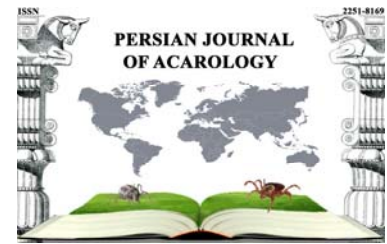




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Article

On the effect of ozonated water on mortality of *Tetranychus urticae* (Trombidiformes: Tetranychidae) on *Capsicum annuum* (Solanaceae) in greenhouse conditions

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ABSTRACT

Pesticide-resistant populations of the two-spotted spider mite, *Tetranychus urticae* Koch, have been reported in many countries. This problem has led to the search for alternative control methods. One of these alternative strategies is aqueous ozone. During a series of preliminary test, the effect of different concentrations of aqueous ozone on mortality of *T. urticae* on pepper (*Capsicum annuum* L.) was evaluated in greenhouse conditions at 25 ± 2 °C; $50 \pm 5\%$ RH and 16:8 (L:D) photoperiod. Then efficacy of ozone to control the pest was evaluated in different experimental conditions including three exposure times (5, 10 and 15 seconds), different time of spraying (8 am, 2 pm and 8 pm), and different ages of the host plant (4, 8 and 12 weeks old). Each experiment was carried out in a randomized complete design with six replications. Based on the screening tests, ozone concentration of 43 g/m^3 was found to give efficient results in terms of mortality rates of the mites and morphological traits of the host plant. The mortality rate of the mites increased with increasing exposure time. Although the interaction effect between ozone concentration and exposure time was significant, in other experiments, no significant difference in the mortality percentage of the mite among either times of application, or ages of the host plant was found. In none of these experiments, no symptom of injury to the host plant foliage such as chlorosis, necrosis, yellowing or malformation was observed after aqueous ozone spraying. Based on these results, aqueous ozone at a specific concentration and conditions can reduce the population density of *T. urticae* without any visible damage on the pepper foliage. However, more research needs to be done before ozonated water can be deployed commercially as a pesticide.

KEY WORDS: Acari; alternative control methods; aqueous ozone; greenhouse pests; pepper; two-spotted spider mite.

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INTRODUCTION

These days environmentally friendly alternatives to agricultural chemicals for plant pests have been required. The use of ozone, a triatomic form of oxygen (O_3), could reduce agricultural chemicals.

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Ozone, an unstable gas with a half-life of about 20 minutes, has many advantages as a sanitizer. For example, ozone is generated on-site, eliminating the need to store or dispose of chemical containers, no residue on treated product and chemical reaction with organic material occurs very quickly, which prevents microorganisms from developing tolerance to ozone (Mendez *et al.* 2003; Isikber and Oztekin 2009). It is a safe method because it decomposes to oxygen, thus leaving no undesirable residue.

Tetranychus urticae Koch is a serious cosmopolitan pest species commonly found on many horticultural and agricultural crops such as pepper, *Capsicum annuum* L. (Chaudhri *et al.* 1985; Tsagkarakou *et al.* 2002). The importance of pepper is due to the nutritional value of its fruits (high content of dry substance, vitamin C and B-complex, minerals, essential oils, carotenoids, etc.) and their various kinds of utilization in the culinary and food industry of different countries. In Iran, the cultivation of pepper as a greenhouse vegetable is growing. Despite its economic, food and medicinal importance, its cultivation is facing many stresses including pests that cause severe yield losses. The short life span, high reproductive potential and development of resistance to many pesticides make chemical control of the pest particularly difficult (Tsagkarakou *et al.* 2002; Fathipour *et al.* 2006). So, a program of integrated pest management is essential for the control of *T. urticae*. Application of aqueous ozone is one of the non-chemical control methods. Ozone and its derivative radicals such as $*O_2^-$, $*HO_2$, $*OH$, $*O_3^-$ enter the pests through spiracles of respiratory system and cause damage. Also, it can affect the structure of DNA (Ebihara *et al.* 2013).

