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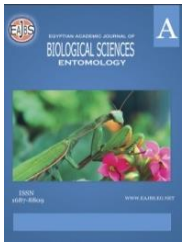

# Effects of Different Aqueous Ozone Exposure Times and Usage Times on Mortality of *Tetranychus urticae* Koch on *Capsicum annuum* L.

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**ABSTRACT**

*Tetranychus urticae* Koch, is a major pest of many plants including greenhouse and field crops in the world. The development of resistance in *T. urticae* populations to pesticides has motivated the search for alternative control methods to suppress the pest. The control of *T. urticae* by ozonated water on pepper (*Capsicum annuum* L.) was tested in greenhouse trials. Ozone at 43 g/m$^3$ concentration with a flow rate of 12.87 g/h ozone was applied as aqueous form at different times (8 am, 2 pm and 8 pm) and different ages of the plant (4, 8 and 12 weeks old) for three exposure times (5, 10 and 15 seconds). The results indicated that in all treatments, *T. urticae* was susceptible to 43 g/m$^3$ ozone concentration. In all cases, differences in mortality percentage of control and ozone treatments were significant, but differences in the mortality percentage among either time of application or duration of exposure time and ages of plant treatments were not significant. Also, no symptom of injury (chlorosis, necrosis, yellowing, or malformation) was observed for any of the pepper's leaves after aqueous ozone spraying. Based on these results, aqueous ozone can reduce the population density of *T. urticae* without any visible damage on the pepper leaves. However, more research needs to be done before ozonated water can be deployed commercially as a pesticide.

## INTRODUCTION

*Tetranychus urticae* Koch (Acari: Tetranychidae) is an important pest all over the world (Tsagkarakou et al. 2002). *T. urticae* causes considerable damage to many horticultural and agricultural crops such as pepper, *Capsicum annuum* L. (Chaudhri et al. 1985). The importance of pepper is due to the nutritional value of its fruits, an excellent source of health-related phytochemical compounds, such as carotenoids, vitamin C, and phenolic compounds. 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. When growth conditions are appropriate, *T. urticae* can increase the reproductive rate very quickly, resulting in a decline of host plant quality (Fathipour et al. 2006). In addition to serious damage and high yield losses; organophosphate-resistant populations of *T. urticae* have been reported in more than 40 countries (Tsagkarakou et al. 2002). These problems have led to the search for alternative methods. One of these alternative strategies is aqueous ozone.

Ozone, a triatomic form of oxygen ($O_3$), is an unstable gas with a half-life of about 20 minutes (Isikber and Oztekin 2009). Ozone is generated on-site, so it needs no special storage. Ozone chemical reaction with organic material occurs very quickly, which prevents microorganisms from developing tolerance to ozone (Mendez et al. 2003). Ozone is a safe method because it decomposes to oxygen, thus leaving no undesirable residue. Ozone and its derivative radicals such as $*O^{-}_2$,$*HO_2$, *OH, $*O^{-}_3$ through spiracles enter the pests respiratory system and cause damage (Ebihara et al. 2013).

Despite studies on the impact of ozone on stored product pests, few studies have been done about the greenhouse pests (for example; Hollingsworth and Armstrong 2005; Takigawa et al. 2011; Ebihara et al. 2013). This can be explained by many studies that have shown the negative effects of ozone gas on plants, may have inadvertently led to an oversight of the prophylactic use of aqueous ozone, to control of greenhouse pests. However, some researchers showed that the phytotoxic properties of gaseous ozone are altered when the ozone is in an aqueous form since aqueous ozone does not interact with plants in the same way as gas ozone (Fujiwara and Fujii 2002). Also, a low concentration of gaseous ozone can stimulate oxidative stress adaptation and systemic acquired resistance responses without visible injury (Pell et al. 1997; Kovalchuck et al. 2003). Due to a lack of practical information in the field, assessing the effect of aqueous ozone on insect-plant interactions is necessary (Trumble et al. 1987).

The extent of damage depends on some factors such as; 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.

Gaseous ozone through the stomata enters the leaf (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 a different time of day. As a result, a reduction in susceptibility of plants to ozone injury is expected.

In the present study, we tested the hypothesis that damage to ozone will be fewer if plants are exposed to ozone at night rather than during the day. The purpose of this study was to investigate the influence of ozonated water on (i) mortality of *T. urticae* at 43 $g/m^3$ ozone concentration; (ii) the possible detrimental effects of ozonated water on morphological features of the host plant (*C. annuum* L.) at different usage times; (iii) to determine the proper ozone exposure time and (iv) the reaction of different ages of the plant to ozone spraying.

