an unstable molecule with short half-life and readily degrades into O_2 and a free oxygen atom. This atom disrupts and kills microorganisms through reaction with the cell membrane and cellular components [5]. Ozone is commonly generated using an electrical discharge. In recent years, the efficacy of ozone as a means of controlling post-harvest grain pests has been evaluated. Insecticidal effects of gaseous ozone against the confused flour beetle (*Tribolium confusum*), the red flour beetle (*Tribolium castaneum*), maize weevils (*Sitophilus zeamais*) and Indian meal moths (*Plodia interpunctella*) have been investigated and ozone was found to be efficacious against stored product insects under both laboratory and field conditions [6-8].

The aim of this study was to evaluate the acaricidal activity of aqueous ozone specifically against *D. gallinae* under laboratory conditions.

Results

The examined groups of mites exposed to aqueous ozone at concentrations of 1, 2, 3, 4, 5 and 10 ppm showed 13.13, 25.99, 36.66, 47.33, 53.99 and 63.99% mortality, respectively. The mortality rates were dependent on ozone concentration, with ascending mortality rate as concentration increased ($p < 0.05$). The mortality rates at 4, 5 and 10 ppm concentrations of aqueous ozone were significantly different than the control group ($p < 0.05$). The highest mortality rate (63.99%) was observed at concentration of 10 ppm (Figure 1).

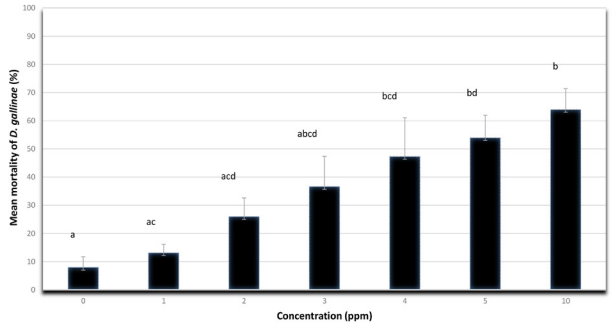

Figure 1.
Mean mortality (%) (± SE) of *Dermanyssus gallinae* exposed to various aqueous ozone concentrations (ppm). Means designated by different letters are significantly different ($p < 0.05$).

Discussion

Huge economic losses imposed by *D. gallinae* infestation across the world and restriction in the use of conventional acaricides, indicates that searching for new control strategies is an important necessity. Because ozone acts as a strong oxidizer, it is highly toxic to living organisms such as microorganisms, fungi, insects and mites [9, 10]. Ozone is thought to kill organisms by oxidation of cell membrane and cellular components such as enzymes, proteins, fatty acids, it can also destruct and damage nucleic acids [11, 12].

Whilst working with aqueous ozone is much safer than gaseous ozone and neither hazardous to skin nor to eyes, a few researches have been conducted on acaricidal/insecticidal activity of ozonized water [13]. Besides these advantages, reaction between ozone and water also results in the formation of highly reactive radicals such as $^*OH$, $^*O_2-$, $^*HO_2$ and $^*O_3-$ which can cause fatal damage to respiratory system of insects and acarians [12]. All ozone derivative radicals can affect adversely cellular membranes and collapse DNA (deoxyribonucleic acid) structure of affected cells, among them hydroxyl free radical is much more powerful [12, 14]. Despite the lack of documented researches about various practical uses of aqueous ozone in livestock and poultry industry, it is already in use in dairy farms and relevant industries [15]. Also, a portable ozone mist system for the pest control in farms has been developed [12].

In this preliminary study, the acaracidal potency of aqueous ozone against *D. gallinae* was demonstrated, with an average mortality of 64% at ozone concentration of 10 ppm. However, to the best of our knowledge, no study has yet investigated the effect of ozone against poultry red mites. Several factors influence ozone effectiveness against a living organism including method of application, concentration, exposure time, type of organism, life stage of organism, temperature and relative humidity [16, 17]. Exposure of *Plodia interpunctella* to 500 ppm gaseous ozone for 60 minutes resulted in 100% mortality [18]. Niakausari et al. (2010) reported complete mortality of adult *Phoe-*

nix dactylifera when exposed to gaseous ozone at concentrations more than 2000 ppm for 2 h [19]. In the present study, based on statistical analysis, increasing in ozone concentration can result in more mortality of *D. gallinae*, however; producing and applying ozonized water with more than 10 ppm concentration due to our technical limitations and safety concerns was not possible. Niakausari et al. (2010) also found that ozone at lower concentrations required a much longer exposure time to be effective [19]. Due to the unstable nature of aqueous ozone, we tried to increase the exposure time by increasing ozone half-life time. Half-life time of ozone is notably affected by temperature and researchers have found that lower temperatures optimize the half-life of aqueous ozone [20]. At 20°C and pH 7, the half-life of ozone in potable tap water is about 24 minutes and in our study drinking tap water at 10°C was used to achieve longer ozone half-life time.

In this study, aqueous ozone showed a dose-dependent acaricidal potency against *D. gallinae*. These findings cannot be extrapolated directly to decreasing the mite population in infested poultry farms and increasing productivity as a consequence of spraying aqueous ozone. Further researches should be done to determine the concentration of aqueous ozone with the highest acaricidal activity, and the least probable adverse effects of aqueous ozone on birds' health in poultry farms at rearing and egg-production.

Materials and methods

Mite collection and preservation

D. gallinae were collected from a naturally infested caged laying poultry farm and were kept at 22°C with a 16:8 light - dark cycle.

Ozone generation

Ozone was produced using a laboratory corona discharge ozone generator (Ozone Tajhiz Co. Mashhad, Iran). The Maximum concentration of 143 g/m^3 was obtained at oxygen flow rate of 1.5 L/min. Gaseous ozone was injected into a water column for producing aqueous ozone. Ozone concentration in the column was determined using a colorimetric kit (CHECK kit ® Comparator ozone, The Trinometer Hd. England). Ozonized water through a valve located at the bottom of water column was poured into a glass beaker for determining the ozone concentration, then desired amount of certain concentration was transferred into a handheld sprayer and used in bioassays.

Bioassay

For this study, six treatment groups and one control group were set up. A petri dish containing over 250 mites was placed in a freezer at 0°C for five minutes to immobilize them. Seven batch of mites (each batch contained 20 mites) were separately transferred to a glass petri dish (90 mm diameter and 15 mm depth) lined with Whatman filter paper by an aspirator. Different aqueous ozone concentrations of 1, 2, 3, 4, 5 and 10 ppm were prepared and 400 µl of desired concentration was sprayed on each treatment group, using a handheld sprayer. The control group was subjected to distilled water with the same procedure. Each Petri dish was sealed using parafilm before the lid was placed on top. The plates were incubated at 22°C for 24 h. Finally, mite mortality was examined under a dissecting microscope, where a mite was considered dead if it did not show any sign of movement when it was agitated with an entomological pin. The bioassay was repeated five times.

Statistical analysis

All data were analyzed using SPSS ver. 22 for Windows (SPSS Inc., Chicago, Illinois) and one-way analysis of variance (ANOVA) was used for comparing mortality rate. Pearson correlation test was used to determine the relationship between ozone concentration and mite mortality. Mortality rates were adjusted using Abbott's formula. *p* values less than 0.05 were regarded statistically significant.

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Author contributions

Conceived and designed the experiments: AM. Performed the experiments: HDB, AM, MHHK, GAK. Analyzed the data: AM. Wrote the paper: AM.

Conflict of interest

The authors declare that there is no conflict of interests.

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