The efficacy of ozone against stored product pests have been reported in many studies (Kells *et al.* 2001; Sousa *et al.* 2008, Isikber and Oztekin 2009; Bonjour *et al.* 2011; Keivanloo *et al.* 2013, 2014), but few studies have been done about the greenhouse pests (for example, Hollingsworth and Armstrong 2005; Ebihara *et al.* 2013; Takigawa *et al.* 2013). This can be explained by some studies over the past 50 years that have shown ozone as a phytotoxic gas which has negative effects on plants (Davison and Barnes 1998; Fuhrer and Booker 2003; Fiscus *et al.* 2005), may have inadvertently led to an oversight of the prophylactic use of aqueous ozone, to control of greenhouse pests. However, some researchers reported that the phytotoxic properties of gaseous ozone and aqueous form are different. Because aqueous ozone does not interact with plants in the same way as ozone gas. In aqueous solution, the mass transfer physics and chemical stability are much different than in the gas state (Fujiwara and Fujii 2002; Sloan and Engelke 2005). Also, plants respond to ozone induced oxidative stress and systemic acquired resistance without visible injury (Sandermann 1996; Pell *et al.* 1997; Kovalchuck *et al.* 2003). More information is needed to clarify the effect of aqueous ozone on plant and pest interactions (Fujiwara and Fujii 2002).

Depending on the ozone concentration, the duration of exposure, previous exposure (adaptation), environmental conditions (wind speed, humidity, temperature), water status, plant genetics, stomatal functioning, cuticular composition and plant developmental stage, the type and severity of injury is different (Heagle 1989; Sandermann 1996; Pasqualini *et al.* 2002).

Ozone gas enters the leaves through the stomata during normal gas exchange (Trumble *et al.* 1987; Malaiyandi and Natarajan 2014). Thus, it is expected that partial stomatal closure, in response to the photoperiod decreases the rate of foliar gas exchange at different time of day. As a result, a reduction in susceptibility of plants to ozone injury is expected. In present study, we tested the hypothesis that ozone damage will be fewer if plants are exposed to ozone at night rather than during the day.

The purpose of this study was to (1) determine a concentration of aqueous ozone that would cause an acceptable mortality rate of the two-spotted spider mite, *T. urticae* on pepper plant at controlled environment; (2) the mortality rate of *T. urticae* at different exposure time to ozonated water under specific ozone concentration and flow rate; (3) the mortality of *T. urticae* at different time of day; (4) the mortality of the mites at different ages of the host plant to ozone spraying and (5) the possible detrimental effects of ozonated water on morphological features of the host plant (*C. annuum* L.).

MATERIALS AND METHODS

Tetranychus urticae culture

The founder population of *T. urticae* was collected from infested cucumber (*Cucumis sativus* L.) in a greenhouse located in Ferdowsi University of Mashhad, Mashhad, Iran in 2016. The mites were reared on pepper plants (*C. annuum* L. cv. Red chili) in the controlled environment (25 ± 2 °C; $50 \pm 5\%$ RH and 16:8 (L:D) photoperiod). After three generations, mites from the stock colony were used for conducting the experiments.

Host plant

The pepper seeds (*C. annuum* L. cv. Red chili) were germinated in plastic trays (26 × 25 cm) with 50 cells containing vermicompost. Three-week-old seedlings were transplanted at plastic pots (12 × 12 cm) filled with a mixture of field soil: perlite: peat: vermicompost at ratios of 1:1:1:1. Plants were kept under controlled conditions at 25 ± 2 °C; $50 \pm 5\%$ RH and a photoperiod of 16: 8h (L:D). The plant pots were irrigated daily. After 8 weeks, plants were used for experimental units except in the case of the effect of different plant age where 4, 8 and 12-week-old plants were used.

Experimental setup

Ozone for the experiment was generated using an ozone generator (from Ozoneab® Company Inc., Iran, <http://www.ozoneab.com> under the license of Tech Trade International, Australia), an oxygen generator (Model: LFY-I-5F-WY from LONGFEI® Company, China), and a container of ozone gas (from Ozoneab® Company). The output of the ozone generator was estimated with a portable ozone analyzer (was obtained from “A Teledyne Technologies® Company”, San Diego, USA. Process Ozone Monitor, Model 454). Aqueous ozone solutions were prepared at the time of experiments. It was produced by forcing ozone gas into a distilled water (with pH 7) container. Aqueous ozone enters the pump through the outlet of the container (with 30 PSI pressure and 4350 cc per minute flow rate). The pump provides the required pressure to throw droplets through the nozzle. Following Ebihara *et al.* (2013) the treatment was performed at the distance of 20 mm between the ozone ejection nozzle and the plants.

