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Javed Rahimi

College of Horticulture,
University of Horticultural
Sciences, Bagalkot, Karnataka,
India

MD Jameel Jhalegar

College of Horticulture,
University of Horticultural
Sciences, Bagalkot, Karnataka,
India

Shankar Meti

Directorate of research,
University of Horticultural
Sciences, Bagalkot, Karnataka,
India

Noorulla Haveri

College of Horticulture,
University of Horticultural
Sciences, Bagalkot, Karnataka,
India

Anand Nanjappanavar

College of Horticulture,
University of Horticultural
Sciences, Bagalkot, Karnataka,
India

Gajanan Kushtagi

College of Horticulture, Koppal
University of Horticultural
Sciences, Bagalkot, Karnataka,
India

Corresponding Author:**Javed Rahimi**

College of Horticulture,
University of Horticultural
Sciences, Bagalkot, Karnataka,
India

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Effect of ozone and sulphur dioxide as postharvest treatment to control Rhizopus rot and quality maintenance of table grape (*Vitis vinifera* L.)

Javed Rahimi, MD Jameel Jhalegar, Shankar Meti, Noorulla Haveri, Anand Nanjappanavar and Gajanan Kushtagi

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Abstract

Table grape is one of the most important fruits which is subjected to fungal decay during post-harvest handling, transit and storage. An investigation was made to evaluate ozone (O₃) fumigation at the concentration of 7274.4μL L⁻¹, 5455.8μL L⁻¹ and 3637.2μL L⁻¹ and sodium metabisulfite (SMB) as Indian grape guard, African grape guard and SMB powder pouches (0.5g/500g fruit) against Rhizopus rot and their effect on quality of table grapes under ambient storage (33±2°C, RH-37±5%, 12 d). The results revealed, ozone at the concentration of 7274.4μL L⁻¹ and 5455.8μL L⁻¹ O₃ recorded significantly least disease severity (0.78; 0.94) and higher retention of berry firmness (82.51; 81.07 N), titratable acidity (0.99; 0.98 %) and least PLW (13.24; 13.58 %), TSS (19.41; 19.57°B) and TSS/Acid Ratio (19.88; 19.88) compared to SMB and inoculated control treatments. In addition, SMB treatments and inoculated control showed high decay by Rhizopus rot on 5th day of storage, therefore they were not evaluated after 5th day till end of storage (12th day). From the above results, we conclude that, ozone is a good alternative to sulfur dioxide treatments in conventional grape production and it could be a suitable technology to use with grapes marketed under ambient chain, where sodium metabisulfite and grape guard release, excess SO₂ in ambient condition, moreover it could be also an acceptable technology to use with grapes marketed under "organic" classification, where the use of SO₂ is prohibite.

Keywords: Ozone, *Vitis Vinefera*, sodium metabisulfite, titratable acidity, TSS/Acid Ratio, Rhizopus rot

1. Introduction

Grape (*Vitis vinifera* L.) is a highly perishable, non-climacteric fruit. Its shelf life is usually shortened by firmness loss, berry drop, discoloration of the rachis, desiccation and fungal rots (Sabir and Sabir, 2009) [35]. Rhizopus rot is one of the most common diseases in grape orchards of Bagalkot, district of Karnataka which appears commonly at the time of harvesting, transporting and marketing and cause losses to the fruits and also this disease is most commonly found in tropical and subtropical regions which causing a rapid and very destructive nested decay of grapes during handling, storage and marketing which known as Rhizopus rot, incited by *Rhizopus stolonifer* Vuillemin (Barkai, 2001) [6]. The most common and commercial method to control decay of the table grapes is the use of SO₂ releasing pad or grape guard (Crisosto *et al.*, 2002) [9]. Despite its excellent effect in controlling decay and preventing stem browning in cold chain, SO₂ application is becoming restrictive in many countries owing to issues associated with sulfite residues, SO₂ emissions, its negative impact on grape quality (Lichter *et al.*, 2008; Fernández-Trujillo *et al.*, 2008) [23, 11] and hairline fissure disorder (Zoffoli *et al.*, 2008) [42]. Recently, there has been increasing consumer pressure to eliminate the use of synthetic fungicides on fresh produces which has increased the need for the development of alternative treatment which is safe, effective, simple, economical and innovative, so as to meet requirement of APEDA, GLOBAL GAP (Good Agricultural Practice), *Codex Alimentarius* and comply with regards to maximum residues level and maximum decay tolerance rules (Feliziani *et al.* 2014) [10]. As on numerous times many table grapes have been blocked in the developed countries because of releasing high concentration of SO₂ from grape guard which inevitably the concentration of SO₂ in the box is conditioned by the relative humidity inside the plastic bag and,