## MATERIALS AND METHODS

### *T. urticae* Stock Colony*:*

The two-spotted spider mite used in experiments were reared on pepper plants (*C. annuum* L. cv. Red chili) in the controlled environment (25 ± 4 °C; 50-60% RH and 16:8 (L:D)) for several generations before conducting any experiment. These mites were originally collected from *Cucumis sativus* L. in a greenhouse at Ferdowsi University of Mashhad, Mashhad, Iran in 2016.

### Host Plant*:*

The pepper seeds (*C. annuum* L. cv. Red chili) were germinated in plastic trays (26 *

25 cm) with 50 cells containing vermicompost. After 3 weeks, individuals of small seedlings were transferred to plastic pots (12 * 12 cm) under controlled conditions at 25 ± 4 ºC; 50-60% RH and 16:8 (L:D). The soil of pots was a mixture of field soil: perlite: peat: vermicompost at ratios of 1:1:1:1 and irrigated daily. After 8 weeks, plants were used for experimental purposes.

**Ozone Generation*:***

The experimental setup consisted of an ozone generator which was obtained 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). Gaseous ozone was generated using a laboratory corona discharge ozone generator from the purified extra dry oxygen feed gas. Aqueous ozone was produced by forcing ozone into a filled water container of 2 liters volume at room temperature. The container was filled with distilled water with pH 7. Aqueous ozone through the outlet of the container enters the pump (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 distance between the ozone ejection nozzle and the plants was 50 mm. The ozone concentration was monitored by a portable ozone analyzer (was obtained from "A Teledyne Technologies Company", San Diego, USA. Process Ozone Monitor, Model 454).

**Bioassays:**

**Usage Times of Ozonation:**

Experiments were carried out at 25 ± 4 ºC, 50–60% RH, 16:8 (L:D). 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 test plants were exposed to 43 $g/m^3$ ozone concentration with 12.87 g/h ozone flow rate, for 10 seconds at 3 different times (8 am, 2 pm and 8 pm) by sprayer. For each treatment, a separate control plant was considered to be sprayed only with distilled water. The experiment was replicated six times. 1 day after treatment, the mortality of mites was assessed. The mite that could not walk was considered as dead (Ay and Kara 2011).

**Exposure Times of Ozonation:**

Infested plants 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 plant was considered to be sprayed only with distilled water. The mortality of mites was assessed after 1 day.

**Age of the Exposed Plants:**

Infested test plants (4, 8, and 12 weeks old) were exposed to 43 $g/m^3$ ozone concentration with 12.87 g/h ozone flow rate, for 10 seconds by sprayer. The control plant was considered to be sprayed only with distilled water. The experiment was replicated six times. The mortality of mites was assessed after 1 day.

In all treatments, plants were assessed for visible damages (chlorosis and necrosis symptoms or malformation on leaves), before and 24, 48, 72 hours after the spraying treatment.

**Data Analysis:**

Analyses were carried out using the statistical program, R 3.3.3 (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. Data were transformed where appropriate to satisfy the assumption of normality and homogeneity of variance for ANOVA.

## RESULTS

**Mortality of *T. urticae* at Different Usage Times of Ozonation**:

Data in table 1 show that *T. urticae* was susceptible to 43 g/m$^3$ ozone concentration. Statistical analyses showed that no significant interactions were found, so data were analyzed separately for each main effect (Table 1). The results showed that differences in the mortality percentage of control and ozone treatments were significant. But differences in the mortality percentage of mites among usage time treatments at the same ozone concentration were not significant (Table 1).

**Table 1**. Summary of ANOVA results for effects of different usage times and ozone on *T. urticae* mortality.

| Source of Variation | F | P |
|---|---|---|
| Ozone concentration (Control, Ozone) | **$F_{1,32}$=307.80** | **<.0001** |
| Usage time (8 am, 2 am and 8 pm) | $F_{2,33}$=0.01 | 0.98 |
| Ozone concentration * Usage time | $F_{2,30}$=0.04 | 0.95 |

Significant P-values (P<0.05) are shown in bold-face type.