Bioassays

Range finding test

Preliminary screening tests were carried out in controlled conditions of 25 ± 2 °C; $50 \pm 5\%$ RH and a photoperiod of 16L: 8D h. To perform these experiments, 30 adult female mites from our stock culture were transferred to each pot plant for oviposition. After 12 hours, all adult mites were removed from the test pot plants. After 14 days, before conducting the experiments the number of mites on each test plant was recorded. To determine a concentration that would cause 25–75% mortality, preliminary concentration-mortality tests followed Robertson and Preisler (1992) were done before conducting main experiments.

Duration of exposure times of ozonation

In similar environmental conditions to screening tests, a homogenous population of *T. urticae* was provided before conducting any experiment. Thirty adult female mites were transferred to each pot plant for oviposition. After 12 hours, all adult mites were removed. After 14 days, before conducting the experiments the number of adults and nymphs on each test plant was recorded. Infested plants with estimated population of mite were exposed to 43 g/m^3 ozone concentration with 12.87 g/h ozone flow rate for three different exposure times of 5, 10 and 15 seconds with 6 replications. The control plants were sprayed only with distilled water. The mortality of mites was assessed after one day. Following Ay and Kara (2011) the mite that could not walk was considered as dead.

Usage times of ozonation

The same as above mentioned method, the effect of application time of ozonated water were carried out at a controlled environment of 25 ± 2 °C; $50 \pm 5\%$ RH and a photoperiod of 16L:8D. Infested test plants were exposed to 43 g/m^3 ozone concentration with 12.87 g/h ozone flow rate, for 10 seconds at three different time of a day (8 am, 2 pm and 8 pm) by a sprayer. For each treatment, the control treatment was sprayed only with distilled water. The experiment was replicated six times. One day after treatment, the mortality of mites was assessed.

Age of the exposed plants

Six combinations of ozone concentration and plant were tested. Infested test plants (4, 8 and 12 weeks old) with determined mite population on them were exposed to 43 g/m^3 ozone concentration with 12.87 g/h ozone flow rate, for 10 seconds by a sprayer. The control plants were sprayed only with distilled water. The experiment was replicated six times. The mortality of mite was assessed after one day.

In all treatments, before and also 24, 48, 72 hours after ozonation, plants were carefully inspected for noticeable damage including chlorosis, necrosis and yellowing symptoms or malformation.

Data analysis

Effect of aqueous ozone on mortality of *T. urticae* on pepper was conducted in a randomized complete design (factorial design). The experimental design had two independent factors i.e., 1. Spraying (with 2 levels: ozone & water) and 2. The time of spraying (with 3 levels) (or age of plants (with 3 levels) or exposure time (with 3 levels)) where the effect of spraying and the time or age plant were evaluated on the mortality rate of spider mites. All analyses were carried out using the statistical program, R (R core team 2017). Data were analyzed by the generalized linear model (GLM) with an ANOVA table built up by sequentially deleting terms from the model. Since no significant interactions were found for the experiment of different usage times and age of the plant, we focused on the main effects and then they were compared by using Tukey's test. Data were transformed by using Shapiro-Wilk normality test where appropriate to satisfy the assumption of normality and homogeneity of variance for ANOVA.

RESULTS

Range finding test

Based on the preliminary concentration-mortality tests, ozone concentration ranging from 14.5 to 43 g/m^3 with 12.87 g/h ozone flow rate was found to give efficient results in terms of mortality rates of the two-spotted spider mite, *T. urticae*, without any visible malformation or colour changes on pepper foliage, the host plant.

