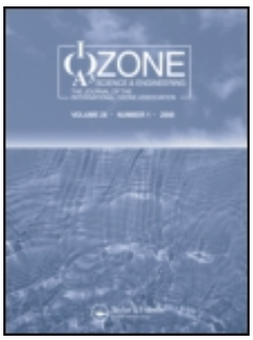

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Application of Ozone Post-Harvest Treatment on Kabkab Date Fruits: Effect on Mortality Rate of Indian Meal Moth and Nutrition Components

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Methyl bromide (MB) was used for years to treat infested stored date fruits; however, MB is due to be phased out by 2015. In this study ozone is used for disinfestations of Kabkab date against Indian meal moth in three life stage. They were exposed to four ozone concentrations (300 ± 10, 1050 ± 40, 2000 ± 40, and 4000 ± 50 ppm) during four periods (2, 4, 6, and 8 h). The findings show 2000 ppm of ozone concentration within 8 h resulted in complete mortality of larvae and adult insects and over 90% mortality of eggs. The proposed ozone treatment is a promising approach replacing application of MB for disinfestations of examined date fruits, as no remarkable changes were observed on pH of the date fruits and its chemical compositions (total phenolic content, anti-oxidant activity and free radicals).

Keywords Ozone, Kabkab Date, Insect Disinfestations, Indian Meal Moth, Chemical Composition, Agri-Food Applications

INTRODUCTION

The date palm (*Phoenix dactylifera* L.) is one of the oldest cultivated fruit trees that has been considered as a main factor of life for populations who live in the dry regions because of its multi-purpose use (Munier 1973). All parts of the palm can be used to provide food, shelters, and timber products (Mahmoudi et al. 2008). Date production is unfortunately accompanied by a substantial increase of loss during picking, storage, commercialization, and conditioning processes. Insects such as the Indian meal moth (*Plodia interpunctella*) are believed to contribute to a major limiting factor for marketing these fruits. To protect dates from pests, particularly during transport and storage and after distribution, various methods have been used. In this regard, methyl bromide (MB) has been and remains the most effective and well-used chemical for postharvest treatments. MB is, however, known to be responsible for ozone depletion and is due to be withdrawn globally by 2015. Although aluminum phosphate tablets (which sublimate poisonous phosphine gas in reaction with water vapor) have replaced MB in many instances, its application has not been without problems too, because on many occasions, it requires a long exposure time (5 d or more) and its indiscriminate use has resulted in the evolution of resistant populations even at high concentrations (Bell 1997, 2000). Therefore, it might be phased out sooner or later.

Date fruit is highly nutritional because they contain different kind of sugars, carbohydrates, fibers and minerals and are rich in phenolic compounds and anti-oxidant activity (Biglari et al. 2009). These important compounds are an integral part of the human diet, and could be helpful against human cancers, arteriosclerosis, ischemia and inflammatory disease, which are partially caused by exposure to oxidative stress (Caillet et al. 2006). Phenolic profile of seven different varieties of ripe date palm fruit were found to contain mainly *p*-coumaric, ferulic and sinapic acids and some cinnamic acid derivatives and also three different isomers of 5-*o*-caffeoylshikimic acid (Mansouri et al. 2005).

Ozone (O_3) is a powerful oxidizer that can eliminate the handling, storage, and disposal problems of conventionally used postharvest pesticides. Its application to food storage is attractive because it can be electrically generated on-site at the time of use, eliminating the need to store and dispose of insecticide packages (Kells et al. 2001), and it leaves no

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residue. It is therefore more environmentally friendly than traditional pesticides such as MB or aluminum phosphate. The above mentioned characteristics make the feasible application of ozone as treatment of stored products more attractive (Guzel-Seydim et al., 2004).

Ozone has been accepted as a GRAS product by the U.S. Food and Drug Administration organization in June 2001 (Guzel-Seydim et al. 2004). It is currently used to disinfect fruits, vegetables, and other foodstuffs and eradicate microorganisms and viruses; as a means to eliminate odors, colors, and pollutants in industrial applications (Henry et al. 2000, Karaca 2010, Steffen et al. 2010). Ozone in its gaseous form has also been considered to have the potential to kill insect pests in commodities. High mortality was achieved for adults of the maize weevil, *Sitophilus zeamais* (Motschulsky), and of the red flour beetle, *Tribolium confusum* (Jacqueline du Val) when exposed to low ozone concentrations ranging from 5 to 50 ppm (Kells et al. 2001). Whereas for *Ephestia kuehniella*, 300 ppm concentration of ozone treatment resulted in complete mortality of adults, pupae and larvae, however eggs were more resistant.