therefore it is hard to control releasing of SO₂ from grape guard in ambient condition even the emission of SO₂ increasing twice or thrice beyond the amount which release in cold storage condition (Nelson and Ahmedullah, 1973) [31]. Hence, its limits the use of SO₂ in ambient condition and present interest centers focus on the use of healthy, safe, simple, and innovative technology to replace SO₂. Therefore, ozone (O₃) is an effective alternative to SO₂ treatment and may represent to be a promising option. Ozone is a highly unstable tri atomic oxygen molecule (O₃) formed by the addition of an oxygen atom to a molecular diatomic oxygen (O₂) that can be generated readily and economically for application to several commodities, including processed horticultural products. It is one of the most powerful oxidants that has several advantages, most prominently the absence of detectable residues on treated products. Since it was recognized as generally regarded as safe (GRAS) in the United States (US Food and Drug Administration, 1997), the application of ozone in the food industry has significantly increased both at experimental and commercial levels. Ozone has been evaluated as a sanitizer in the food industry aiming at inactivation of microorganisms, as a removal agent of toxic substances such as mycotoxins and pesticide residues, as well as for the extension of postharvest life of horticultural produce through the ozone-mediated oxidation of ethylene (Minas *et al.*, 2010) [27]. Our objective was to evaluate the efficacy of ozone and sulphur dioxide against *Rhizopus* rot, a post-harvest disease in grape *cv.* Manik Chaman and maintain fruit quality under ambient condition (33±2°C, RH-37±5%).

2 Materials and methods

2.1 Inoculum preparation

Grape diseased samples which showed typical symptoms of *Rhizopus* rot were collected from Honnakatti village of Bagalkot District, Karnataka during the month of January, 2020. *R. stolonifer* isolated through tissue isolation method in which small pieces of diseased sample were placed in 1% solution of sodium hypochlorite for surface sterilization for 30 seconds and rinsed thrice in distilled water; thereafter the bits were placed on PDA holding petri-plates and incubated for 96 h at 25±1°C (Lisker *et al.*, 1996) [25]. Isolated culture was sub cultured on PDA slants and allowed to grow at 25±2°C for 6 days in BOD incubator. Such slants were preserved in refrigerator (4°C). For spore suspension predation the spores were gently removed from an actively growing culture with 20 ml sterile distilled water per each petriplates in laminar air flow chamber. Then the resulting suspension was diluted with sterilized distilled water by serial dilution method to get 10⁻⁵ (Lisker *et al.*, 1996, Aneja, 2007; Hernández *et al.*, 2006) [25, 3, 16] were used as inoculum.

2.2 Ozonation system

Ozonation System is an electrically operated device that produces Ozone. It takes Oxygen (O₂) as feed gas and uses Corona Discharge method to convert it to Ozone (O₃). We used SEONICS Ozone Generator for ozone generation. When purified oxygen from oxygen concentrator enters ozone generator it splits the normal oxygen molecules into single atoms by Corona Discharge method. These atoms then attach to other O₂ molecules in the air to form ozone (O₃) in gram per cubic meter. Then it needs to be converted to microliter per liter. Therefore, purified oxygen passes through the oxygen concentrator to the ozone generator liter per minute (LPM) which range from 0.5 to 5 LPM, which here one liter concentrated oxygen /min is used to produce 51.96 g ozone

per meter cubic (51.96 g/m³ /min = 51.96 mg/liter /min = 51.96 µl L⁻¹ min⁻¹).