Mortality of Tetranychus urticae at different usage time of ozonation

The results showed that there was no significant interaction between ozone concentration and usage time, so, data were analyzed separately for each main effect ($F_{2, 30} = 0.04$, $P = 0.95$). Also, no significant differences in the mortality of *T. urticae* at different usage times were found ($F_{2, 33} = 0.01$, $P = 0.98$) (Table 2). Statistical analyses showed that the mortality rate of *T. urticae* was significant between 0 and 43 g/m^3 ozone concentration treatments ($F_{1, 32} = 307.80$, $P < 0.0001$). Forty-three (43 g/m^3 aqueous ozone concentration caused a mortality of 60.86 ± 3.14 percentage of *T. urticae* compared with 0 ozone concentration (2.90 ± 0.71) (Table 1).

Mortality of Tetranychus urticae at different exposure times of ozonation

The results of this experiment showed that the interaction effect between ozone concentration

and exposure time was significant ($F_{2, 30} = 34.00$, $P < 0.0001$). Also, differences in the mortality percentage of 0 and 43 g/m^3 ozone concentration treatments were significant ($F_{1, 32} = 137.38$, $P < 0.0001$). The mortality of *T. urticae* at 43 g/m^3 ozone concentration at three exposure times (5, 10 and 15 seconds) was 26, 57.6 and 73.52% respectively (Fig. 1). As it is shown in Figure 1, the mortality percentage of the mites increased with increasing exposure time. Statistical analyses showed that differences in the mortality percentage of the mites among exposure time treatments at the same ozone concentration were not significant ($F_{2, 33} = 2.21$, $P = 0.12$).

Table 1. The effect of ozone concentration on mortality rate of *T. urticae* on pepper (*Capsicum annuum* L.) in a controlled environment.

Ozone concentration (g/m^3)	Mean \pm SE
0	$2.90 \pm 0.71^{\text{b}*}$
43	$60.86 \pm 3.14^{\text{a}}$

* Columns with the same letter were not significantly different based on Tukey's test at $\alpha = 0.05$

Table 2. The effect of usage times on mortality rate of *T. urticae* on pepper (*Capsicum annuum* L.) at 0 and 43 g/m^3 ozone concentration in a controlled environment.

Time	Mean \pm SE
8:00 am	$30.74 \pm 9.24^{\text{a}*}$
2:00 pm	$31.78 \pm 8.88^{\text{a}}$
8:00 pm	$33.12 \pm 9.40^{\text{a}}$

* Columns with the same letter were not significantly different based on Tukey's test at $\alpha = 0.05$

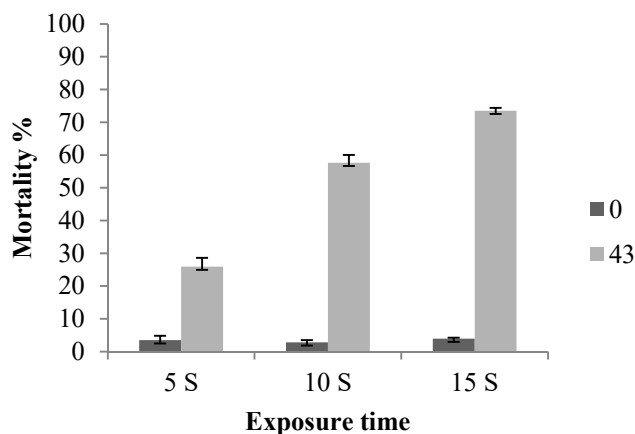


Figure 1. Interaction effect between ozone concentration (0 and 43 g/m^3) and exposure time (5, 10 and 15 s) on mortality percentage (mean \pm SE) of *T. urticae* on pepper (*Capsicum annuum* L.) in a controlled environment.

Mortality of *Tetranychus urticae* at different age of the exposed plants

Statistical analyses showed no significant interaction between ozone concentrations and age of the host plant, so data were analyzed separately for each main effect ($F_{2, 30} = 1.54$, $P = 0.23$) (Figs. 2, 3). Although the difference in the mortality% of the mites between 0 and 43 g/m^3 ozone concentration treatments was significant ($F_{1, 32} = 483.19$, $P < 0.0001$), the mortality% of mites among 3 age of the exposed plants (4, 8 and 12-week-old plants) was not significant ($F_{2, 33} = 0.08$, $P = 0.91$).