For *Tribolium confusum*, this treatment resulted in very low mortality of adults, pupae and eggs, ranging from 4.2 to 14.1% while only larva had a high mortality of 74% (Isikber and Oztekin 2009). Two hours at 4000 ppm ozone exposure, for the eggs of both *P. interpunctella* and *Oryzaephilus surinamensis* leaded to 80% mortality of eggs (Niakousari et al. 2010). An important issue, however, is to determine if ozone or any of the above mentioned control strategies, has any detrimental effects on chemical composition or bioactive components in dates.

The aim of this study was to determine the best combination of ozone concentration and time of exposure in mortality of all three stages of Indian meal moth in the date fruits and to examine the influence of gaseous ozone on the pH, total phenolic content, anti-oxidant activity, and free radical production. As far as our awareness no such study has ever previously been conducted.

MATERIALS AND METHODS

Materials

The dates

Dates (*Phoenix dactylifera* L. Kabkab) at uniform size (average size: 27 mm high and 16 mm wide) and maturity (Tamar stage) without wounds, rot or infestation were used. Dates (thereafter is called fruits) were harvested at Tamar stage, which is the latest stage of maturity and kept refrigerated (4 ± 1 °C) until use. These fruits, obtained from a specifically controlled palm tree field in Boushehr province, in Iran, which was grown organically under the direct supervision of the Iran Agriculture Organization, were free from chemicals not only during cultivation but also after its harvesting.

Test Insect

Tests were carried out with three life stages of Indian meal moth (P. interpunctella) (adult insects, larvae, and eggs). Insects were maintained and inbreeded in containers (30 × 20 × 8 cm) at 27 ± 2 °C and 60% ± 5% relative humidity (r.h.) and photoperiod of 10:14 (dark: light) hours on date fruit with small quantity of yeast, glycerol and honey as a diet (Sauer and Shelton 2002).

Oxygen compressor

A laboratory Integra oxygen compressor (model 6323A-OM-5) was provided by SeQual Co., United Kingdom. This electronic oxygen compressor separates oxygen from ambient air through its inlet filter. The compressor was designed to produce a flow of 0.5 to 7.5 L/min.

Ozone generator

The ozone generator with corona discharge technology (model COW-020) was provided by Sahand Engineering Research Company – Tabriz – Iran. The generator nominal capacity was 35 g gaseous ozone per hour (oxygen input).

Ozone analyzer

To calibrate the ozone generator and measure the ozone concentration of the test chamber, a flame photometer ozone analyzer (Ozone Analyzer BMT 964, BMT Messtechnik, Berlin, Germany) was used. A 200-L volume test chamber (70 × 50 × 55 cm) was made of Plexiglas (corrosion resistance) and was equipped with inlet and outlet valves, a small ventilation fan (speed about 1400 rpm) to ensure a uniform distribution of gas within the chamber, a temperature indicator and a relative humidity meter.

Method

Experimental design

Based on the previous studies, it was decided to examine the effect of four ozone concentrations (300 ± 10, 1050 ± 40, 2000 ± 40, and 4000 ± 50 ppm) during four exposure time (2, 4, 6, and 8 h) on the mortality of Indian meal moth (adult insects, larvae, and eggs). Additionally, any alteration in the date fruit chemical compositions and micronutrients, under the above experimental conditions, were investigated. The aim was to obtain an optimum ozone time-concentration in order to achieve the highest mortality and the lowest micronutrients degradation. A control group of untreated adult insects, larvae, and eggs were also included in the experimental setup. Untreated date samples (control) were concurrently examined for their chemical compositions and micronutrients.