In this study we used 9 liters three LDPE airtight boxes (connected to ozone generator by 6mm Outer Dia tube connector) for first three treatments (1, 2, 3) in which 2 liter volume space of each box was occupied by fruits. Hence, to get total concentration of ozone, 7 liter volume of each box was multiplied with 51.96mg/liter/minute and different period of time *i.e.* 10min, 15min and 20min leading to a final concentration of ozone were 3637.2 µl L⁻¹/10min, 5455.8 µl L⁻¹/15min and 7274.4 µl L⁻¹/20min, respectively.

3.2.3 Sulphur dioxide

In this investigation grape guards made in India and Africa were used and to attain 10 ppm MRL in 500g grapes, we put SMB powder with CAS No. 40180 K05, packed in pouches of cloth bag at 0.5g per 500g of fruit, which was according to the export standards proposed by APEDA (Anon, 2019) [4].

2.4 Fruit

In this experiment, eight treatments with three replications and Two Factorial CRD statistical design was employed. The Cultivar Manik Chaman of table grapes (*Vitis vinifera* L.) was used throughout this study. The fruits were harvested from Main Horticultural Research and Extension Centre, University of Horticultural Sciences, Bagalkot in March 2020, and they were brought immediately after harvest to the cold storage of Post-harvest technology department and pre-cooled at 5°C for 12 h, later on physico-chemical observation in the laboratory of Post-Harvest Technology department was done. The bunches were weighed in 0.5 kg for each sample then the bunches were disinfected by immersing in 1% sodium hypochlorite for 2 min and rinsed by distilled water and dried thereafter the bunches have been inoculated by *Rhizopus stolonifer* spores suspension 10⁻⁵ and they were kept in laminar air flow for 20 min to evaporate excess water; packed in 8 different treatments (3637.2µl L⁻¹ ozone/10min, 5455.8 µl L⁻¹ ozone/15min, 7274.4 µl L⁻¹ ozone/20min, Indian grape guard, African grape guard, SMB 0.5g in pouch of cloth bag, control inoculated and control un-inoculated) and carried to ambient storage the first 3 treatment exposure to ozone after 24 h in 2 days interval for 12 days in ambient storage; ozone was applied by Seonics Ozone generator in LDPE 9 liter air tight boxes.

2.5 Observations recorded

2.5.1 Disease severity (DS)

Disease severity was assessed by 0-5 scale: 0 → No disease, 1 → 5 % disease, 2 → >5- 15% disease, 3 → >15 - 30% disease, 4 → >30- 60% disease and 5 → >60% disease (Feliziani *et al.*, 2014) [10] 500g in CFB box

2.5.2 Berry firmness

Fruit firmness was determined using texture analyser using Stable Micro Systems, UK by puncture test. The table grape berries were puncture by using cylindrical 2 mm probe by programmed setting. Firmness was defined as maximum force (kgf) required during test, which was expressed in Newton (N).

2.5.3 Physiological loss in weight (PLW)

To determine the physiological loss in weight (PLW), table grape bunches from each replication were weighed at beginning of storage which was recorded as initial weight. On subsequent dates of observation during storage, the fruits

were reweighed and recorded as P final weight on every 2 days intervals. PLW was calculated by using following formula and expressed in percentage.

$$\text{Physiological loss in weight (\%)} = \frac{\text{Initial weight (g)} - \text{Final weight (g)}}{\text{Initial weight (g)}} \times 100$$

2.5.4 Total soluble solids (°Brix)

The juice extracted by crushing the pulp of the table grapes and strained through muslin cloth was used for measuring total soluble solids. Total soluble solids were estimated using FISHER Digital Refractometer. The results were expressed as degree brix.