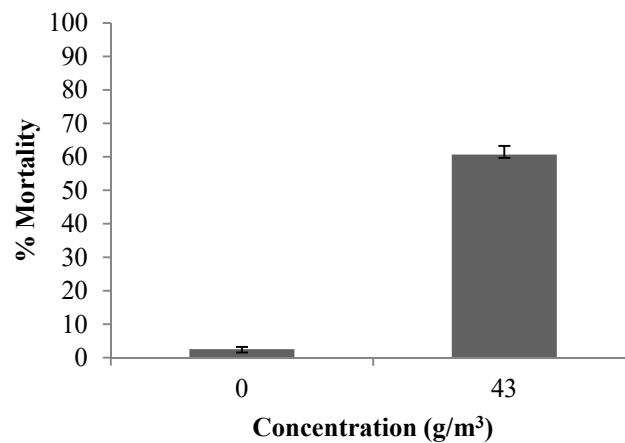


Figure 2. The effect of ozone concentrations (0 and 43 g/m³) on mortality rate (mean ± SE) of *T. urticae* on pepper (*Capsicum annuum* L.) in a controlled environment.

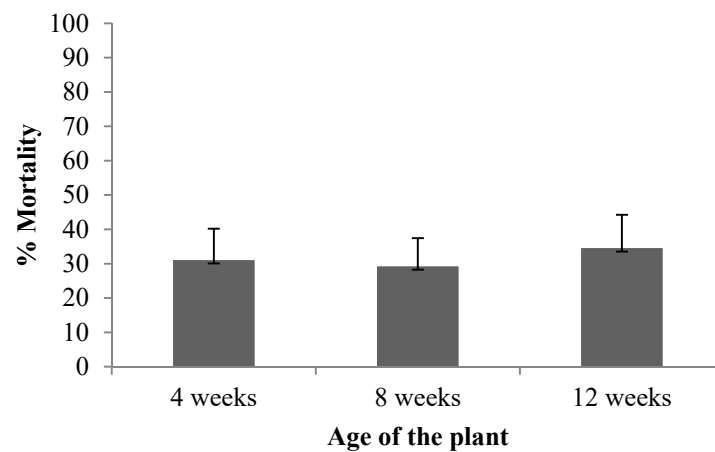


Figure 3. The effect of age of the plant (4, 8 and 12 weeks old) on mortality rate (mean ± SE) of *T. urticae* on pepper (*Capsicum annuum* L.) at 0 and 43 g/m³ ozone concentration in a controlled environment.

Damage on plants

According to results, no visible injury or morphological disorder such as chlorosis and necrosis symptoms or malformation was observed for any of the plants treated with ozonated water at a concentration of 43 g/m³ in all three experiments: a) time of sprayings (8 am, 2 pm and 8 pm), b) duration of exposure times (5, 10 and, 15 seconds), and c) age of the exposed plants (4, 8 and 12 weeks old) which they were assessed 24, 48, and 72 h after the spraying.

DISCUSSION

This study showed that aqueous ozone at a concentration of 43 g/m³ has potential of reducing the active populations of *T. urticae* up to 73.52 % without any visible injury or morphological disorders such as chlorosis or necrosis symptoms and malformation to pepper, the host plant. This preliminary results support the works by others that ozonated water has the potential to control some pests. For example, Hollingsworth and Armstrong (2005) reported that a 30-min. treatment of ozone at \approx 200 ppm in 100% CO₂ at 37.8 °C caused 47.9 and 98.0% mortality of *Pseudococcus longispinus* Targioni Tozzetti, and *Frankliniella occidentalis* Pergande, respectively. Keivanloo *et al.* (2013) found that *Plodia interpunctella* Hübner was susceptible to 2, 3 and 5 ppm aqueous ozone concentration. They

reported mortality rate of 5-day-old larvae increased from 0.00 at control treatment to 88.3 after 2 hours of exposure to ozone at 5 ppm concentration. Takigawa *et al.* (2013) observed that when *Uroleucon nigrotuberculatum* Olive was exposed to 86 g/m³ for 5 seconds, the survival rate of the aphids was 3.3%. Also, Ebihara *et al.* (2013) reported that a concentration of 68 g/m³ of ozone-mist treatment for 30 seconds resulted in 98% mortality on *U. nigrotuberculatum*.

