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Evaluation of the efficacy of ozonated olive oil for controlling the growth of *Alternaria alternata* and its toxins

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ABSTRACT

Toxigenic fungi infect fruits and vegetables either during harvest or storage and create mycotoxins as secondary metabolites, which pose a serious threat to human and animal health throughout the food chain. Therefore, the objective of this study was to determine the inhibitory effect of OZO against the growth and spore germination of the *Alternaria alternata* fungal strain. Additionally, evaluation of the synthesis inhibition of *Alternaria* toxins (ATs), among which are alternariol (AOH), alternariol-9-methyl ether (AME), and tenuazonic acid (TeA) in the potato dextrose broth (PDB) medium and orange fruit after harvest. The results indicated that the inhibition zone was 29.0 ± 1.2 mm at 20 mg/L of OZO. The MIC and MFC values were recorded at 0.186 and 1.57 mg/mL, respectively. In this regard, OZO prevented conidia germination at 98.8% with the treatment of 5 mg/mL. OZO at 20 mg/mL was efficacious in producing a high loss in ATs production in the PDB medium, reaching 73.4, 76, and 67.1% for AOH, AME, and TeA, respectively. In addition, OZO prevents the biosynthesis of AOH and AME during the storage of orange fruits compared with the positive control sample. In contrast, 20 mg/mL reduced TeA accumulation and the appearance of *Alternaria* brown spot (ABS) in orange. To the best of our knowledge, this is the first report that studies OZO to control ATs in vitro in orange fruits.

1. Introduction

Olive oil has mainly monounsaturated fatty acids such as oleic acid as predominant (65–85%), linoleic acid, and palmitic acid (up to 18–21%). Olive oil is a source of many bioactive substances, such as tocopherols or phenolic compounds [1]. The ozone mainly interacts with double carbon-carbon bonds in unsaturated fatty acids, which yields many oxygenated compounds such as peroxides, aldehydes, and ozonides [2]. These components cause the increase of ozonated olive oil (OZO) biological activity, like antimicrobial activity. The amounts of these components are affected by several factors, including the time of the ozonation process, temperature, ozone concentration, flow, type, and quality of the oil [3,4]. Several studies have reported that OZO has antimicrobial activity dependent on the Criegee Mechanism [5–7]. On the other hand, we did not find studies that investigated with the effect of OZO on the production of toxins. As a result, this study will look into the efficacy of OZO as an anti-mycotoxin, specifically against *Alternaria* toxins (ATs). ATs are one of the main mycotoxins naturally occurring in fruits, vegetables, and cereals. ATs include more than 70 secondary metabolites. But the major and frequent of ATs contaminants include alternariol (AOH), alternariol monomethyl ether (AME), and

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tenuazonic acid (TeA), which are produced by several *Alternaria* species like *Alternaria alternata*, *A. citri, A. tenuis, A. tenuissima,* and others [8–10]. According to previous studies, the toxins of AOH, AME, and TeA are mutagenic, genotoxic, and toxic to cells, so they pose a serious health risk to humans and animals. In China, ATs are one of the most important risk factors for human esophageal cancer [11–13]. The threshold of toxicological concern value for ATs was defined by the European Food Safety Authority (EFSA) at 2.5 ng/kg body weight/day for AOH and AME and increased to 1500 ng/kg body weight/day for TeA. The Bavarian Health and Food Safety Authority set a TeA maximum limit in infant food based on sorghum/millet at 500 μg/kg [14]. The European Commission is now considering limit levels for ATs in foods, although there is no legislation in place as of yet. AOH, AME, and TeA were three ATs that were the subject of a draught EU Commission recommendation that was released in June 2019. Fruits and vegetables have a higher moisture content after harvest, therefore, *Alternaria* infections become more prevalent in this peroid [15,16]. Therefore, this study aimed to evaluate the inhibitory effect of OZO against the growth and conidia germination of the *Alternaria alternata* strain. As well as inhibiting the production of ATs with this strain in the potato dextrose broth (PDB) medium. Finally, control for ATs in post-harvest orange fruits.

2. Materials and methods

2.1. Preparation of ozonated olive oil

Ozonated olive oil (OZO) is obtained after ozonation of olive oil (500 mL) using an ozone generator, Model OZO 6 VTTL (OZO MAX LTD, Shefford, Quebec, Canada; http://www. ozomax.com) that produces 30 g/h from dry air feed. 500 mL of olive oil was poured into a 1000 mL flask, and the flask was plugged with a silicone stopper with two holes in it. One hole was for the ozone line, and the other was for tubing connected to the ozone destruction unit. This ozonation process was applied for 8 h a day for about three weeks at room temperature (22–25 °C).

2.2. Chemical characterization of ozonated olive oil

2.2.1. Acidity and iodine values

According to American Oil Chemists' Society (AOCS) (1998 a, b), the acidity value of OZO was calculated. The acidity index is calculated as the amount of potassium hydroxide required to neutralize the free fatty acid in 1 g of OZO. While, the iodine value was calculated as the number of grams of iodine that is a measure of OZO's unsaturation rate [17,18].

2.2.2. Peroxide value

The peroxide value of OZO was calculated as described by AOCS (1998c), indicating the amount of peroxide present in the substance. Its measured in milliequivalent and is stated as the amount of active oxygen per kilogram of OZO (mmol/kg) [19,20].

2.2.3. Viscosity

A vibrating viscometer (AND SV-10 Japan) was used to measure the viscosity of OZO between 24 and 40 °C. A typical trend can be a helpful tool for quick quality control evaluation throughout the entire ozonation process and timing the process to achieve the required ozonation level for the sample is also crucial [3,20].

2.2.4. P-anisidine value

OZO's *P*-anisidine value was calculated in accordance with AOCS (2011). In order to calculate the aldehyde ratio, free hydroxylamine is added to the aldehyde carboxylic group. The result is given in mmol/g [21].