2.5.4 Titratable Acidity (%)

The acidity was determined in terms of tartaric acid by diluting the juice extracted from five grams of sample and filter through muslin cloth and made up to known volume with distilled water (25ml) then titrated against standard sodium hydroxide solution (0.1N), using phenolphthalein indicator. The appearance of light pink colour was recorded as the end point. The result was explained in terms of per cent acidity of the fruits (Ranganna, S., 1986).

$$\text{Acidity (\%)} = \frac{\text{Titre value} \times \text{N of NaOH} \times \text{Vol. made up} \times \text{Eq. weight of acid}}{\text{Vol. of aliquot} \times \text{Vol. of sample taken} \times 1000} \times 100$$

2.5.5 TSS to Acid ratio

The TSS to Acid ratio was determined by taking ratio of total soluble solids to titratable acidity and calculated by using formula (Srivastava and Sanjeev Kumar 2002).

$$\text{TSS to Acid ratio} = \frac{\text{Total soluble solids}}{\text{Titratable acidity}}$$

2.6 Statistical analysis

The data disease severity, physico-chemical parameters were recorded and subjected to Two Factorial Completely Randomized Design analysis. Statistical analyses were performed using Web Agri Stat Package (WASP) Version 2 and Operational Statistics (OPSTAT). Significant differences among means at $P \leq 0.01$ for in-vitro and $P \leq 0.05$ were determined by post hoc tests using Duncan's multiple range test.

3. Result and Discussion

The infected table grape samples collected from different places of Bagalkot showing typical symptoms of Rhizopus rot were selected for isolation of *Rhizopus stolonifer*. From the diseased grape samples, three isolates of *R. stolonifer* were isolated and identities of isolates were confirmed as *R. stolonifer* by morphological and microscopic observation of colony, mycelia and spores. The Colonies were very fast growing with dense cottony white mass then changed to reddish gray-brown; fluffy topography with black heads after sporulation; in addition mycelia with their constituents (rhizoids, stolons, sporangiophores, columella, sporangia, and sporangiospores) were incubated on PDA for 96h. Sporangiospores were observed in brownish black colour with globose shape. Similar morphological features of *Rhizopus stolonifer* were reported by Hernández *et al.* (2006) [16] and Bullerman (2003) [7].

3.1 Disease Severity (DS)

Rhizopus rot (*R. stolonifer*) usually starts at the base of mature berries as a soft, very watery rot that partially or completely decays infected berries. Longitudinal fissures are produced, and a black mould develops along the fissures. The skin of the berry turns light gray (Bullerman 2003) [7]. The observations of the study indicated that fruits treated with ozone had significantly least disease severity (Table 1) *i.e.*, 7274.4 $\mu\text{L L}^{-1} \text{O}_3$ (0.78 DS), 5455.8 $\mu\text{L L}^{-1} \text{O}_3$ (0.94 DS) and 3637.2 $\mu\text{L L}^{-1} \text{O}_3$ (1.06 DS) exhibited better results when compared to inoculated control which showed a DS of 5 on 5th day of storage itself. The reduced severity of the disease in ozone treatments could be due to destruction of *R. stolonifer* by the progressive oxidation of essential cellular constituents by the ozone (Khadre *et al.*, 2001, Zeynep *et al.*, 2003 and Silva *et al.*, 2010). Our results corroborate with those of Sarig *et al.* (1996) [25], who reported a supply of 8 mg ozone/min for 30 to 40 minutes was sufficient to eliminate decay caused by *Rhizopus stolonifer* in inoculated grapes and further Gabler *et al.* (2010) [14] also studied the combination of short-term fumigations (60 minutes) with high concentrations of ozone during pre-cooling of table grapes and suggested that 2500 or 5000 $\mu\text{L/L} \times \text{h O}_3$ were equally effective in reducing gray mould by approximately 50% after 7 days of storage at 15°C Thompson Seedless.