Mortality experiments procedure (Bioassays)

To investigate the efficacy of ozone on infested dates, adult insects, larvae, and eggs of Indian meal moth were placed in

plastic Petri-dishes (diameter 6 cm × height 1 cm) top covered with 20 mesh cloth net. Petri dishes containing Indian meal moths were placed in a depth of 10 cm from surface in a porous fruit basket (packaging) designed to contain about 400 g fruits. The basket was placed at the height of 20 cm from the bottom of the chamber. For each run (concentration/time) 12 adult insects, 20 larvae, and 50–60 eggs of *P. interpunctella* were examined (Niakousari et al. 2010). For all 48 runs (4 ozone concentrations, 4 exposure time and 3 replicates), samples (insects, larvae and eggs) not treated with ozone were used as controls to estimate the proportion of mortality due to natural death.

Four concentration of ozone (300 ± 10, 1050 ± 40, 2000 ± 40, and 4000 ± 50 ppm, as shown on ozone analyzer LED) and four exposure times (2, 4, 6, and 8 h) were used to achieve optimum time-concentration of ozone (based on highest mortality). After each treatment, eggs, larvae and adults were held at 27 ± 2 °C and r. h. of 60 ± 5% on the same diet used until examined for mortality. Mortality counts for adults for 1 day after exposure, for larvae had failed to adults 5 days after exposure, and egg hatch was counted 7 days after treatment. A stereomicroscope with binocular head (ZTX-E-W, Pro Way Optics and Electronics Co., China) was used to count non-hatched and hatched eggs.

In the present study, all results were presented as "corrected mortality" which was evaluated based on an equation proposed by Abbott (1925) (Eq. [1]). Abbott's formula is a simple mathematical equation to correct for control mortality in bioassays. This is a formula to adjust for mortality not associated with insecticide treatment such as the natural mortality in an untreated control group, or mortality occurring from a blank spray used as a check.

$$\text{Percent of insects mortality} = \frac{(\text{No. of insects mortality in control sample} - \text{No. of insects mortality in treated sample}) \times 100}{(100 - \text{No. of insects mortality in control sample})} \quad [1]$$

Bioactive components analysis

Total of 60 samples (48 samples treated with ozone according to the conditions previously stated and 12 control samples (untreated: 2, 4, 6, 8 h, and 0 ozone concentration)) were studied for total phenolic contents (Singleton and Rossi 1965), anti-oxidant activity (Benzie and Strain 1999) and presence of free radicals (Sanchez-Moreno et al. 1998). All measurements were performed in three replicates.

Total phenolic content (TPC) measurement

A hundred grams of fruit was crushed and dry-blended for about 4 min using a blender (FU-618, Japan). The paste was then thoroughly mixed with 300 mL of a solvent containing methanol-water (4:1, v/v). The extraction was carried out at room temperature (20 ± 2 °C) for 5 h using an orbital shaker (Biglari et al. 2008; Mansouri et al. 2005). The extracts were then filtered and centrifuged (RC-5 super speed refrigerated, made by Sorval Company, USA) at 4000 g for 20 min. The supernatant was analyzed for TPC based on method described by Singleton and Rossi (1965), using a UV spectrophotometer and Folin-Ciocalteau reagents. The total phenolic contents were expressed as mg Gallic acid equivalents (GAE)/100 g fresh dates. The calibration curve was drawn with Gallic acid standard.

Anti-oxidant activity measurement by Ferric Reducing Anti-oxidant Power (FRAP)

The anti-oxidant capacity of methanolic extracts was determined using modified FRAP assay of Benzie and Strain (1999).The FRAP reagent contained 2.5 mL of a 10 mM tripydyltriazine (TPTZ) solution in 40 mM HCl plus 2.5 mL of 20 mM $FeCl_3.6H_2O$ and 25 mL of 0.3 M acetate buffer at pH 3.6. Analysis was carried out by mixing 3 mL of freshly prepared FRAP reagent which was warmed up to 37 °C with 20 μL of methanolic extract. The reaction mixture was then incubated at 37 °C for 4 min and the absorbance at 593 nm was determined with reference to reagent blank contained distilled water. In this analysis aqueous solutions of known Fe (II) concentrations in the range of 100–2000 μM ($FeSO_4.7H_2O$) were used for calibration.