2.2.5. pH value

Using a pH meter model (3510 pH Metre, Jenway), the pH value of OZO was evaluated at room temperature (24 °C).

2.3. Evaluation of the antifungal activity of OZO

The potential antifungal activity of OZO against *A. alternata* was performed using the agar well diffusion method, the minimal inhibitory concentration (MIC) and the minimum antifungal concentration (MFC) were also evaluated.

2.3.1. Preparation of inoculum

The fungal strain used in this study *Alternaria alternata* (*A. alternate* ITEM 752) was grown at 25 °C for 7 days on potato dextrose agar (PDA) medium until the mycelial growth covered the entire dishes, from which, the conidial suspension was obtained in 10 mL (0.85% NaCl, 0.1% Tween 80). Then it was diluted to 10^{6} conidia/mL using a Thomas counting chamber.

2.3.2. Agar well diffusion method

Freshly prepared fungal suspension was streaked on the surface of (PDA) medium. Following inoculation, wells measuring 5 mm in diameter were drilled into the PDA surface and filled with three different OZO concentrations 5, 10, and 20 mg/mL. As a negative control, wells containing 100 μL of 10% DMSO were employed. Plates were incubated at 25 °C for 72 h, after which the diameter of the wells and the zone of inhibition were measured [22].

2.3.3. Determination of MIC and MFC

According to Luz et al. [23], the MIC and MFC of OZO against *A. alternate* were evaluated using the microdilution technique. Various concentrations of OZO, ranging from 0.0 to 2.5 mg/mL, were prepared. Then, 100 μL of a 10^6 conidia/mL suspension of the fungal strain was added as an inoculant. For three days, inoculated microplates were incubated at 25 °C. Following incubation, the MIC was established as the OZO concentration at which no discernible fungal growth was noticed. To determine MFC, 10 μL of each concentration with no visible fungal growth was seeded on PDA plates. The MFC value was determined after 3 days of incubation at 25 °C. The minimum fungicidal concentration (MFC) is defined as the lowest concentration resulting in a 99.9% reduction in the starting inoculum.

2.3.4. Effect of OZO on A. alternata conidia germination

The effect of OZO at different concentrations (1, 2, 3, 4 and 5 mg/mL) was studied using the slide technique according to Abbas et al. [24]. *A. alternata* strain was cultivated on PDA medium until sporulation. After that, each concentration of OZO was placed on a dried, clean glass slide as a film. The film was covered with a drop of *A. alternata* suspension 10^6 conidia/mL. Similarly, control treatment was performed using sterilized distilled water instead of the OZO. Each concentration was replicated using three slides. Each slide was put on a glass rod and incubated for 24 h at 25 °C in a Petri dish. For each replication (slide), four microscopic fields were studied (x = 10 × 40). According to equation (1), conidia germination (%) was calculated

$$\textbf{Conidia germination}\ (\%) = \left(\frac{\boldsymbol{A-B}}{\boldsymbol{A}}\right)\boldsymbol{x100}$$

where A is the percentage of germinated conidia in the control, and B is the percentage of germinated conidia in the treatment.

2.4. Effect of OZO on ATs produced by A. alternata in PDB medium

Conical flasks containing 50 mL of potato dextrose broth (PDB) were autoclaved at 121 °C for 15 min, cooled down, and inoculated with 1 mL conidial suspension of *A. alternata* (10^6/mL) to investigate the impact of OZO on the toxin produced by *A. alternata*. After that, the inoculated flasks were treated with OZO at 5, 10, and 20 mg/mL and incubated for 14 days at 25 °C. The AOH, AME, and TeA were extracted by sonication for 20 min with 10.0 mL of ethyl acetate that contained 1% formic acid. Three mL of extracts were then evaporated to dryness at 50 °C under a gentle nitrogen stream for determination by high-performance liquid chromatography-UV (HPLC-UV) according to Meena et al. [25,26]. The percentage of inhibition ATs for each type (AOH, AME, and TeA) was calculated as following equation (2):

The percentage of inhibition (%) $= \frac{\mathbf{C-T}}{\mathbf{C}}\ \mathbf{X100}$

Where: C is the concentration of (AOH, AME, and TeA) for each separately in the positive sample inoculated by spores of *A. alternata* only. T is the concentration in the sample containing spores of fungus and OZO (treatment sample).

2.5. Control of A. alternata growth and its toxins in orange fruits

The orange fruits were washed with sterilized water and then with 70% ethanol solution for 30 s and 2% sodium hypochlorite (NaOCl) for 5 min, then washed twice with sterilized distilled water, and then let to dry in a laminar flow hood. Injuring fruits through perforations (3 mm deep) were made with a needle for each point as follows:

Group (A): Orange fruits inoculated with 100 μL conidial suspension of *A. alternata* (positive control sample).
Group (B): Orange fruits inoculated with *A. alternata* and treated with 20 mg/mL of OZO.
Group (C): Orange fruits inoculated with *A. alternata* and treated with commercial disinfection (Ortosol 20%)
Group (D): Non sterilized and un treated orange fruits.
Group (E): Non sterilized and un inoculated orange fruits, but treated with 20 mg/mL OZO. Finally, all fruit was sealed in polyethylene-lined plastic boxes and incubated for 14 days at 25 °C.

2.6. Determination of ATs in orange fruit

On the basis of the method stated by Meng et al. [27], sample preparation was carried out. The samples were shaked for homogeneity. Then, 10 mL of acetonitrile containing 1% formic acid was added to 2.5 mL of juice in a 50 mL centrifuge tube. After being vortexed for 30 s, the ultrasonic extraction process was taken 40 min. The slurry was then immediately given 0.5 g of anhydrous magnesium sulphate and 0.5 g of sodium chloride,and forcefully agitated for 30 s. After centrifugation at 4500 rpm for 10 min, 5 mL of the supernatant was transferred into 10 mL centrifuge tubes and dried by nitrogen stream at 40 °C. The residues were re-dissolved in 1 mL acetonitrile/water containing (5 mmol/L) ammonium acetate (20/80 v/v) and passed through a 0.22 μm membrane filter to be ready for analysis.

