Original Research Article

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Effect of ozone treatment on microbial and quality alteration of onions during 2 months storage

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Abstract

Storing and preserving onion using pesticides is common in Brebes District. The study determined the use of ozone to reduce microorganisms and to maintain the onion quality during months of post-harvesting. There were three chambers with 2.5 m^3 in size, and with a capacity of 20 kg onion used for this study. Each chamber received different treatment for 2 months; chamber I: no treatment; chamber II: pesticide; chamber III: ozone. The ozone treatment maintained better temperature and humidity for preservation. Ozone treatment was also observed to maintain low level of mass damage (1.8%) as compared to control and pesticide (6.5% and 2.4%). The protein, ash, fat and vit B1 of onions were remained high in ozone-treated chamber. Total Plate Count (CFU/mL) and the Mold Yeast Count (colonies/mL) decreased on day 30 and 60 after pesticides and ozone treatment. This study showed the importance of ozone treatment to maintain better quality of onion after months of storage as compared to pesticide treatment. Therefore, ozone can be a potential use to replace the pesticide for preservation at post-harvesting.

Keywords

Pesticide, ozone, agriculture, onion farm

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INTRODUCTION

The district of Brebes, Central Java Province has a population of 1,781,379 people, 70% of those work in the agricultural sector. About 40% of the total population are farmers and farm workers, with most of them work in shallot plantation. A total area of onion plantation was 250 km^2, and the production of onion was 297,609.9 tons according to previous study (Budiyono B et al., 2017). The farmers and farm workers sprayed 560 to 1,588 L per ha (0.01 km^2) of the pesticide solution on the onion crops for 15–20 times during the onion plantation season. de Putter and Witono (2013) identified that organophosphate, carbamates, and pyrethroid were the most common pesticide used (de Putter and Witono, 2013).

Farmers spray pesticides on onion to prevent pest attacks and losses. Pesticides can also prevent the spoilage of onions bulb caused by fungi. *Penicillium digitatum, Fusarium oxysporum, Rhizopus stolonifer, Alternaria alternate, Aspergillus niger*, and *Saccharomyces cerevisiae* were observed in rotten onion bulbs (Samuel and Ifeanyi, 2015). The most recent studies also showed that *Fusarium sp* caused spoilage the onion bulbs at post-harvest and during storage (Chang et al., 2018; Ji et al., 2018).

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Preserving onions after harvesting period is very common in Brebes. Total 16.7% of 84 parents of the school children in the district of Brebes stored onion at home. They kept onion bulbs and onion products on the kitchen ceiling and sprayed with the pesticide (Budiyono B et al., 2017). The use of pesticide for storage and preservation of onions bulbs is a potential source to contaminate the house residents. The contamination might happen when the residues of pesticide fall down along with the dust from the onion to the kitchen floor and/or table. The house residents, especially school children, are at risk of inhaling the pesticide's residues (Lu et al., 2000). The previous study revealed that three types of di-alkyl phosphate metabolites were detected in the urine of 30–50% school children. It was suspected that one of the sources of pesticide exposure came from their house (Budiyono B et al., 2017).

Therefore, the potential exposure of pesticides to house residents has to be reduced by replacing the use of pesticide to preserve the onion bulbs. There are several methods for killing fungi on onion bulbs, and one of them is ozonation method. The previous studies showed that ozone could prevent the mycelia growth and sporulation of the crop yields (Chang et al., 2018; Pandiselvam et al., 2017; Timoshkin et al., 2012) and could degrade pesticide residues (Pandiselvam et al., 2020). Ozone destroys microorganisms by the progressive oxidation of vital cellular components (Pandiselvam et al., 2017; Torlak et al., 2013). The ozone method causes a reduction of contamination of microflora in dried onion (Brodowska AJ and Smigielski, 2013; Pandiselvam et al., 2017). This method does not change in taste, flavor, and appearance (Pandiselvam et al., 2017; Torlak et al., 2013) and no residues in fruits and vegetables are left (Guzel-Seydim et al., 2004; Pandiselvam et al., 2017). This study aimed to investigate the use of ozone treatment to maintain the quality of onion and to kill the microbes that induce the decay of onion during the preservation after the harvesting period.