Free radical measurement

To estimate antiradical ability of sample extracts, radical scavenging activity was measured by DPPH (1, 1-diphenyl-2-picrylhydrazyl) according to Sanchez-Moreno et al. (1998). Two g of date sample was crushed and homogenized with 10 mL of methanol (50%). The extract was then centrifuged (7000 g, 25 min, at 40 °C) and the precipitate were washed with 15 mL of methanol. The produced supernatant was mixed with first extract aliquot. The methanolic extract volume was reduced to 20 mL by rotary evaporator and 3.9 mL methanolic DPPH (0.025 g/L) was added to 100 μL of the concentrated extract. The absorbance of the resulting solution was measured at 515 nm during intervals to reach a steady point. The methanol without DPPH and the methanol contain DPPH without extract was used respectively as blank and control samples. The inhibitory percent was measured by this method (Deepa et al. 2006). The scavenging percent was determined by Eq. [2].

$$\text{Radical scavenging percent of extract Scavenging (\%)} = 100 - [(OD_{sample}/OD_{control}) \times 100] \quad [2]$$

pH measurement

To determine the possible effect of gaseous ozone on fruits, the pH measurement was carried out by addition of 50 mL of distilled water to 10 g of paste of date samples. The pH

of homogenized mixture was then measured by a pH meter (model CG-824, Germany).

Statistical Analysis

All data were subjected to analysis of variance (ANOVA). Mean comparisons were performed using Post Hoc Multiple Comparison Duncan test to examine if differences between treatments were significant at $p < 0.05$. All analysis was performed with SPSS software package version 15.0 for Windows.

RESULTS AND DISCUSSION

Mortality Experiments

The results indicated that 90% of the adults were killed if they exposed to any ozone concentration above 300 ppm for 4 h. Extending the exposure time to 6 h ensure complete mortality of adult Indian meal moth (Table 1).

The mortality data obtained in the present work were comparable to those published by previous workers. During 5 d of ozonation with 5 ppm ozone, 100% of adult flour beetle and saw-toothed grain beetle were killed (Mason et al. 1997). About 92–100% of Indian meal moth larva and adult flour beetles died in 50 ppm of ozone concentration during 3 d (Kells et al. 2001). To get rid of Indian meal moth and flour beetle in ozone concentration of 500 ppm and 200 ppm, 5 h was needed (Leesch and Tebbet 2001).

In this study at low concentration of 300 ppm, after 8-h exposure, just under 70% of the larvae were killed. At about 1000 ppm ozone, at least 6 h of exposure was required to ensure mortality of over 90%. Ozone concentration of 2000 and 4000 ppm at 4 and 2 h exposure time, respectively, brought about mortality of approximately 90%. Ozone concentration of 1000 ppm or more at 8 h exposure ensured complete mortality of larvae (Table 1). Low mortality rate of larvae at low concentration or short exposure time could be due to low penetration capability of gaseous ozone. Another reason for this low mortality rate was due to the fact that ozone needs much longer exposure time to enter the respiratory system of larvae and/or react with the larvae cell system (Isikber and Oztekin 2009).

Niakousari et al. (2010) expressed that complete mortality of adults and larvae of Indian meal moth may only be achieved if samples were exposed to ozone concentration of 4000 ppm for 4 h. They also expressed that only 30% of the eggs underwent transformation into larvae if samples were exposed to ozone environment of 4000 ppm for 4 h. In fact, the eggs were more tolerant against ozone exposure. There were not any other published data on mortality of eggs of Indian meal moth or other insects by ozone or any other disinfestations agents. Most researchers merely stated that eggs are more tolerant. Fumigation with MB, which typically takes between 24–48 h, may not also secure complete destruction of eggs (Niakousari et al. 2010). Through this investigation it has been indicated that the mortality for eggs was even lower, as after 8 h exposure only about 25 and 50% of eggs were not hatched on exposure to 300 and 1000 ppm ozone, respectively (Table 1).