2.7. Statistical analysis

General Linear Model procedure of the SPSS ver. 18 (IBM Corp, NY) was used to for statistically analyzed. The significance of the

Table 1
Some physicochemical analysis for OZO.

Parameters				
Peroxide value (mmol-mEq/kg)	Acidity value (mg KOH/g)	Iodine value (g Iodine/100 g)	Viscosity (24–40 °C) (centipoise)	pH (24 °C)
1280 ± 2.57	**8.73 ± 0.55**	**4.11 ± 0.25**	**1150 ± 3.5**	**1.4 ± 0.12**

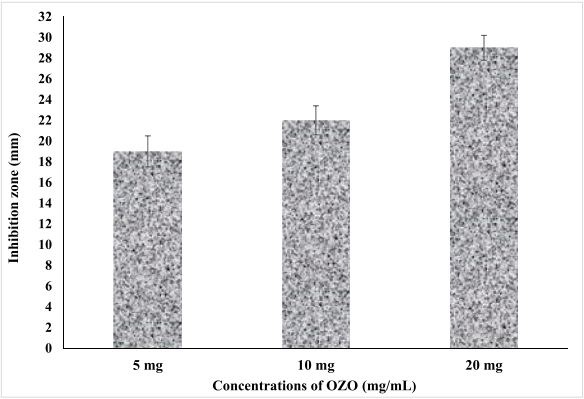

Fig. 1. Inhibition zone (mm) of OZO against *A. alternata* strain.

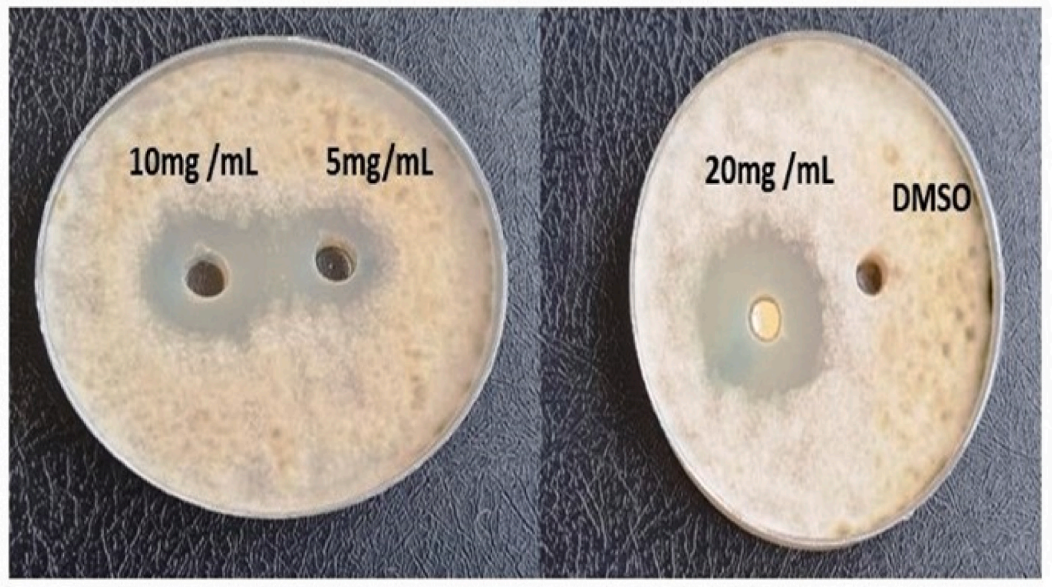

Fig. 2. Antifungal activity of OZO on the growth of *A. alternata* strain using agar well diffusion method.

differences among treatment groups was determined by Waller–Duncan k-ratio. All statements of significance that were depended on the probability of *P*-value ≤ 0.05 was considered to be statistically significant. Values represent averages ±standard deviations for triplicate experiments.

3. Results and discussion

3.1. Characterization of olive oil after ozonation process

Olive oil contains unsaturated fatty acids, phospholipids, sterols, vitamins, carotenoids, and polyphenols, which can interact with ozone gas through the existence of C–C double bonds and cause a change in the physicochemical properties of the oil afterward. For example, the peroxide value, iodine value, density, and viscosity of the oil are all affected by the degree of ozonation [2]. The results

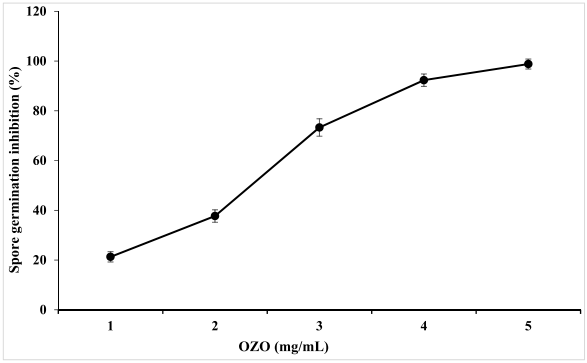

Fig. 3. Effect of different concentrations of OZO on conidia germination of *A. alternata.*

reflected that the OZO has a high peroxide value and acidity value of 1280 (mmol-mEq/kg) and 8.73 (mg KOH/g), respectively, according to the results shown in (Table 1). Moreover, the viscosity increased to 1150 cP, while the pH decreased to 1.4. The increase in the value of peroxide denotes the existence of substances with an oxidizing activity that was produced as a result of ozone treatment [4]. Elimination of the double bonds that have reacted with ozone is correlated with an increase in van der Waals contacts, which are revealed by viscosity. Additionally, the polymerization that can take place during ozonation procedures produces polymeric peroxide, increasing viscosity [2,7]. On the other hand, the ozonides and aldehydes that derive from the hydrolysis reactions of the oxygenated products increase the acidity of the medium [28]. Oils can be ozonated through a procedure called the Criegee reaction, which explains how ozone interacts with bonds in an unsaturated oil to produce an unstable primary ozonide, which then breaks down into ozonized derivatives like cyclical trioxolane, one of the highly effective therapeutic ozone compounds. Exposure of oil to ozone for a long time led to increased peroxide and acidity levels. Due to the creation of novel carbonyl compounds such as aldehyde, 1,2,4-trioxolane, and chain scission at the C═C bonds of the fatty acid chains during the ozonation process [4,29].