MATERIALS AND METHODS

The specification of the ozone equipment

The ozone generator is D'Ozone with the dielectric barrier discharge (DBD). The dimension of D'Ozone is 80 cm in length, 50 cm wide, and 50 cm height. The steel hollow pipes with 2.5 cm in diameter and 2 meters length were used. The pipes are built as frame of the chambers and the pipes are inter-connected to each other to ensure the ozone flows all over the chambers. The device uses 100 Watt of electrical power and the oxygen is taken from ambient air with a flow rate of 20 L/min. The ozone flows through a hole in the stainless steel' pipe from the chamber frame. The ozone flows into the chamber, when it is tightly closed. The air outside the chambers was minimized to interrupt the ozone concentration in the chambers. The Personal Ozone Monitor™ Models 202 is used to measure the concentration of ozone (2B Technologies USA).

The onion sample preparation

On the first day, the farmers collected the red onions to a particular place in the farming area, and they brought the onions home on the second day. Two days post-harvest, a total of 100 kg of red onions (no treatment) from the District of Brebes were sent to the Center for Plasma Research Laboratory (CPR), Faculty of Science and Mathematics at the Integrated Laboratory, Universitas Diponegoro, Semarang, Central Java. A total of 100 kg of onion received was a gross mass (containing of onion bulbs, roots, and straws). The hay mass was subtracted to obtain net mass. Total 40% of the total weight was obtained as net mass from 100 kg of onions (n = 60 kg).

Treatment

Onions were put in three separate chambers equipped with storage shelves. The dimension of each storage chamber is 1-m length × 1 m in wide × 2.5 m in height (or 2.5 m^3). The chamber can load onion up to 100 kg. However, in this study, we loaded each chamber with 20 kg of onions. Each chamber was designed to receive different treatment. The chamber 1 was left without treatment as a control group and chambers 2 and 3 were treated with pesticides (fungicide), and ozone, respectively. Total 3 g of pesticide was dissolved into 1.5 L water for 20 kg of onions. The calculation mimics the concentration used by the farmers to preserve the onions in their homes. The concentration of ozone in chamber 3 was set to 216 ppm, and with a flow rate 20 L/min. The ozone generator was left on 4 ± 6 h/day to produce ozone of approximately 259.2 g/h. The density of ozone in a space of 2.5 m^3 was estimated as 622.08 g/m^3. In the normal condition in the field, the farmers use to store the onions in small ties and keep them hold on to wooden ribs in storage chambers. This condition was done for 2 months before the onions are re-planted. The treatment in this study adapted the storage system carried out by farmers in their homes. Meanwhile, ozone was given 5 days a week and 6 hours per day to ensure the availability of ozone in the chamber.

The physical parameters: temperature, and relative humidity (RH) of the chambers, were observed daily for 2 months (60 days) by Digital Thermometer Hygrometer (model number HTC1, Xuzhou Sanhe Automatic Control Equipment Co., Ltd, CHN). Whilst, the damage mass of onion bulbs was measured on day 30 and on day 60.

Mass-damaged and mass depreciation

Calculation of percentage of damaged mass and mass depreciation were determined with the equation of Gorrepati K et al., 2018 (Gorrepati K, 2018). Mass depreciation was measured at day 30 and day 60 (Gorrepati K, 2018). The percentage of damaged-mass was calculated based on amount of damaged-mass divided by initial mass. Whilst, percentage of depreciation mass was calculated using a formula of measured of undamaged-mass subtracted by total of damaged-mass divided by initial mass.

Microorganism identification

The physical parameters of microorganism growth were measured using the total number of bacterias (Total Plate Count/TPC), and mold and yeast count (Total Fungal Count/TFC and Mold Yeast Count/MYC). The study also identified the types of fungi on the bulb of onions. Samples were taken on day 0 of storage, day 30 or first month of storage, and day 60 or second month of storage.

Isolation of fungi on the bulb of onions was performed on day 0 and day 30. Briefly, the onion samples were rinsed three times using sterile distilled water and dried. The bulbs, stems, and leaves were taken and cut into 4–10 mm piece size. The samples were placed on a medium of PDA with chloramphenicol, four pieces per petri dish, and incubated at room temperature. After incubation, fungal colonies were identified. Each fungus colony was transferred to PDA (with chloramphenicol) media and made in a sloped position to obtain a pure culture of each fungal colony.