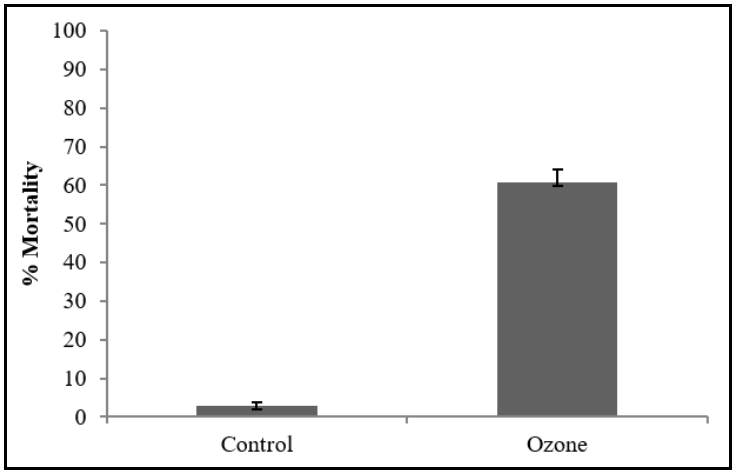


**Fig. 1-** The main effect of ozone and control on mortality rate of *T. urticae*

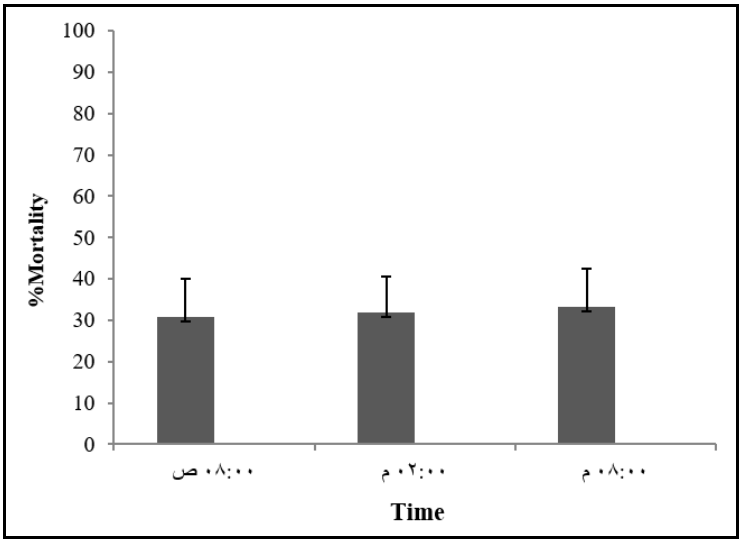


**Fig. 2-** The main effect of usage times on mortality rate of *T. urticae*

**Mortality of *T. urticae* at Different Exposure Times of Ozonation:**

Mortality of *T. urticae* at 43 g/m$^3$ ozone concentration and 3 exposure times (5, 10, and 15 seconds) is summarized in table 2. Data show that the mortality percentage increased with increasing exposure time (Fig. 3). The results showed that differences in the mortality percentage of control and ozone were significant. But differences in the mortality percentage of each time treatments at the same ozone concentration were not significant. Statistical analyses showed that the interaction effect between ozone concentration and the exposure time was significant (table 2).

**Table 2**. Summary of ANOVA results for effects of aqueous ozone on *T. urticae* mortality at different exposure times.

| Source of Variation | F | P |
|---|---|---|
| Ozone concentration (Control, Ozone) | **$F_{1,32}$=137.38** | **<.0001** |
| Exposure time (5, 10 and 15 seconds) | $F_{2,33}$=2.21 | 0.12 |
| Ozone concentration * Exposure time | **$F_{2,30}$=34.00** | **<.0001** |

Significant P-values (P<0.05) are shown in bold-face type.