In addition, SMB exposed treatments (Indian grape guard, African grape guard, SMB powder 0.5g/500g grapes) and inoculated control were spoiled on day 5th itself and were not evaluated further (Table 1). The earlier spoilage may be attributed to the fast growing habit of *R. stolonifer* and also the hairline cracks (physical injury) occurring on berries due to excess emission of sulfur dioxide (SO₂) at high ambient temperature, all this prompted to the increase in disease severity incidence, water loss. We find support to our hypothesis from researcher like Mustonen (1992) [28] and Sabir and Sabir (2009) [35] who reported that the amount of SO₂ released is also affected by the temperature and the effective use of these pads depends on a good cool-chain being maintained. Zoffoli and Latorre (2011) [41] reported that hairline cracks in berries are an expression of phytotoxicity due to overexposure to sulfur dioxide (SO₂) characterized by the development of small, fine, longitudinal, linear cracks, almost undetectable to the naked eye. Juice exudation from the split zone makes the berry skins wet and sticky finally it causes the berry to serious water loss. Therefore, in our experiment as well we could notice that grape guard and SMB pouches did not have influence on Rhizopus rot of table grapes in ambient condition probably due to the physical injury caused by overexposure to sulfur dioxide (SO₂). On the other hand, the excess sulphur dioxide (SO₂) produced was insufficient to inhibit the growth of *R. stolonifer*, but was enough to cause the physical injury and might have supplemented the increase in disease severity.

3.2 Berry firmness

Fruit softening is attributed to cell wall degradation components, mainly pectin, due to action of specific enzymes such as polygalacturonase (PG), Pectin methyl esterase (PME). Berry firmness of was significantly declined by increase in storage period from initial (96.10 N) to 12th day (63.88 N) (Table 2). Which could be attributed to the softening of fruits with the rising activity of peel softening enzymes like PG, PME (Lazan *et al.*, 1993; Feng *et al.*, 2000; Jeong *et al.*, 1998; Khan and Singh, 2008; Zhang, 2005) [19, 37, 39] and Sarig *et al.*, 1996 [25] reported that ozone treatments did not adversely affect the firmness of some cultivars even after 80 minutes of exposure.

Ozone fumigated table grapes, *i.e.*, 7274.4 $\mu\text{L L}^{-1} \text{O}_3$ (82.51 N) showed significantly highest firmness of berries and the lowest firmness was recorded in inoculated control (73.19 N). Other treatments, *i.e.*, 5455.8 $\mu\text{L L}^{-1} \text{O}_3$ (81.07 N), 3637.2 $\mu\text{L L}^{-1} \text{O}_3$ (79.32 N) and uninoculated control (79.19 N), exhibited appreciative result. In addition, ozone treatments significant compared to SMB treatments

The difference in the results of treatments of ozone and SMB could be owing to the varying level of delaying action of enzymes like PG, Cellulase and PME. To our astonishment was the result of ozone treatment which had a better shelf life as well as firmness till its 12th day of storage, which was only 5 days in SMB treatment, which may be due to the powerful oxidizing power of ozone gas, able to promote a double effect: it directly causes microbial cell membrane disruption, enzyme inactivation, and nucleic acid damage and induces, a defense response that delay or reduces fruit decay. Further ozone exposure might have led to a clear decline in pectin methylesterase (PME) activity, pectin solubilization and depolymerization. Changes of other debasing enzymes of the cell wall or alterations in cell wall cross-linkages could have also contributed to cell walls firmness. Moreover, Hong and Gross (1998)^[17] was of opinion that, oxidizing agents, for instance ozone, might lead to oxidation of feruloylated cross-linkages or phenolic cross-linkages among cell wall pectin, structural proteins or other polymers, and causing the variation in the firmness of the product. Correspondingly, Zhao *et al.* (2013)^[40] observed that higher firmness was maintained in pear fruit treated with different ozone concentrations under room temperature.

Concerning to SMB treatments as they exhibited steep decrease in grape firmness, it might be assigned to over emission of SO_2 owing to ambient condition, nevertheless, it might be triggered fine cracks on berries which impelled the virulence of disease due to penetrating and biofilm establishment *R. stolonifer* in ambient high temperature, thereby, grape guard in ambient temperature release more sulphur dioxide firstly it cause physical damage to grape berries which increase physiological process which lead the berries to senescence, secondly, it pave the way for *R. stolonifer* to penetrate in berries and finally cause huge losses of stored grape. Whatever little retention of fruit firmness in SMB treated fruits may be because, it might have slowed down metabolism and brought down the breakdown of insoluble protopectins into soluble pectin and prolonged the firmness intern shelf life. Narayanasamy, P., 2006^[40]; Muñoz *et al.*, 2003 stated that the efficacy of using SO_2 generators at low temperature for the control of decay due to *B. cinerea* in table grapes was assessed. A temperature of 4°C in combination with SO_2 treatment was the most effective in preserving fruit quality of two cultivars Italia and Red Globe. High temperatures increased the weight loss, stem colour, decay, and SO_2 residue in berries. Nonetheless, the untreated control fruits showed lesser and steep decline in firmness which may be because of faster degradation of cell wall components and drastically sever decay incidence and prompt forming of biofilm of *R. stolonifer*.