Fungal colonies from emerging pure cultures were replanted to a new PDA with chloramphenicol medium on a plate for macroscopic identification. The identification was based on the colony surface texture and pigmentation (Sharma and Pandey, 2010). Briefly, object glass was prepared by following with alcohol cleaning and flaming. A drop of lactophenol/methylene blue was positioned on the object glass. Cultures of fungi were taken and placed on top of prepared objects glass, followed by a drop of alcohol. Samples were covered with cover glass and flamed briefly. The type of hyphae, spore size, spore shape, and color spores were identified microscopically according to the key book (Samson et al., 2014). Observations were performed by microscopy at 200× and 400× magnifications.

Quality proximate of onions

The percentage of weight of the carbohydrate, water, protein, fat, ash, and vitamin B1 was measured for the quality check of onions as described elsewhere (Nielsen, 2003).

Statistical analysis

Data were analyzed using a GraphPad Prism (GraphPad Software, San Diego, CA, USA). Chi-square was used to assess the differences between means. Values of $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

The temperature and humidity of ozone chamber

In this study, the mean temperature from week 1–10 in the control chamber (chamber I) was 25.71 °C (24.7–27.1 °C), the pesticide chamber (chamber II) was 25.73 °C (25–26.8 °C), and the ozone chamber (chamber III) was 25.92 °C (24–27.3 °C) (Supplemental Table 1). This study's temperature of onion storage chambers falls within the recommended standard temperature range (25–30 °C) (Lu, 2003). The mean relative humidity of the ozone, pesticide, and control chambers were 70.58 ± 6.69, 65.71 ± 5.13, and 65.58 ± 6.05 (Supplemental Table 2). These findings showed that the humidity of the onion storage chambers in this study was also within the recommended standard moisture range, 60–75% (Sentana, 1998; Tripathi and Lawande, 2019). This finding highlighted that ozone treatment maintained the better temperature and humidity for preserving the onion.

Temperature and humidity are essential for fungal growth. Controlling the temperature and relative humidity of storage chambers is important to ensure the condition of the onion bulbs during the storage period. The storage room in a traditional way is usually between 25 °C and 30 °C, with relative humidity around 60–80%. However, storing onions at high temperatures (in a tropical climate) of over 25 °C and at humidity 75–85%, is necessary for minimizing water loss (Lu, 2003). To obtain the optimal temperature and humidity, the ventilation is playing important role in achieving good storage condition (Priya et al., 2014). The two most used preservation technologies for minimally processed vegetables are in a low temperature and in controlled atmospheres (Ragaert et al., 2007). In cold and traditional (75% and 25 °C) storage conditions, the humidity of medium-sized onions showed no significant change (Malek S and Heidarisoltanabad, 2015). High humidity (>85%) can trigger the growth of fungi and bacteria more rapidly on the onion bulbs causing the rotten onion faster (Mishra et al., 2014).

The mass-change of onions after the treatment

There was a different of mass-damaged in the control, pesticide and ozone chamber after one and 2 months of storage (Table 1). The total mass-damaged was higher in control chamber (6.5%) or without treatment as compared to pesticide (2.4%) and ozone (1.8%) chamber after 2 months of storage. The depreciation of onion was observed higher with the pesticide treatment (32%) and without treatment (22.2%) than in ozone chamber (19.1%) (Table 2). These findings are in line with the previous study that gaseous ozone can safely control the decay of onion bulbs during storage and removes pesticide residues (Vijay Rakesh Reddy et al., 2022). Organic matter broken down by ozone produces safer elements and ozone is converted

Table 1. The damaged-mass of onion bulbs after treatment.

Storage time (month)	Damaged-mass (kg)			Percentage of total damaged-mass (%)		
	Control (Chamber I)	Pesticide (Chamber II)	Ozone (Chamber III)	Control (Chamber I)	Pesticides (Chamber II)	Ozone (Chamber III)
1	1.1	0.3	0.2	5.3	1.5	1.2
2	0.2	0.2	0.1	1.2	0.9	0.6
Total of damaged-mass	1.3	0.5	0.4	6.5	2.4	1.8
Remaining of undamaged-mass	18.7	19.5	19.6			

Table 2. The depreciation of onion bulbs after treatment.