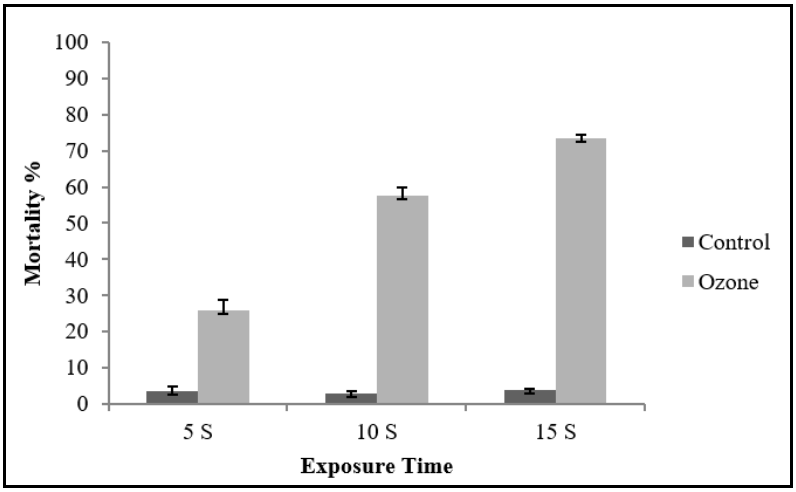


**Fig. 3.** Effects of aqueous ozone on mortality (mean ±SE) of *T. urticae* at different exposure times.

**Mortality of *T. urticae* at Different Age of The Exposed Plants**:

Statistical analyses showed that no significant interactions were found, so data were analyzed separately for each main effect (table 3) (Fig. 4 and 5). The results showed that differences in the mortality percentage of control and ozone treatments were significant. But differences in the mortality percentage of mites among different age of the exposed plants at the same ozone concentration were not significant (table 3).

**Table 3.** Summary of ANOVA results for effects of different age of the exposed plants and ozone on *T. urticae* mortality.

| Source of Variation | F | P |
|---|---|---|
| Ozone concentration (Control, Ozone) | **$F_{1,32}$=483.19** | **<.0001** |
| Age of plant (4, 8 and 12 weeks old) | $F_{2,33}$=0.08 | 0.91 |
| Ozone concentration * Age of plant | $F_{2,30}$=1.54 | 0.23 |

Significant P-values (P<0.05) are shown in bold-face type.