Although ozone gas has known and well characterized phytotoxic effects such as foliar reddening, bronzing, chlorosis and necrosis (Heagle 1989; Fuhrer and Booker 2003; Fiscus *et al.* 2005), surprisingly, at present study no symptom of injury was observed on any of the pepper's leaves. Our findings indicate that when ozonation is carried out under controlled conditions, 43 g/m³ aqueous ozone do not cause any visible injury to the leaves of pepper, and this may be explained by either low ozone concentration or short time of ozone exposure. More concentration or more exposure time spraying may cause visible damage, but the effects of these conditions were not investigated in this study. Although a few studies have been reported some foliar injuries, at least in cases of some plants such as soybean, tomato and alfalfa the appearance of foliar symptoms of damage does not necessarily lead to decreases in measurable growth or yield (Tingey *et al.* 1973; Oshima *et al.* 1975; Tingey and Reinert 1975; Heagle *et al.* 1979).

The results presented here support those of Heagle *et al.* (1986, 1987) who observed no visible acute foliar injury in soybean and tobacco, respectively. Graham *et al.* (2009) reported that a dose of 62.5 µmol.L⁻¹ (or greater) of aqueous ozone on *Salix integra* Thunb., *Weigela florida* Thunb., *Spiraea japonica* L.f., *Hydrangea paniculata* Seib. and *Physocarpus opulifolius* L. Maxim. had negative effects on the growth parameters of the host plants but 31.2 µmol.L⁻¹ (or less) of aqueous ozone did not have any risk to plant growth. Also, Fujiwara *et al.* (2011) showed that when *Cucumis melo* L., *Lycopersicon esculentum* M., *Citrullus lanatus* M., *Cucumis sativus* L., *Capsicum annuum* L. and *Solanum melongena* L. seedlings exposed to 4.0 and 8.0 mgL⁻¹ aqueous ozone, no visible injury was observed.

According to some studies, young plants are more susceptible to ozone gas injury than older plants. Similarly, it is expected that host plants to be more susceptible to ozonated water spraying in the early growth stages than in the old growth stages (Fujiwara *et al.* 2011). The findings of Ebihara *et al.* (2013) support this suggestion who observed remarkable damage to young seedlings of tomato and eggplant. However, in their study, old eggplants did not show any noticeable injury. In contrast to these findings, Costa *et al.* (2001) reported that old leaves showed greater injury than young leaves. Also, Keen and Taylor (1975) reported that primary leaves of 9-day-old plants were relatively tolerant to ozone injury. At present study, no relationship was found between a specific ozone concentration and age of host plant on susceptibility of the pepper as host to ozone damage. None of 4, 8 and 12 weeks old plants showed any injury symptoms.

Reiling and Davison (1995) showed that 70 nl/l ozone for 7 h/d reduced the stomatal conductance in *Plantago major* L. within a few hours after fumigation. Ebihara *et al.* (2013) reported that the ozone-mist treatment caused serious damage on the stomata of tomato and eggplant. In contrast, Malaiyandi and Natarajan (2014) showed that when *V. unguiculata* L. exposed to up to 60 ppbv, the number of epidermal cells and stomata increased. Our study showed that usage time of ozonation at concentration of 43 g/m³ did not have any negative effects on the pepper plant. As stomata regulate ozone uptake it is possible that some of this variation in plant response to ozone is the result of differences in stomatal behavior.

Although Schreiber (1978) reported that long exposure times to lower gaseous ozone concentrations were more injurious than short exposures to higher ones in *Phaseolus vulgaris* L. Heagle (1989) observed that the concentration factor is more important in causing plant response than the exposure time.