Storage time (month)	Undamaged-mass (kg)		
	Control (Chamber I)	Pesticide (Chamber II)	Ozone (Chamber III)
0	20.0	20.0	20.0
Remaining of undamaged-mass	18.7	19.5	19.6
2	14.3	13.1	15.8
Difference	4.4	6.4	3.8
Percentage of depreciation (%)	22.2%	32.0%	19.1%

automatically to oxygen when released to the environment. It does not leave toxic residues and hazardous chemicals that negatively affect the environment (Botondi et al., 2021; Pandiselvam et al., 2017).

The quality of onion after the treatment

The protein, ash, fat, and vit B1 were remained high after 2 months of storage with ozone compared to control and pesticide treatment chamber (Figure 1). The ozone treatment successfully maintained the quality of onion better than with pesticides and without treatment. However, many factors may affect the product quality when using ozone. A previous study reported that the products' weight, appearance, and quality depend on ozone concentration and exposure time (Botondi et al., 2021; Pandiselvam et al., 2020). Thus, in this study, we optimized the weight of onion to be preserved in our chamber 2.5 m^3 size with 216 ppm ozone for 2 months storage.

Total microbial count, and mold and yeast count (MYC)

The total microbial count on the onions on day 0 (before storage), day 30, and day 60 was 2.0×10^5 CFU/mL, $> 3.0 \times 10^7$ CFU/mL, and 5.2×10^9 CFU/mL, respectively. The total microbial on the onions after pesticide treatment (after 6 hours), day 30, and day 60 were 5.6×10^4 CFU/mL, 1.1×10^6 CFU/mL, and 4.8×10^8 CFU/mL, respectively. The total count of microbial on the onions after ozone treatment (after 6 h), day 30, and day 60 were 1.6×10^4 CFU/mL, 1.5 $\times 10^5$ CFU/mL, and 2.8×10^7 CFU/mL, respectively. The results showed that the total number of bacteria decreased in pesticides and ozone treatment groups as compared to un-treatment chamber (Table 3).

The results of examination of total mold and yeast count (MYC) in onions at day 0; day 30; and day 60 were of 52×10^5 colonies/mL; 30×10^6 colonies/mL; and 18×10^7 colonies/mL, respectively. Whilst, total 84×10^4 colonies/mL; 17×10^6 colonies/mL; and 11×10^7 colonies/mL were observed in the pesticide treatment, at first 6 h treatment, day 30 and day 60, respectively. The ozone treatment on first 6 h; day 30; and day 60 resulted 24×10^4 colonies/mL; 14×10^5 colonies/mL; and 6×10^7 colonies/mL, respectively. It showed that on the bulbs of onion, the number of MYC decreased after pesticide and ozone treatment. The number of MYC on the onion bulbs decreased significantly more than the pesticide treatment (Table 4). This finding is in line that ozone destroys microorganisms by the progressive oxidation of many essential cellular components (Kaavya et al., 2021; Torlak et al., 2013). Ozone inactivates microorganisms rapidly through a reaction with intracellular enzymes, nucleic material, and parts of their cell envelope, spore coats, or viral capsids (Khadre et al., 2001; Sujayasree et al., 2022). There are two mechanisms to destroy microorganisms by ozone: first, ozone oxidizes sulfhydryl groups and amino acids of enzymes, peptides, and proteins to shorter peptides and causes protein damage (Davies, 2016; Khanashyam et al., 2022; Sharma and Pandey, 2010; Sujayasree et al., 2022; Vijay Rakesh Reddy et al., 2022). The second, ozone reacts with the double bonds of unsaturated fatty