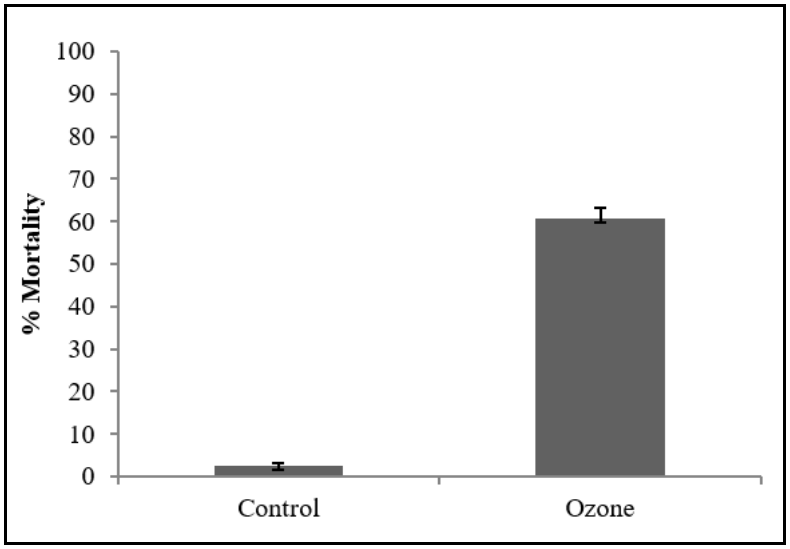


**Fig. 4-** The main effect of ozone and control on mortality rate of *T. urticae*

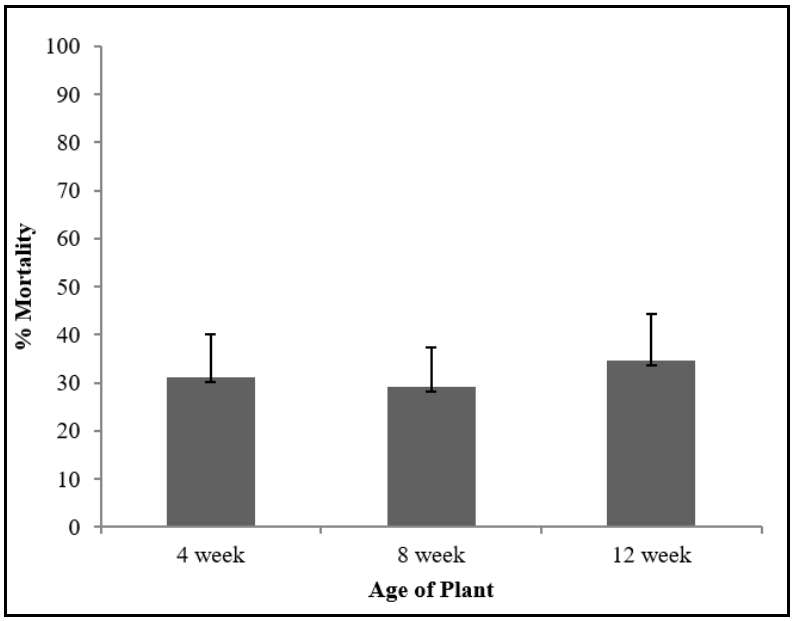


**Fig. 5-** The main effect of age of plant on mortality rate of *T. urticae*

**Damage on Plants:**

Our result showed that, at controlled conditions and 43 g/m3 concentration, the time of use aqueous ozone is not important. Because no visible injuries or morphological disorders such as chlorosis and necrosis symptoms or malformation was observed for any of the plants during 3 different times (8 am, 2 pm and 8 pm), exposure times (5, 10 and 15 seconds) and 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 *T. urtice* was susceptible to 43 g/m$^3$ ozone concentration. The results presented by others reported that ozonated water has the potential to control some pests. Takigawa *et al.* (2013) showed that when *Uroleucon nigrotuberculatum* was exposed to 86 g/m$^3$, the survival rate of the aphids was 3.3% for 5 seconds of treatment time. Also, Ebihara *et al.* (2013) observed ozone-mist treatment (68 g/m$^3$ for 30 seconds) for *U.*

*nigrotuberculatum* caused 98% mortality. Hollingsworth and Armstrong (2005) reported that 200 ppm ozone for 30 minutes caused 47.9 and 98.0% mortality of *Pseudococcus longispinus*, and *Frankliniella occidentalis*, respectively. Keivanloo *et al.* (2013) found *Plodia interpunctella* was susceptible to 2, 3, and 5 ppm aqueous ozone concentration.

Although previous studies have shown the negative effects of gaseous ozone such as reduced photosynthetic capacity, foliar reddening, and necrosis (Heagle 1989; Fuhrer and Booker 2003; Fiscus *et al.* 2005), surprisingly, at present study no symptoms of injury were found on pepper's leaves. Our findings indicate that when ozonation is performed under controlled conditions, the pepper leaves do not show any visible damages at 43 $g/m^3$ aqueous ozone, 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. On the other hand, the appearance of foliar symptoms of damage does not necessarily lead to decreases in measurable growth or yield. It has been shown in some plants such as; *Glycine max*, *Lycopersicon esculentum*, *Medicago sativa*, and *Triticum aestivum* (Tingey *et al.* 1973; Oshima *et al.* 1975; Tingey and Reinert 1975; Heagle *et al.* 1979).

The present results support those of Heagle *et al.* (1986) and Heagle *et al.* (1987) who showed that no visible acute foliar injury occurred in soybean and tobacco respectively. Graham *et al.* (2009) reported that 62.5 $\mu mol.L^{-1}$ (or greater) ozonated water was a negative effect on the growth parameters; but, 31.2 $\mu mol.L^{-1}$ (or less) ozonated water did not have any risk to plant growth. Also, Fujiwara *et al.* (2011) observed no visible damage to seedlings with 4.0 and 8.0 $mgL^{-1}$ aqueous ozone.

In different studies, there was a variation in an exposure time of aqueous ozone. In the study of Graham *et al.* (2009) aqueous ozone was used for 7.5 minutes daily for 6 weeks. Takigawa *et al.* (2013) sprayed ozone on *U. nigrotuberculatum* for 5 seconds. Keivanloo *et al.* (2013) found that mortality percentage was increased with an increase in 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 for 30 and 60 seconds. Malaiyandi and Natarajan (2014) used 15 minutes twice a day. Heagle (1989) observed that the concentration factor is more important in causing plant response than the exposure time. But Schreiber (1978) showed that long exposure times to lower gaseous ozone concentrations were more injurious than short exposures to higher ones in *Phaseolus vulgaris*.

Ebihara *et al.* (2013) reported that the ozonation treatment caused serious injury on the stomata. On the other hand, Malaiyandi and Natarajan (2014) found that when *Vigna unguiculata* exposed to up to 60 ppbv, the number of epidermal cells and stomata increased. Our study showed that usage time of ozonation did not have the negative effect on the pepper plant.