To accomplish approximately 90% mortality of eggs, samples were required to be exposed to ozone concentration of 2000 ppm and 6 h. At exposure to 2000 ppm and 8 h only about 5% of eggs were hatched after 7 d incubation. At a higher concentration of 4000 ppm and 8 h exposure, the increase in mortality was not much higher (about 4% of eggs (2–3 out of 60 eggs) were hatched after 7 d incubation). This

TABLE 1. Corrected Percent Mortality (Based on Abbott's 1925 equation) of Indian Meal Moth Adult Insects, Larva and Eggs Exposed to Various Ozone Concentration-Time Combinations[1,2,3]

Life stage	Time (h) Conc. (ppm)	2	4	6	8
Part I	300	$44.44 \pm 19.24^{a,A}$	$88.89 \pm 19.24^{b,A}$	$100.00 \pm 00.00^{b,A}$	$100.00 \pm 00.00^{b,A}$
	1050	$78.78 \pm 19.24^{a,B}$	$100.00 \pm 00.00^{a,A}$	$100.00 \pm 00.00^{a,A}$	$100.00 \pm 00.00^{a,A}$
	2000	$100.00 \pm 00.00^{a,B}$	$100.00 \pm 00.00^{a,A}$	$100.00 \pm 00.00^{a,A}$	$100.00 \pm 00.00^{a,A}$
	4000	$100.00 \pm 00.00^{ab,B}$	$100.00 \pm 00.00^{ab,A}$	$100.00 \pm 00.00^{a,A}$	$100.00 \pm 00.00^{a,A}$
Part II	300	$0.00 \pm 00.00^{a,A}$	$0.00 \pm 00.00^{a,A}$	$6.67 \pm 11.55^{a,A}$	$66.67 \pm 11.55^{b,A}$
	1050	$0.00 \pm 00.00^{a,A}$	$40.00 \pm 20.00^{b,B}$	$93.33 \pm 11.55^{c,B}$	$100.00 \pm 00.00^{c,B}$
	2000	$0.00 \pm 00.00^{a,A}$	$86.67 \pm 11.55^{b,C}$	$100.00 \pm 00.00^{c,B}$	$100.00 \pm 00.00^{c,B}$
	4000	$93.33 \pm 11.55^{a,B}$	$100.00 \pm 00.00^{a,C}$	$100.00 \pm 00.00^{a,B}$	$100.00 \pm 00.00^{a,B}$
Part III	300	$5.25 \pm 0.09^{a,A}$	$9.89 \pm 0.69^{b,A}$	$9.58 \pm 1.07^{b,A}$	$24.59 \pm 0.71^{c,A}$
	1050	$19.37 \pm 1.25^{a,B}$	$26.16 \pm 1.01^{b,B}$	$18.68 \pm 0.59^{a,B}$	$51.17 \pm 1.00^{c,B}$
	2000	$73.66 \pm 2.72^{a,C}$	$81.05 \pm 1.57^{b,C}$	$85.47 \pm 4.36^{b,C}$	$94.40 \pm 5.34^{c,C}$
	4000	$85.13 \pm 0.53^{ab,D}$	$88.09 \pm 1.12^{b,D}$	$82.41 \pm 0.87^{a,C}$	$96.42 \pm 4.58^{c,C}$

[1]All measurements were performed in triplicate.

[2]Significant difference ($p < 0.05$) between data is expressed by different letters (A, B, C, and D); and lower-cased letters (a, b, and c) are within rows and capital letters are within columns.

[3]Although Part I indicates Adult insects; Part II indicates Larvae; and Part III indicates Eggs.

TABLE 2. Total Phenolic Content (TPC) of Date Fruits (mg (GAE)/100 g fresh date) Exposed to Various Ozone Concentration-Time Combinations [1,2,3]

Time (h) Conc. (ppm)	2	4	6	8
Untreated	$1.34 \pm 0.03^{a, A}$	$1.34 \pm 0.03^{a, B}$	$1.34 \pm 0.03^{a, AB}$	$1.34 \pm 0.03^{a, A}$
300	$1.35 \pm 0.07^{a, A}$	$1.35 \pm 0.01^{a, B}$	$1.36 \pm 0.05^{a, AB}$	$1.30 \pm 0.07^{a, A}$
1050	$1.33 \pm 0.06^{a, A}$	$1.27 \pm 0.04^{a, A}$	$1.33 \pm 0.04^{a, AB}$	$1.27 \pm 0.01^{a, A}$
2000	$1.29 \pm 0.16^{a, A}$	$1.33 \pm 0.03^{a, B}$	$1.42 \pm 0.09^{a, B}$	$1.32 \pm 0.11^{a, A}$
4000	$1.30 \pm 0.11^{a, A}$	$1.23 \pm 0.03^{a, A}$	$1.21 \pm 0.15^{a, A}$	$1.19 \pm 0.11^{a, A}$