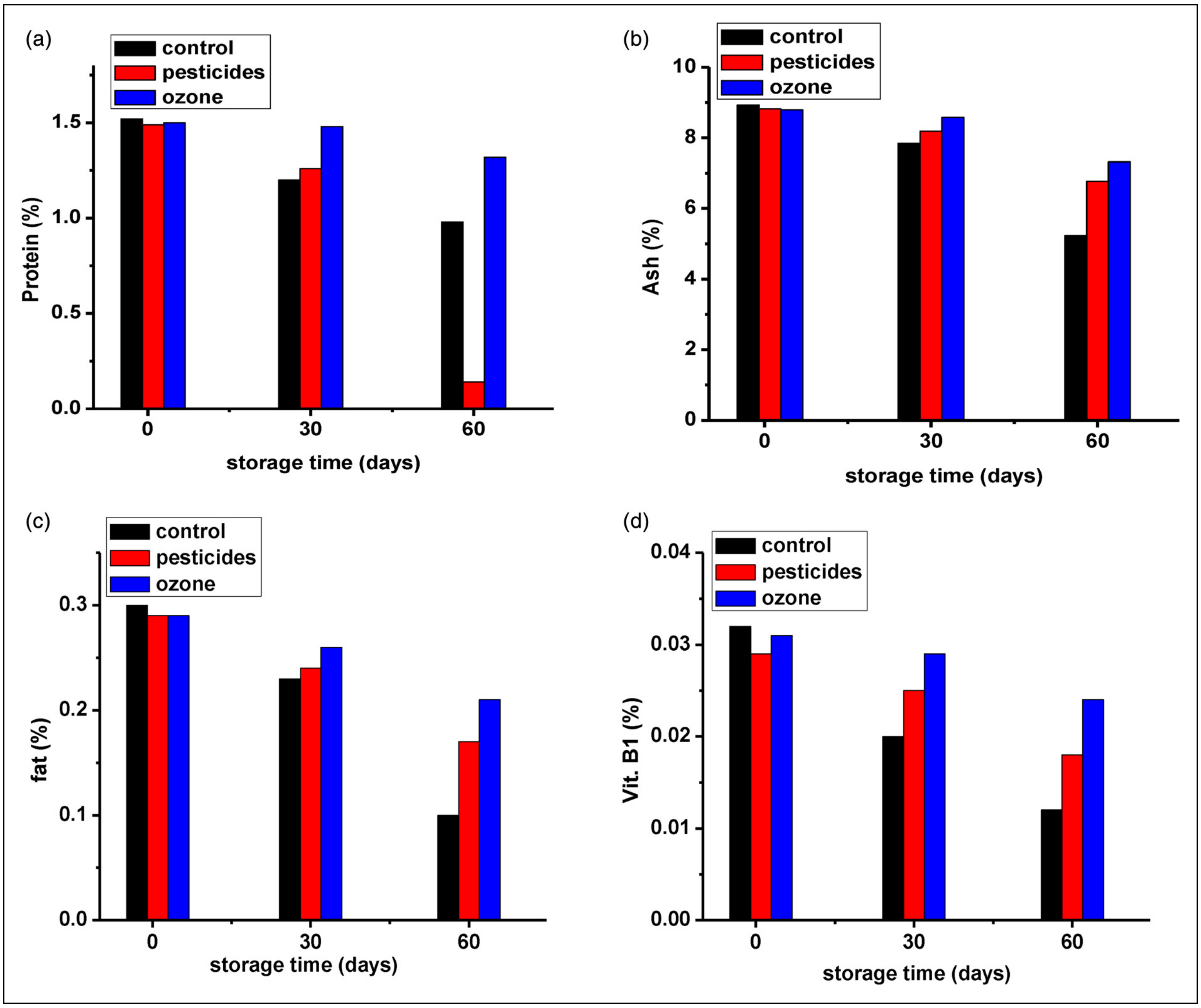

Figure 1. The quality of The Onion bulbs after ozone treatment: (a). The percentage of carbohydrate (KH), (b). The percentage of Water (W), (c). The percentage of protein (P), (d). The percentage of ash (A), E). The percentage of fat (F), F). The percentage of vitamin B1 (Vit. B1).

Table 3. The total microbial examination on the bulbs of onion (control).

	Total Plate Count (CFU/mL)		
Treatment	Day 0	Day 30	Day 60
Control	$2.0{\times}10^5$	$7.8{\times}10^7$	$5.2{\times}10^9$
Pesticides	$5.6{\times}10^4$	$1.1{\times}10^6$	$4.8{\times}10^8$
Ozone	$1.6{\times}10^4$	$1.5{\times}10^5$	$2.8{\times}10^7$

acid (lipids) in the cell membrane, causing cell contents leakage and destruction (Khanashyam et al., 2022; Reis and Spickett, 2012; Sujayasree et al., 2022; Udomkun et al., 2017) and the ozone prevents microorganism resistance (Khadre et al., 2001; Śmigielski and Radzimierska, 2019).

Table 4. The total mold and yeast on onion bulbs.