3.3 Physiological loss in weight (PLW)

Physiological loss in weight is one of the important elements accountable for quantitative in conjunction with qualitative loss of produce, which bring on desiccation and detract product saleability (Kader, 2002)^[20]. Sample weight loss happens due to water loss and the reduction in stored materials in the course of respiration process. Physiological

loss in weight from any product is governed mostly by the protective surface of the commodity, the quantity of air moving over it, and the difference in vapor pressure between the commodity and the air. As a matter of fact, the grape berries have a relatively impervious skin so they do not give up water readily in normal condition, and consequently moisture is lost mostly over the stems. Similarly, in grape, water loss increases later berry skin come under physical, chemical or microbial impairment (Harvey, 1959)^[34].

In this investigation on table grapes inoculated with *Rhizopus stolonifer* treated by ozone and Sodium Meta Bisulfite (SMB) under ambient storage (33±5°C, RH-37±5%) (Table 3), we observed that ozone treated fruits at 7274.4 $\mu\text{L L}^{-1} \text{O}_3$ (13.24 %), 5455.8 $\mu\text{L L}^{-1} \text{O}_3$ (13.58); 3637.2 $\mu\text{L L}^{-1} \text{O}_3$ (18.71%) showed significantly lower PLW compared to SMB treatments, SMB powder 0.5g /500g grapes (15.74 %), African grape guard (17.36%); Indian grape guard (18.26%) and the control treatments (19.83 %; 15.62 %). The positive effect of treatments might be assigned to decrease in rate of respiration and other physiological activity as a result of inactivation of enzymes by oxidation. Our findings are in corollary with authors like Geransayeh *et al.* (2012)^[15] and Artes-Hernandez *et al.* (2003)^[5] who reported ozone treatment decreased the weight loss of grapes samples noticeably. Glowacz *et al.* (2015b) observed similar results in red bell peppers, but without any significant effect on weight loss, texture, and fruit colour. Meanwhile, SMB treated fruits on 5th day *i.e.*, SMB powder 0.5g/500g grapes (19.78 %), African grape guard (22.08 %) and Indian grape guard (23.04%) (Table 5). showed more PLW compared to ozone it might be because of SMB release excess sulphur dioxide in ambient condition, might have caused injuries of tissue, bleaching of colour, followed by sunken areas where accelerated water loss would have occurred, which prompted physiological loss in weight. Same results were reported by Ryall and Harvey, 1959, Zoffoli *et al.*, 2008 and Chervin *et al.*, 2012)^[34, 42]. Moreover, all SMB exposed treatment exhibited to some extent lesser PLW compared to inoculated control it could be because of its excess decay of berries and accelerated physiological process on grape in ambient condition.

3.4 Total soluble solids (°Brix)

A large portion of the soluble solids in grapes is sugars (sucrose, glucose, and fructose) that account for more than 90% of TSS at harvest (Muñoz-Robredo *et al.*, 2011). Our study has revealed a steady increase in TSS with the increase in storage period from initial day (17.00°B) to 12th day (21.98°B) under ambient condition (33±5°C, RH-37±5%) (Table 4), which can be assigned to the hydrolysis of starch to sugar in addition to hydrolysis of sucrose to fructose and glucose, in addition transpiration due to respiration also lead to increase in TSS.