3.2. Inhibitory effect of OZO against fungal growth

The OZO displayed an inhibitory effect on the growth of *A. alternat*a strain at three concentrations of 5, 10, and 20 mg/mL were 19.0 ± 1.5, 22.0 ± 1.4, and 29 ± 1.2 mm, respectively shown in Figs. 1 and 2. This effect may be due to many compounds like ozonides, aldehydes, hydroperoxide, polyperoxides and diperoxides which formed during the ozonation process through ozonated unsaturated fatty acids in olive oil by ozone gas. These molecules are responsible for antimicrobial activity. In addition, oxidative stress for OZO is an inhibitor of aerobic microorganisms [30]. Influence of the oxidation products on fungal strains by oxidation of cellular components such as sulphydryl groups and amino acids of enzymes, peptides, proteins and polyunsaturated fatty acids, and oxidation of the cell membrane. By directly oxidizing phospholipids and lipoproteins, ozonated oil breaks down a cell's membrane, changing its chemical makeup, preventing it from exchanging with the environment, and inducing cell envelope disintegration, which leads to cell lysis and death [31].

3.3. Evaluation of MIC and MFC for OZO

Data revealed that the MIC and MFC for OZO against *A. alternata* strain were 0.186 and 1.57 mg/mL, respectively. Geweely (2006) reported that the OZO was effective against many fungi such as *A. fumigatus* and *Epidermophyton floccosum* at MIC 1.45 and 2.0 mg/mL, respectively [1]. According to MFC data, the activity of OZO is due to toxicity rather than a metabolic interruption, as is the case for traditional antimicrobial agents. OZO significantly reduces the activity of the enzymes needed for many biological activities, including growth [1]. It was revealed that ozonized oil has a potent inhibitory effect on the formation of mycelia. This behavior can be explained by its oxidative effects on fungus cells, which are harmful to the genetic material, enzymes, intracellular proteins, and spore envelopes [32].

3.4. Efficacy evaluation of OZO against A. alternata (conidia germination and production of ATs)

3.4.1. Reduce conidia germination

The OZO at concentrations 1, 2, 3, 4, and 5 mg/mL was evaluated for 24 h at 25 °C against the germination ofA. alternata spores. According to the results in Fig. 3, conidia germination reduced to 21.3, 37.3, 73.3, 92.3, and 98.8%, respectively. The conidia produced by *A. alternata* are easily dispersed in the air and endure light and desiccation without losing viability for a long time. Mycelia and spores can serve as inoculant to infect food [33]. In light of this, OZO is beneficial in combating spores. The OZO damages the spores through deterioration of the cell surface of the spore, which includes peroxidation of the membrane phospholipids due to contact with OZO. According to Hatzipapas et al. [34], ozone oxidizes the phospholipids in the cell membrane, which causes the surface to become brittle and fluid. Additionally, ozonated oil completely prevents conidial germination because the OZO targets the anatomical structures of *A. alternata* and disrupts vital cellular functions. On the other hand, many fungi have distinctive colors because of conidia.

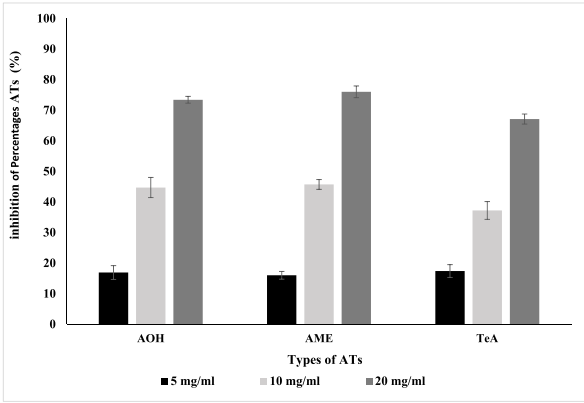

Fig. 4. The inhibition percentages of three types of ATs treated by OZO in the PDB media.

Table 2
ANOVA analysis for concentrations of OZO on the percentages of inhibition ATs in PDB medium.

Source	SS	df	MS	F	*P*
Intercept	51903.053	1	51903.053	3914.585	0.000000
Con. of OZO	13856.596	2	6928.298	522.540	0.000000
Types of ATs	144.962	2	72.481	5.467	0.014
Con. of OZO* Types of ATs	107.569	4	26.892	2.028	0.133
Error	238.660	18	13.259		
Total	66250.840	27			

SS, sum of squares; df, degree of freedom; MS, mean square; *P*, probability at confidence 0_95.