[1]All measurements were performed in triplicate.
[2]Significant difference ($p < 0.05$) between data is expressed by different letters (A, B, C, and D); and lower-cased letters (a, b, and c) are within rows and capital letters are within columns.
[3]GAE indicates Gallic Acid Equivalents.

could be due to low penetration ability of ozone when applied to biological substances. No tests were carried out to examine the productivity of eggs, larvae and adults after exposure to the above dosage of ozone.

Bioactive Components Analysis

Control samples (untreated fruit) as well as ozonated samples were examined for phenolic content. The phenolic content for untreated date was 1.34 ± 0.03 [mg (GAE)/100 g fresh date] (Table 2). Eight hours' exposure to gaseous ozone at concentration of 4000 ppm, about 11.2% downward trend in phenolic content, in comparison with control ones, was observed however this had no statistically meaningful diversity ($P < 0.05$). At 2000 ppm and 8 h exposure time (indicated to be optimized concentration-time combination in terms of mortality of all three stages of insects) only 2% decreasing trend in phenolic content was observed. There were not much published data on the phenolic content changes of date fruits by ozone treatment or other means of treatment such as aluminum phosphate or MB, thus no comparison was possible.

Anti-oxidant activity of untreated Kabkab date was about 475.25 (μmol FRAP/100 g fresh date) (Table 3). Although in some of the data (treated or untreated samples) a variation of about 19% was observed, the difference among data were not significant ($p < 0.05$) (Table 3). The highest decrease in anti-oxidant activity of ozonated samples was for samples treated for 8 h of 4000 ppm ozone (19% decreasing trend). Based on the present data, it may be concluded that exposing date to ozone had no adverse effect on its anti-oxidant activity, i.e., ozone is not capable of oxidizing bonded anti-oxidant constituents in date. This perhaps was due to fairly short time of exposure, low penetration capability of gaseous ozone or physicochemical characteristic of Kabkab date. It has been reported that a 180 g/min ozone flow rate during 135 min cannot change a complex structure of lycopene in tomato slices (Malone 2003).

To examine the influence of ozone on formation of free radical in Kabkab date, let us consider the situation when maximum ozone concentration at maximum time of exposure, i.e., 4000 ppm and 8 h is applied to date. The data in Table 4 indicated this value for treated fruits (4000 ppm, 8 h) was about 3.12%, while this value for untreated samples was 2.4%. This was a very small variation in free radicals.

Though the pH has impact on phenolic content resistance and also on ozone destruction, it has been achieved that the pH has no meaningful changes by ozonation (Table 5). Table 5 shows that the pH of untreated fruit was about

TABLE 3. Anti-oxidant Activity of Date Fruits (μmol [FRAP]/100 g Fresh Date) Exposed to Various Ozone Concentration-Time Combinations[1,2,3]

Time (h) Conc. (ppm)	2	4	6	8
Untreated	$473.74 \pm 74.39^{a,A}$	$477.73 \pm 14.77^{a,B}$	$473.29 \pm 16.87^{a,B}$	$475.74 \pm 56.57^{a,B}$
300	$464.15 \pm 10.24^{b,A}$	$471.77 \pm 4.40^{b,B}$	$458.08 \pm 14.37^{b,AB}$	$440.37 \pm 6.87^{a,B}$
1050	$474.55 \pm 12.89^{b,A}$	$469.72 \pm 8.35^{b,B}$	$456.22 \pm 11.57^{ab,AB}$	$443.14 \pm 14.81^{a,B}$
2000	$465.00 \pm 18.71^{b,A}$	$461.17 \pm 7.68^{b,AB}$	$447.43 \pm 11.80^{b,AB}$	$427.50 \pm 7.38^{a,AB}$
4000	$464.77 \pm 16.30^{b,A}$	$446.40 \pm 13.16^{b,A}$	$434.47 \pm 19.17^{b,A}$	$380.02 \pm 49.13^{a,A}$

[1]All measurements were performed in triplicate.
[2]Significant difference ($p < 0.05$) between data is expressed by different letters (A, B, C, and D); and lower-cased letters (a, b, and c) are within rows and capital letters are within columns.
[3]FRAP indicates Ferric Reducing Anti-oxidant Power.