	Total mold and yeast (MYC) (colony/mL)		
Treatment	Day 0/6 h	Day 30	Day 60
Control	$52{\times}10^5$	$30{\times}10^6$	$18{\times}10^7$
Pesticide	$84{\times}10^4$	$17{\times}10^6$	$11{\times}10^7$
Ozone	$24{\times}10^4$	$14{\times}10^5$	$6{\times}10^7$

The isolation of fungus

The results showed that the fungus found in onions before storage were *Aspergillus sp.* and *Fusarium sp.* Some species of *Aspergillus sp.* were also found in all three chambers after 30 days of storage (Table 5). *Aspergillus sp.* was found on most part of onion (stems, bulbs, and leaves) before being stored and after the pesticide and ozone treatment. However,

Table 5. Identification of fungi on onion bulbs on day 0 and day 30.

Treatment day-0	Fungus	Treatment day 30	Fungus
Control	*a. Aspergillus* sp. (stems) *b. Aspergillus* sp.(bulbs) c. *Aspergillus* sp. (leaves) d. *Fusarium* sp. (leaves)	Control	a. *Aspergillus* sp. (stems) b. *Aspergillus* sp. (bulbs) c. *Aspergillus* sp. (leaves) d. *Fusarium* sp. (stems) e. *Fusarium* sp. (leaves)
		Pesticides	a. *Aspergillus* sp. (stems) b. *Aspergillus* sp.(bulbs) c. *Aspergillus* sp. (leaves) d. *Fusarium* sp. (stems) e. *Fusarium* sp. (leaves)
		Ozone	a. *Aspergillus* sp. (stems) b. *Aspergillus* sp. (bulbs) c. *Aspergillus* sp. (leaves)

Fusarium sp. was not found on any part of onion after the ozone treatment, but was found in control and pesticide treatment group (Table 5).

Fusarium sp. was considered as potent pathogenic fungi on both wound and fresh onion. Anthracnose, purple spots, Cercospora leaf spot, Peronospora leaf rot, rotten bulbs, fusarium wilt, and leaf blight are the most common diseases found in onion plants and products (Mishra et al., 2014). The ozone density in the chamber was 622.08 g/m^3. This ozone density was satisfying to reduce the number of total microbial, mold, and fungus, and maintaining the quality of onion bulbs. Several studies have shown that ozone density provides various efficacy. A previous study revealed that the average ozone concentration of 1.6 ppm for 70 min reduced 90% of the microbial load in a classroom's air with a 600 m^3. The ozone concentration and time exposure are important aspects, with the correct dose of reducing the bacteria, viruses, fungi, and molds by 99% and inactivating >99.8% of viruses on plastic surfaces (Cristiano, 2020). Ozone with a dosage of 11 mg/g within 15 min reduced Fusarium growth by 24–36% and a concentration of 49 mg/min within 5 min, 96% of fungal spores were inactivated, and 13.8 g/m^3 gaseous ozone for 15 min inactivated all fungi (Sujayasree et al., 2022).

It is necessary to consider the costs benefits of treating onions using ozone as compared to pesticides. As it requires treatment for 6 h/day, total cost is estimated to equal with 0.05 USD/day. Thus, for 60 days, the cost to be incurred is 3.3 USD. Meanwhile, the price of a 500 g fungicide is approximately 5.18 USD. The fungicide solution is 10 g for 100 kg onions or ±3.3 g per 20 kg onions (3.3/500*5.18 USD), so it cost 0.03 USD/day. If a fungicide treatment is carried out for 60 days, the pesticide use is worth of 2.05 USD. The chamber with the same dimensions can load up to 100 kg, the need for fungicide is 5-fold higher or 10.25 USD. It is concluded that using ozone to preserve onions is cheaper than using fungicides.

CONCLUSION

The study revealed that the Fusarium sp on onion was absence, and total microbial, mold, and yeast were reduced with Ozone treatment compare to pesticides treatment at day 30. The quality of proximate the onions is better using ozone than pesticide treatment, without significantly decreasing the proximate of onions. The ozone replacement is expected to reduce the use of pesticides and to prevent the risk of exposure to pesticides among house residents. However, further studies are needed to correlate the health impacts of the ozone method.

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DECLARATION OF CONFLICTING INTERESTS

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SUPPLEMENTAL MATERIAL

Supplemental material for this article is available online.

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