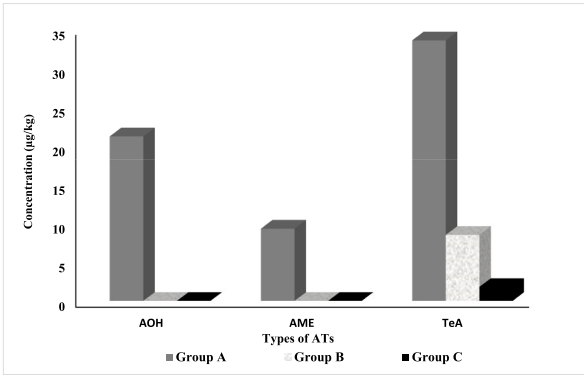

Fig. 5. Concentrations of ATs in infected orange fruits by *A. alternata*. (Group A) without any treatment; (Group B) treated with 20 mg/mL OZO; and (Group C) treated with commercial disinfection.

As a result, some fungal species may have changed color as a result of ozonized oil's impact on conidia [35].

3.4.2. In vitro evaluation for OZO on the amount of ATs produced by A. alternata in PDB medium

The results displayed inFig. 4 show the percentages of inhibition ATs including (AOH, AME, and TeA) produced by *A. alternata* in the PDB medium, which was treated with three concentrations of OZO. The data reflected that the percentages of inhibition of AOH were 16.9, 44.7, and 73.4% at 5, 10, and 20 mg concentrations of OZO, respectively. The highest inhibition percentages recorded with AME at 10 and 20 mg/mL were 45.7 and 76%, respectively. On the other hand, the same concentrations of 10 and 20 mg/mL from OZO gave the lowest inhibition with TeA of 37.2 and 67.1%. The ANOVA analysis for the effect of OZO at three concentrations on the percentage inhibition of three types of ATs produced by *A. alternata* in the PDB medium was displayed in Table 2. According to the results, OZO may influence the activities of many of the enzymes that are needed by the *A. alternata* strain in the metabolism process and thus the production of toxins. The authors suggest that OZO may have affected the genome sequences that play a significant role in ATs pathway genes. So, the production of ATs decreased as a consequence of a reduction in gene expression regulation levels. Therefore, it will be important to investigate how OZO treatments affect gene expression in the toxic *A. alternata* strain in the future. So, the study of variations in biochemical pathways and expression genes by OZO may confer a better understanding. In general,

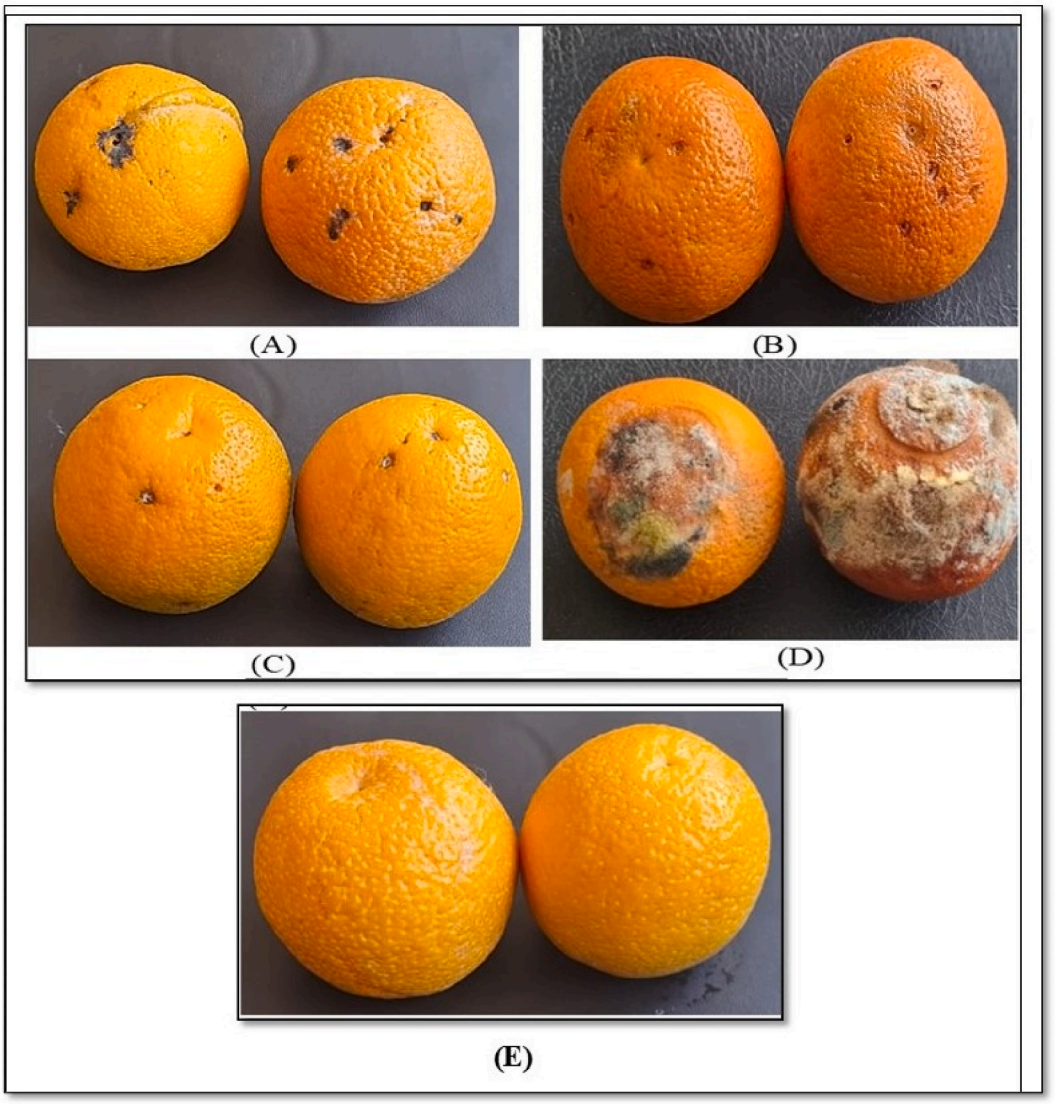

Fig. 6. Orange fruits after 14 days of incubation at 25 °C. (A) Inoculated with spore suspension of *A. alternata* (positive control); (B) orange fruits inoculated with *A. alternata* and treated by 20 mg/mL OZO; (C) orange fruits inoculated with A. *alternata* and treated by commercial disinfection (Ortosol, 20%); (D) fruits without sterilization and untreated, (E) Orange fruits without sterilization and free from (*A. alternata*) spores, but treated by 20mg/mL OZO. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

alkaloids, terpenes, and polyketides are the common pathways of fungal secondary metabolism. AOH is biosynthesized in the polyketide pathway [36–38]. According to Saha et al. [39] AOH and AME production were associated with the timing of the expression of two polyketide synthase (PKS) genes, pksJ and pksH. PksH downregulation impacted pksJ expression, which had an indirect impact on the generation of AOH.