In different studies, a variation in the exposure time of plant/pest to ozone can be seen. In study by Graham *et al.* (2009) aqueous ozone was used for 7.5 minutes daily for six weeks on *S. integra*, *W. florida*, *S. japonica*, *H. paniculata* and *P. opulifolius*. Takigawa *et al.* (2013) sprayed ozone on

the aphid, *U. nigrotuberculatum* for 5 seconds. Keivanloo *et al.* (2013) found that mortality percentage of *P. interpunctella* was increased by increasing of exposure time. In their study, *P. interpunctella* was exposed to ozonated water for 30, 60, 90, and 120 minutes. Ebihara *et al.* (2013) sprayed on the leaves of tomato and eggplant for 30 and 60 seconds. Malaiyandi and Natarajan (2014) used 15 minutes twice a day for *Vigna unguiculata* L.

Overall, the results indicate that ozonated water can be an alternative to agricultural chemical pesticides for *T. urticae* on pepper. Based on present study, ozone, particularly at low concentrations, required much longer exposure times to be effective. This suggests the recommended concentration of ozone may need to be increased if 100% mortality is desired. This could be related to the unstable nature of ozone, its short half-life, its high reactivity, or its conversion to O₂ during the ozonation process. More experiments with higher ozone concentrations, different exposure times, or a combination of both, may increase its effectiveness against *T. urticae* and the other pests.

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بررسی تاثیر ازن محلول در آب بر مرگومیر *Tetranychus urticae* (Trombidiformes: Tetranychidae) روی گیاه *Capsicum annuum* (Solanaceae) در شرایط گلخانه

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چکیده

بروز مقاومت به آفت‌کش‌ها در جمعیت‌های کنه تارتن دو لکه‌ای، *Tetranychus urticae* Koch، از کشورهای مختلفی گزارش شده است. این مسئله منجر به پژوهش درباره روش‌های جایگزین شده است. در بررسی حاضر، ابتدا تاثیر غلظت‌های مختلف ازن محلول در آب بر مرگومیر کنه تارتن دو لکه‌ای روی گیاه فلفل (*Capsicum annuum* L.) در شرایط گلخانه با دمای 25 ± 2 درجه سلسیوس، رطوبت نسبی 50 ± 5 درصد و دوره روشنایی ۱۶:۸ ساعت (تاریکی:روشنایی) مورد آزمایش قرار گرفت. پس از تعیین غلظت مناسب، در آزمایش‌های جداگانه کارایی ازن در کنترل آفت در مدت زمان‌های متفاوت در معرض بودن (۵، ۱۰ و ۱۵ ثانیه)، ساعت‌های مختلف ازن‌پاشی در شبانه روز (۸ صبح، ۲ ظهر و ۸ شب) و سنین مختلف گیاه (گیاه ۴، ۸ و ۱۲ هفته‌ای) ارزیابی شد. هر آزمایش در قالب طرح کاملاً تصادفی با شش تکرار انجام شد. نتایج اولیه نشان داد که غلظت ۴۳ گرم بر متر مکعب ازن محلول در آب قادر است مرگومیر قابل قبولی در جمعیت کنه تارتن دو لکه‌ای بدون ایجاد آسیب قابل رویتی به گیاه ایجاد کند. میزان مرگومیر آفت با افزایش مدت در معرض بودن افزایش یافت. گرچه اثر متقابل غلظت و مدت در معرض بودن بر مرگومیر کنه معنی‌دار بود، اما در آزمایش‌های دیگر، اثر متقابل غلظت و زمان‌های کاربرد ازن و همچنین سن گیاه معنی‌دار نبود. گیاهان تیمار شده، در آزمایش‌های مختلف، هیچ آسیب قابل رویتی مانند کلروز، نکروز، زردی و یا بدشکلی روی شاخ و برگ گیاه فلفل مشاهده نشد. براساس این نتایج، ازن محلول در آب با غلظت معین و شرایط تعریف شده می‌تواند تراکم جمعیت کنه تارتن دو لکه‌ای را بدون آسیب ظاهری به گیاه فلفل کاهش دهد. با این حال، پیش از اینکه ازن محلول در آب به صورت تجاری به عنوان یک سم مورد استفاده قرار گیرد، باید پژوهش‌های بیشتری انجام شود.

واژگان کلیدی: زیررده کنه‌ها؛ روش‌های جایگزین مبارزه؛ ازن محلول در آب؛ آفات گلخانه؛ فلفل؛ کنه تارتن دو لکه‌ای.

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