The result showed that the TSS of ozone treated grapes at 7274.4 $\mu\text{L L}^{-1} \text{O}_3$ (19.41°B) 5455.8 $\mu\text{L L}^{-1} \text{O}_3$ (19.57°B) and 3637.2 $\mu\text{L L}^{-1} \text{O}_3$ (20.35°B) increased but at significantly slower rate than SMB powder 0.5g /500g grapes (20.56°B), Indian grape guard (20.70°B) and African grape guard (20.83°B) and control inoculated (21.28°B) during ambient storage (33±5°C, RH-37±5%). It might be because of lower water loss, the slower rate of respiration and ethylene evolution as a consequence, the conversion of sucrose into glucose and fructose might have been delayed. Our results are in agreement with Cayuela *et al.* 2010^[8] and Geransayeh *et al.* 2012^[15] who reported increase in TSS in ozone treatment

throughout storage. On the contrary, in the majority of available literatures, it is reported that significant differences in total soluble solids content between ozonated and untreated samples was not noticed. On the contrary, Alegria *et al.* (2009) [1] noted a significant reduction of total soluble solids content in ozonated carrots, explained by a leaching process, once carrots were treated with water containing ozone (Perez *et al.*, 1999) [32]. Nonetheless, our work was in accordance with Ali *et al.* (2014) who reported higher total soluble solids, sweetness and better appearance in ozone-treated papaya fruit. Shalluf (2012) revealed a significant increase in glucose and fructose levels in tomato fruit following exposure to ozone. Similarly, Glowacz *et al.* (2015b) reported that continuous ozone exposure resulted in increased levels of glucose and fructose sugars in red bell peppers.

Similarly, SMB treatments (21.77°B), (22.10°B) and (21.93°B) on day 5th of storage showed significantly higher increase in TSS. Here, very least control of decay by SMB impregnated pad in ambient temperature, driven excess¹ releasing of SO₂ due to bolster of decay through fine cracks in berries and it led *R. stolonifer* to quick penetration and nesting in grape berries which provoked loss of water and finally extreme increase in TSS of SMB treatments. Hence, all these events like spoilage, high temperature, water loss, all together played remarkable role in the upturn of TSS in both SMB treated fruits and inoculated control (22.70°B) upto 5 days of storage. Our findings find relevance with that of Nelson and Ahmedullah, 1973 [31], Laszlo *et al.*, 1981 [22]. Similarly, Narayanasamy, P., 2006 [30] stated that a temperature of 4°C in combination with SO₂ treatment was the most effective in preserving quality of fruit of two cultivars Italia and Red Globe and he further revealed that high temperatures increased the weight loss, stem colour, decay, and SO₂ residue in berries

3.5 Titratable acidity (TA %)

Several harvested products contain a significant quantity of organic acids, many of which play a central role in metabolism. Most organic acids are found only in trace amounts; however, some acids such as malic, citric and tartaric tend to be found in abundance in some plant tissues. Thus, in grapes tartaric acid available in abundance, in food products, organic acids may impart a significant portion of the characteristic flavour (Kays, 1998) [21]. In the current study, titratable acidity showed significant decreasing trend in relation to progress in storage period, from initial (1.11%) upto 12th day (0.80%) (Table 5). This might be owing to the fact that the organic acids which are being utilized by means of a substrate in respiration process or there could be the conversion taking place to sugars (Islam *et al.*, 1996) [18]

With respect to ozone treatments, *e.i.*, 7274.4 µl L⁻¹ O₃, 5455.8 µl L⁻¹ O₃, 3637.2 µl L⁻¹ O₃ with 0.99%, 0.98%; 0.90% of titratable acidity respectively showed significantly highest TA retention it might be because of reduced respiratory enzymatic activity due to ozone through oxidation of sulfhydryl groups in amino acids of enzymes (Victorin, 1992; Zhang, 2005) [39] since, organic acids are the substrate of respiration in fruits and vegetable respiration, thereby, ozone has linear effect in holding of titratable acidity.

In addition to SMB treatments (SMB powder 0.5g /500g grapes -0.84%, African grape guard -0.84% and Indian grape

guard - 0.81%) and inoculated control (0.79%) showed significantly higher decline in TA on 5th day of storage compared to ozone exposed treatments, it might be because of releasing of more sulphur dioxide from SMB in ambient due to high temperature and moisture loss, which might have provoked for high rate of respiration and other physiological process in table grapes, coincident with disease severity in SMB and inoculated control treatments. Likewise, uninoculated and untreated control treatment with 0.92% TA exhibited to some extent linear titratable acidity retention with ozone treatments probably because of no occurrence of decay incidence noticed.