3.5. OZO as an anti-Alternaria toxin in post-harvest oranges contaminated by A. alternata

OZO at a concentration of 20 mg/mL prevented the formation of AOH and AME in orange fruits; the TeA concentration was 8.53 μg/kg. While positive control sample (group A) concentrations from AOH, AME, and TeA were 21.28, 9.33, and 33.5 μg/kg, respectively, in contrast, the group (C) did not show any amounts of AOH or AME, although TeA recorded 1.83 μg/kg, as illustrated in Fig. 5. *A. alternate* infects orange fruits in various regions of the world, which results in *Alternaria* brown spots. This fungal strain grows while the fruits are stored in a cold environment, becomes noticeable during the marketing stage, and causes postharvest losses. Farmers use synthetic fungicides to control *Alternaria* diseases, which cause the appearance of resistant fungi, oncogenic risk, handling hazards, and threats to the environment. As a result, many nations around the world have limits or outright bans on the use of chemical fungicides on fruits. Nowadays, a lot of research is focused on offering farmers better substitutes for chemical fungicides that don't

have harmful impacts on the environment [40,41]. As shown in (Fig. 6) treating groups B and E with OZO, reduced the appearance of *Alternaria* brown spot (ABS) disease compared to untreated fruit. On the other hand, it is possible to use or develop OZO to be used as an alternative to industrial antifungals, therefore it can be used to spray plants in the field (during cultivation) to protect against fungal infection and thus control the disease of ABS in fruits. The fungicide activity of OZO is caused by the slow release of peroxides that oxidize phospholipids and lipoproteins, which are components of the fungal cell wall, damaging their integrity to infiltrate inside the cell, oxidize glycoproteins and glycolipids, and block enzymatic function. These processes work together to restrict growth, which mostly happens during certain periods [42,43]. The ozonation process of olive oil produced unstable compounds including 1.2.3 trioxolane and molozonide. Because of this, in accordance with the Criegee Mechanism, it tends to rearrange to (1.2.4-trioxolane), which is more stable throughout the formation of carbonyl oxide as an intermediate compound that reacts with the aldehyde to form the stable ozonide. Orange peel tissue and fungal mycelium that already contain water will interact with carbonyl and hydroperoxide, decomposing into a peroxide H_2O_2 thats is classified as a Reactive Oxygen Species (ROS) and a carbonyl fragment classified as a Lipid Oxidation Product (LOP). Both ROS and LOP are generally harmful to fungal growth [44–46]. This hypothesis is backed by the theory that ozone causes aldehydes, peroxides, and hydroxyl radicals to be formed in the cytoplasmic membrane during the oxidation of polyunsaturated fatty acids, which then assault cytoplasmic enzymes. Oxidation of sulfhydryl groups (SH to S–S) in enzymes causes the fungi lose their ability to grow and then die. Additionally, the concurrent breakdown of nucleic acids and enzymatic activities may indicate that nucleic acids were involved in the creation of enzymes [47].

4. Conclusion

Our data revealed the antifungal activity of OZO against *A. alternata*. In addition, the inhibitory activity of OZO was increased by increasing its concentrations. Also, OZO reduced conidia germination by 98.8% at 5 mg/mL, furthermore 20 mg/mL of OZO gave the highest inhibition of ATs production being 73.4%,76% and 67.1% for AOH, AME, and TeA respectively. Moreover, OZO control *A. alternata* infection in orange fruits as compared to the commercial disinfectant. Thus, OZO can be used as an alternative fungicide for controlling fungal diseases.

Author contribution statement

Amira E. Sehim: Performed the experiments; Analyzed and interpreted the data. Rasha Y. Abd Elghaffar: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data. Amany M. Emam: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data. Tarek A. El-Desouky: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] N.S.I. Geweely, Antifungal activity of ozonized olive oil (Oleozone), Int. J. Agric. Biol. 8 (5) (2006) 670–675.
[2] P. Guerra-Blanco, I. Chairez, T. Poznyak, M. Brito-Arias, Kinetic analysis of ozonation degree effect on the physicochemical properties of ozonated vegetable oils, Ozone: Sci. Eng. 43 (6) (2021) 546–561, https://doi.org/10.1080/01919512.2020.1868972.
[3] M.F. Diaz, R. Hernández, G. Martínez, G. Vidal, M. Gómez, H. Fernández, R. Garcés, Comparative study of ozonized olive oil and ozonized sunflower oil, J. Braz. Chem. Soc. 17 (2006) 403–407.
[4] S. Moureu, F. Violleau, D. Ali Haimoud-Lekhal, A. Calmon, Ozonation of sunflower oils: impact of experimental conditions on the composition and the antibacterial activity of ozonized oils, Chem. Phys. Lipids 186 (2015) 79–85, https://doi.org/10.1016/j.chemphyslip.2015.01.004.
[5] V.F. Georgiev, T.T. Batakliev, M.P. Anachkov, S.K. Rakovski, Study of ozonated olive oil: monitoring of the ozone absorption and analysis of the obtained functional groups, Ozone Sci. Eng. 37 (2015) 55–61.
[6] M. Montevecchi, A. Dorigo, M. Cricca, L. Checchi, Comparison of the antibacterial activity of an ozonated oil with chlorhexidine digluconate and povidone-iodine. A disk diffusion test, New Microbiol. 36 (3) (2013) 289–302.
[7] W. Zakrzewski, M. Dobrzynski, J. Nowicka, M. Pajaczkowska, M. Szymonowicz, S. Targonska, P. Sobierajska, K. Wiglusz, W. Dobrzynski, A. Lubojanski, S. Fedorowicz, Z. Rybak, R.J. Wiglusz, The influence of ozonated olive oil-loaded and copper-doped nanohydroxyapatites on planktonic forms of microorganisms, Nanomaterials 10 (10) (2020) 1997, https://doi.org/10.3390/nano10101997.
[8] E. Ugazio, V. Tullio, A. Binello, S. Tagliapietra, F. Dosio, Ozonated oils as antimicrobial systems in topical applications. Their characterization, current applications, and advances in improved delivery techniques, Molecules 25 (2020) 334.