Based on some studies, young plants are more susceptible to gaseous ozone damage than older plants. Therefore, it is expected to be more susceptible to ozonated water spraying in the early growth stages than in the later growth stages (Fujiwara *et al.* 2011). Whereas at present study, 4, 8- and 12-weeks old plants did not show any injury symptoms. Keen and Taylor (1975) reported that primary leaves of 9-day-old plants were relatively tolerant to ozone injury. 13-day-old plants showed greater toxicity, whereas 16-day-old plants were injured less. Costa *et al.* (2001) reported that old leaves showed greater injury than young leaves. In contrast to these results, Ebihara *et al.* (2013) observed remarkable damage to young seedlings of tomato and eggplant. However, large eggplant did not show a noticeable injury.

Overall, according to these results, it seems that ozonated water can be used in management programs of some agricultural pests in some conditions. Also, the application of aqueous ozone can reduce agricultural chemicals. However, many questions still remain regarding

stomatal controlled mass transfer, the internal distribution of ozone, direct versus indirect effects, and physiological systems affected within the leaf and plant proper (Graham *et al.* 2009). More experiments with higher ozone concentrations or different exposure times may increase effectiveness against *T. urticae* and the other pests.

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## REFERENCES

Ay, R. and Kara, F. E. (2011) Toxicity, Inheritance and Biochemistry of Clofentezine Resistance in *Tetranychus urticae*. *Insect science*, 18: 503-511.

Chaudhri, W. M.; Akbar, S. and Rasool, A. (1985) Studies on biosystematics and control of mites of field crops, vegetable and fruit plants in Pakistan, third ed. University of Agriculture, Faisalabad, Pakistan.

Costa, S. D.; Kennedy, G. G. and Heagle, A. S. (2001) Effect of Host Plant Ozone Stress on Colorado Potato Beetles. *Environmental Entomology*, 30(5): 824-831.

Durrant, W. E. and Dong. X. (2004) Systemic acquired resistance. *Annual Review Phytopathology* 42: 185–209.

Ebihara, K.; Mitsugi, F.; Ikegami, T.; Nakamura, N.; Hashimoto, Y.; Yamashita, Y.; Baba, S.; Stryczewska, H. D.; Pawlat, J.; Teii, Sh. and Sung, T-L. (2013) Ozone-mist Spray Sterilization for Pest Control in Agricultural Management. T*he European Physical Journal Applied Physics*, 61: 24318.

Fathipour, Y.; Ahmadi, M. and Kamali, K. (2006) Life table and survival rate of *Tetranychus urticae* (Acari: Tetranychidae) on different bean varieties. *Iranian Journal of Agricultural Sciences*, 37(1): 65-71.

Fiscus, E. L.; Booker, F. L. and Burkey, K. (2005) Crop Responses to Ozone: Uptake, Modes of Action, Carbon Assimilation and Partitioning. *Plant, Cell & Environment*, 28: 997–1011.

Fuhrer, J. and Booker, F. (2003) Ecological Issues Related to Ozone: Agricultural Issues. *Environment International*, 29: 997–1011.

Fujiwara, K. and Fujii, T. (2002) Effects of Spraying Ozonated Water on the Severity of Powdery Mildew Infection on Cucumber Leaves. *Ozone: Science & Engineering*, 24(6): 463-469.

Fujiwara, K.; Hayashi, Y. and Park, J. S. (2011) Spraying Ozonated Water under Well-Ventilated Conditions Does Not Cause Any Visible Injury to Fruit-Vegetable Seedlings. *Ozone: Science & Engineering*, 33: 179–182.

Graham, T.; Zhang, P.; Zheng, Y. and Dixon, M. A. (2009) Phytotoxicity of Aqueous Ozone on Five Container-grown Nursery Species. *Hortscience*, 44(3): 774–780.

Heagle, A. S.; Spencer, S. and Letchworth, M. B. (1979) Yield Response of Winter Wheat to Chronic Doses of Ozone. *Canadian Journal of Botany*, 57(19): 1999-2005.

Heagle, A. S.; Heck, W. W.; Lesser, V. M.; Rawlings, J. O. and Mowry, F. L. (1986) Injury and Yield Response of Cotton to Chronic Doses of Ozone and Sulfur Dioxide. *Journal of Environmental Quality*, 15: 375-82.