TABLE 4. Corrected Percent Free Radical of Date Fruits (%Free Radical/ g Fresh Date) Exposed to Various Ozone Concentration-Time Combinations[1,2]

Time (h) Conc. (ppm)	2	4	6	8
Untreated	$2.40 \pm 0.15^{a,AB}$	$2.40 \pm 0.17^{a,A}$	$2.43 \pm 0.06^{a,D}$	$2.40 \pm 0.79^{a,AB}$
300	$2.40 \pm 0.32^{a,AB}$	$2.18 \pm 0.17^{a,A}$	$1.98 \pm 0.17^{a,B}$	$2.36 \pm 0.42^{a,AB}$
1050	$2.16 \pm 0.44^{a,A}$	$2.04 \pm 0.38^{a,A}$	$1.73 \pm 0.14^{a,A}$	$1.80 \pm 0.04^{a,A}$
2000	$2.16 \pm 0.21^{a,A}$	$2.43 \pm 0.15^{bc,A}$	$2.20 \pm 0.04^{ab,C}$	$2.60 \pm 0.02^{c,BC}$
4000	$2.82 \pm 0.40^{ab,B}$	$2.22 \pm 0.17^{a,A}$	$2.86 \pm 0.16^{ab,E}$	$3.12 \pm 0.66^{b,C}$

[1]All measurements were performed in triplicate.
[2]Significant difference ($p < 0.05$) between data is expressed by different letters (A, B, C, and D); and lower-cased letters (a, b, and c) are within rows and capital letters are within columns.

TABLE 5. The pH of Date Fruits Exposed to Various Ozone Concentration-Time Combinations[1,2]

Time (h) Conc. (ppm)	2	4	6	8
Untreated	$5.58 \pm 0.06^{a,AB}$	$5.59 \pm 0.03^{a,AB}$	$5.59 \pm 0.06^{a,AB}$	$5.60 \pm 0.03^{a,AB}$
300	$5.64 \pm 0.09^{a,AB}$	$5.73 \pm 0.07^{a,B}$	$5.60 \pm 0.09^{a,AB}$	$5.73 \pm 0.10^{a,B}$
1050	$5.54 \pm 0.04^{a,A}$	$5.64 \pm 0.06^{b,AB}$	$5.74 \pm 0.06^{bc,AB}$	$5.76 \pm 0.05^{c,B}$
2000	$5.67 \pm 0.07^{a,B}$	$5.72 \pm 0.08^{a,B}$	$5.67 \pm 0.10^{a,AB}$	$5.78 \pm 0.04^{a,B}$
4000	$5.64 \pm 0.03^{a,AB}$	$5.71 \pm 0.02^{ab,AB}$	$5.75 \pm 0.09^{b,B}$	$5.80 \pm 0.01^{b,B}$

[1]All measurements were performed in triplicate.
[2]Significant difference ($p < 0.05$) between data is expressed by different letters (A, B, C, and D); and lower-cased letters (a, b, and c) are within rows and capital letters are within columns.

5.59 when it turned to 5.8 after the harsh treatment of 8-h exposure to 4000 ppm concentration of ozone. Due to pH rise, the number of OH^- radicals increased and as a result the ozone destruction increased. Actually the OH^- radicals caused the intensified ozone decomposition (Eq. [3]). So, the ozone is destroyed faster in alkali medium than the acidic one (Guzel-Seydim et al. 2004).

Ozone destruction in alkali medium is seen in Equation [3]:

$$O_3 + OH^- \longrightarrow H_2O + O_2 \qquad [3]$$

$$O_3 + HO^-{}_2 \longrightarrow OH^- + 2O_2$$

CONCLUSION

Through this investigation it was concluded that 2000 ppm gaseous ozone within 8 h exposure time appeared to be an optimum condition for date fruit disinfestations. It was the optimized concentration-time combination in terms of both reaching the highest mortality of Indian meal moth through all three life stages and having the least detrimental impact on micronutrients and chemical parameters of the date.

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