[9] V. Ostry, Alternaria mycotoxins: an overview of chemical characterization, producers, toxicity, analysis and occurrence in foodstuffs, World Mycotoxin J. 1 (2008) 175–188, https://doi.org/10.3920/wmj2008.x013.
[10] A. Patriarca, Alternaria in food products, Curr. Opin. Food Sci. 11 (2016) 1–9, https://doi.org/10.1016/j.cofs.2016.08.007.
[11] S. De Berardis, E.L. De Paola, G. Montevecchi, D. Garbini, F. Masino, A. Antonelli, D. Melucci, Determination of four Alternaria alternata mycotoxins by QuEChERS approach coupled with liquid chromatography-tandem mass spectrometry in tomato-based and fruit-based products, Food Res. Int. 106 (2018) 677–685.
[12] M. Solfrizzo, Recent advances on Alternaria mycotoxins, Curr. Opin. Food Sci. 17 (2017) 57–61, https://doi.org/10.1016/j.cofs.
[13] S. Chen, S. Qiang, Recent advances in tenuazonic acid as a potential herbicide, Pestic. Biochem. Physiol. 143 (2017) 252–257, https://doi.org/10.1016/j.pestbp.2017.01.003.
[14] S. Fraeyman, S. Croubels, M. Devreese, G. Antonissen, Emerging Fusarium and Alternaria mycotoxins: occurrence, toxicity and toxicokinetics, Toxins 9 (2017) 228.
[15] European Food Safety Authority (EFSA), Scientific opinion on the risks for animal and public health related to the presence of Alternaria toxins in feed and food, EFSA J. 9 (2011) 2407.
[16] H. Rizwana, N.A. Bokahri, S.A. Alsahli, A.S. Al Showiman, R.M. Alzahrani, H.A. Aldehaish, Postharvest disease management of Alternaria spots on tomato fruit by Annona muricata fruit extracts, 10.1016, Saudi J. Biol. Sci. 28 (4) (2021) 2236–2244.
[17] AOCS, Acid value. AOCS official test method Cd 3a-63 for, in: D. Firestone5th (Ed.), Official Methods and Recommended Practices of the American Oil Chemists' Society, American Oil Chemists' Society, Champaign, IL, 1998.
[18] AOCS, Iodine value of fats and oils. WIJS method. AOCS official method Cd 1-25, in: D. Firestone5th (Ed.), Official Methods and Recommended Practices of the American Oil Chemists Society, American Oil Chemists' Society, Champaign, IL, 1998.
[19] AOCS, Peroxide value. AOCS official method Cd 8-53, in: D. Firestone5th (Ed.), Official Methods and Recommended Practices of AOCS, American Oil Chemists' Society, Champaign, IL, 1998.
[20] V. Travagli, I. Zanardi, G. Valacchi, V. Bocci, Ozone and ozonated oils in skin diseases: a review, Med. Inf. (2010) 1–9, https://doi.org/10.1155/2010/610418.
[21] AOCS, P-anisidine value. AOCS method Cd 18-90 (11): official method, in: D. Firestone 6th (Ed.), Official Methods and Recommended Practices of the American Oil Chemists' Society, American Oil Chemists' Society, Champaign, IL, 2011.
[22] T.A. El-Desouky, Evaluation of effectiveness aqueous extract for some leaves of wild edible plants in Egypt as anti-fungal and anti-toxigenic, Heliyon 10 (2) (2021), e06209, https://doi.org/10.1016/j, 7.
[23] Luz C, Izzo L, Ritieni A, Mañes J and Meca G. 2020. Antifungal and antimyco- toxigenic activity of hydrolyzed goat whey on Penicillium spp: an application as biopreservation agent in pita bread. LWT–Food Sci. Technol., 118:108717.
[24] E. Abbas, A. Osman, M. Sitohy, Biochemical control of Alternaria tenuissima infecting post-harvest fig fruit by chickpea vicilin, J. Sci. Food Agric. 100 (7) (2020) 2889–2897, https://doi.org/10.1002/jsfa.10314.
[25] M. Meena, A. Zehra, M.K. Dubey, M. Aamir, V.K. Gupta, R.S. Upadhyay, Comparative evaluation of biochemical changes in tomato (Lycopersicon esculentum mill.) infected by Alternaria alternata and its toxic metabolites (TeA, AOH, and AME), Front. Plant Sci. 7 (2016) 1408, https://doi.org/10.3389/fpls.2016.01408.
[26] M. Meena, P. Swapnil, R.S. Upadhyay, Isolation, characterization and toxicological potential of Alternaria-mycotoxins (TeA, AOH and AME) in different Alternaria species from various regions of India, Sci. Rep. 18 (1) (2017) 8777, https://doi.org/10.1038/s41598-017-09138-9, 7.
[27] J. Meng, W. Guo, Z. Zhao, Z. Zhang, D. Nie, E.K. Tangni, Z. Han, Production of Alternaria toxins in yellow peach (Amygdalus persica) upon Artificial inoculation with Alternaria alternate, Toxins 15 (9) (2021) 656, https://doi.org/10.3390/toxins13090656, 13.