Heagle, A. S.; Flagler, R. B.; Patterson, R. P.; Lesser, V. M.; Shafer, S. R. and Heck, W. W. (1987) In Jury and Yield Response of Soybean to Chronic Doses of Ozone and Soil Moisture Deficit. *Crop Science*, 27(5): 1016-1024.

Heagle, A. S. (1989) Ozone and Crop Yield. *Annual Review of Phytopathology,* 27(1): 397–423.

Hollingsworth, R. G. and Armstrong, J. W. (2005) Potential of Temperature, Controlled Atmospheres, and Ozone Fumigation to Control Thrips and Mealybugs on Ornamental Plants for Export. *Journal of Economic Entomology*, 98(2): 289-298.

Isikber, A. A. and Oztekin, S. (2009) Comparison of susceptibility of two stored-product insects, *Ephestia kuehniella* Zeller and *Tribolium confusum* to gaseous ozone. *Journal of Stored Products Research,* 45(3): 164-159.

Keen, N. T. and Taylor, O. C. (1975) Ozone Injury in Soybeans. *Plant Physiology,* 55: 731-733.

Keivanloo, E.; Sadeghi Namaghi, H.; Haddad Khodaparast, M. H. and Hatefi, S. (2013) Susceptibility of immature stagesof the Indian meal moth, *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae) to ozonated water. *Journal of Crop Protection*, 2(4): 443-451.

Kovalchuck, I.; Filkowski, J.; Smith, K. and Kovalchuck, O. (2003) Reactive oxygen species stimulate homologous recombination in plants. *Plant Cell Environment*, 26: 1531–1539.

Kim, J-G.; Yousef, A. E. and Dave, S. (1999) Application of Ozone for Enhancing the Microbiological Safety and Quality of Foods: a review. *Journal of Food Protection*, 62(9): 1071-1087.

Malaiyandi, M.; and Natarajan, M. (2014) Impact of Ozone on Morphological, Physiological, and Biochemical Changes in Cow Pea (*Vigna unguiculata* [L.] Walp.). *Ozone: Science & Engineering,* 36: 36–42.

Mendez, F.; Maier, D. E.; Mason, L. J. and Woloshuk, C. P. (2003) Penetration of ozone into columns of stored grains and effects on chemical composition and processing performance. *Journal of Stored Product Research*, 39: 33-44.

Oshima, R. J.; Taylor, O. C.; Braegelmann, P. K. and Baldwin, D. W. (1975) Effect of Ozone on the Yield and Plant Biomass of a Commercial Variety of Tomato. *Journal of Environmental Quality*, 4(4): 463-464.

Pell, E.; Schlagnhaufer, C. and Arteca, R. (1997) Ozone-induced oxidative stress: Mechanisms of action and reaction. *Physiolagia Plantarum,* 100: 264–273.

R Core Team (2011) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria, ISBN 3-900051-07-0. URL http://www.R-project.org/.

Schreiber, U. (1978) Chlorophyll fluorescence assay for ozone injury in intact plants. *Plant Physiology*, 61: 80-84.

Takigawa, K.; Ueno, K.; Nagatomo, T.; Mitsugi, F.; Ikegami, T.; Ebihara, K.; Nakamura, N.; Hashimoto, Y. and Yamashita, Y. (2013) Experiment of Pest Control with Portable Ozone Mist Device. Paper presented at the 21st Proceedings of International Plasma Chemistry Society (IPCS 21), Queensland, Australia, August 4 – 9.

Tingey, D. T.; Fitesf, R. C. and Wickliff, C. (1973) Ozone Alteration of Nitrate Reduction in Soybean. *Physiolagia Plantarum,* 29: 33–38.

Tingey, D. T. and Reinert, R. A. (1975) The Effect of Ozone and Sulphur Oxides Singly and in Combination on Plant Growth. *Environmental Pollution,* 9(2): 117-125.

Trumble, J. T.; Hare, J. D.; Musselman, R. C. and McCool, P. M. (1987) Ozone-induced Changes in Host-plant Suitability: Interactions of *Keiferia lycopersicella* and *Lycopersicon esculentum*. J*ournal of Chemical Ecology,* 13(1): 203-18.

Tsagkarakou, A.; Pasteur, N.; Cuany, A.; Chevillon, C. and Navajas, M. (2002) Mechanisms of Resistance to Organophosphates in *Tetranychus urticae* (Acari: Tetranychidae) from Greece. *Insect Biochemistry and Molecular Biology,* 32(4): 417–424.