[28] N. Rodrigues De Almeida Kogawa, E. José De Arruda, A.C. Micheletti, M. De Fatima Cepa Matos, L.C. Silva De Oliveira, D. Pires De Lima, N.C. Pereira Carvalho, P. Dias De Oliveira, M. De Castro Cunha, M. Ojeda, et al., Synthesis, characterization, thermal behavior, and biological activity of ozonides from vegetable oils, RSC Adv. 5 (2015) 65427–65436.
[29] K. Varol, A.N. Koc, M.A. Atalay, I. Keles, 2017. Antifungal activity of olive oil and ozonated olive oil against Candida spp. and Saprochaete spp, Ozone: Sci. Eng. 39 (2017) 462–470.
[30] G. Celenza, R. Iorio, S. Cracchiolo, S. Petricca, C. Costagliola, B. Cinque, B. Segatore, G. Amicosante, P. Bellio, Antimycotic activity of ozonized oil in liposome eye drops against Candida spp, Trans. Vis. Sci. Techn. 9 (8) (2020) 4, https://doi.org/10.1167/tvst.9.8.4.
[31] Zahra Amri, Sarra Ben Hamida, Dbeibia Amal, Asma Ghorbel, Abdelkarim Mahdhi, Mansour Znati, Indu Ambat, Esa Haapaniemi, Mika Sillanpää, Hammami Mohamed, Physico-chemical characterization and antibacterial activity of ozonated pomegranate seeds oil, ozone, Sci. Eng. 42 (6) (2020) 531–538, https://doi.org/10.1080/01919512.2020.1735993.
[32] A. Phaephiphat, W. Mahakarnchanakul, Surface decontamination of Salmonella typhimurium and Escherichia coli on sweet basilby ozone microbubbles, Food Sci. Technol. Res. 4 (1) (2018) 1–12.
[33] M.G. Mustafa, 1990. Biochemical basis of ozone toxicity, Free Radic. Biol. Med. 9 (3) (1990) 245–265, https://doi.org/10.1016/0891-5849(90)90035.
[34] P. Hatzipapas, K. Kalosaka, A. Dara, C. Christias, Spore germination and appressorium formation in the entomopathogenic Alternaria alternata, Mycol. Res. 106 (2002) 1349–1359, https://doi.org/10.1017/S0953756202006792.
[35] J. Wiley, Essential Microbiology, West Sussex PO19 8SQ, England, Southern Gate, Chichester, 2005, p. 481p.
[36] M. Pagès, D. Kleiber, F. Violleau, Ozonation of three different fungal conidia associated with apple disease: importance of spore surface and membrane phospholipid oxidation, Food Sci. Nutr. 8 (10) (2020) 5292–5297, https://doi.org/10.1002/fsn3.1618.
[37] N. Keller, G. Turner, J.W. Bennett, Fungal secondary metabolism-from biochemistry to genomics, Nat. Rev. Microbiol. 3 (2005) 937–947.
[38] F.S. Zghair, B.T. Mohamed, S.M. Neda, Molecular assay of polyketide synthase gene of Alternariol (AOH) produce by Alternaria alternata, Int. J. Innovat. Appl. Stud. 9 (2014) 1124–1127.
[39] D. Saha, R. Fetzner, B. Burkhardt, J. Podlech, M. Metzler, H. Dang, C. Lawrence, R. Fischer, Identification of a polyketide synthase required for alternariol (AOH) and alternariol-9-methyl ether (AME) formation in Alternaria alternata, PLoS One 7 (2012), e40564.
[40] P.K. Mishra, V. Thawani, Characterization of Alternaria alternata isolated from Calotropis gigantea plant leaf, Int. J. Curr. Microb. Appl. Sci. 5 (10) (2016) 459–466.
[41] A.K.D. Anal, V. Kumar, A. Varma, 2021. Ecophysiology of Alternaria alternata causing diseases in litchi (litchi chinensis), Erwerbsobstbau 63 (2021) 163–173, https://doi.org/10.1007/s10341-021-00554-.
[42] F.M. Gabler, Joseph L. Smilanick, Monir F. Mansour, Hakan Karaca, Influence of fumigation with high concentrations of ozone gas on postharvest gray mold and fungicide residues on table grapes, Postharvest Biol. Technol. 55 (2) (2010) 85–90, 10.1016/j.
[43] T.A. El-Desouky, A.M.A. Sharoba, A.I. El-Desouky, H.A. El-Mansy, Khayria Naguib, Effect of ozone gas on degradation of aflatoxin B1 and Aspergillus flavus fungal, Environ. Analy. Toxic. 2 (2) (2012), 1000128.
[44] N.U. Soriano, V.P. Migob, M. Matsumura, Functional group analysis during ozonation of sunflower oil methyl esters by FTIR and NMR, Chem. Phys. Lipids 126 (2) (2003) 133–140.
[45] W.A. Pryor, G. Squadrito, M. Friedman, The cascade mechanism to explain ozone toxicity: the role of lipid ozonation products, Free Radical Biol. Med. 19 (6) (1995) 935–941.
[46] M.A. Mehlman, C. Borek, Toxicity and biochemical mechanism of ozone, Environ. Res. 42 (1987) 36–53.
[47] C. Franco, Ozone degradation of ribonucleic acid (RNA). Poly, Deg. Stab. 89 (2005) 274–281.