3.6 TSS/Acid Ratio

The TSS/Acid ratio is a key constituent of flavour and it rise indicate maturity and ripening of fruit (Marriott, 1980). In this study, TSS/Acid ratio increased in all treatments with the increase in storage period and simultaneously depletion of organic acids occurs due to respiration, where organic acids might be used as a substrate in respiration process or their conversion to sugars (Islami *et al.*, 1996) [18]. TSS/acid ratio significantly increased with the increase in storage period, from initial day (15.32) to 12th day (28.67) during ambient storage condition (33±5°C, RH-37±5%) as shown in (Table 6). With respect to different treatments, 7274.4 µl L⁻¹ O₃ (19.88), 5455.8 µl L⁻¹ O₃ (20.33 and 3637.2 µl L⁻¹ O₃ (22.95) showed significantly lower TSS/acid ratio compared to SMB powder 0.5g /500g grapes (24.51), (20.56°B), Indian grape guard (25.43) and African grape guard (24.95), and also inoculated control (26.23). It could be owing to retention of TSS and TA and similarly, reduction of respiratory enzyme activity by its oxidation through ozone (Horvitz *et al.*, 2013; Zhang, 2005) [39]. However, the rate of respiration was inhibited by treatment with water containing ozone at 0.08 and 0.18 ppm. They concluded that the efficacy of respiration rate inhibition increased with the increase in ozone concentration in water, showing that aqueous ozone was able to retard tissue metabolism. This is in agreement with the study of Toivonen and Stan (2004) [38], they revealed that in which it is described that ozonation reduces respiration, once samples washed in water containing ozone have lower respiration than the ones that have not been treated. Liew and Prange (1994) [24] found that a slight increase in CO₂ production in carrots treated with ozone, depending on doses and storage time. In several studies, it was obvious that the respiration rate was spurred immediately after the ozone treatment, but only for a short period. This fact may indicate that products undergo physiological injury as a result of the oxidative stress caused by ozone exposure (Aguayo *et al.*, 2006, Forney *et al.*, 2007; Minas *et al.*, 2012) [2, 12, 26]. Inversely, Forney *et al.* (2003) [13] observed that although 0.2 ppm of gaseous ozone did not affect respiration and ethylene production, a concentration of 0.7 ppm stimulated respiration and ethylene production in broccoli, causing physiological damage.

In addition, SMB powder 0.5g /500g grapes (27.97), African grape guard (28.60) and Indian grape guard (29.70) and control inoculated (30.29) recorded significantly highest TSS/Acid ratio on 5th day of storage). It could be due to increased disease severity and metabolic respiration in table grapes due to storage in ambient condition in fact high temperature prompt excess emission of SO₂ from for SMB incorporated pad. As Laszlo *et al.* (1981) [22] conducted a research on effect of temperature on the emission of SO₂ from gas generators for grapes and revealed that releasing of SO₂

Releasing of SO₂ from grape guard in ambient condition increasing twice or thrice beyond the amount which release in cold storage condition (Nelson and Ahmedullah, 1973) [31].

increasing with increase in temperature, thereby high temperature provoked more emission of SO₂ from SMB which caused injuries in products and prompted the physiological and biochemical activities horticultural crops.

Meanwhile, T₈- with 22.97 showed to somewhat linear, minimum TSS/Acid ratio under ambient storage (33±5°C, RH-37±5%).

Table 1: Effect of ozone and sodium metabisulfite on disease severity (DS) of table grapes inoculated with *Rhizopus stolonifer* under ambient condition (33±2°C, RH-37±5%)

Treatments	Disease Severity (DS)						Mean
	Days of storage						
	Initial	3	5	7	9	12	
3637.2µl L ⁻¹ O ₃ /10min	0.00	0.67	1.00	1.33	1.67	1.67	1.06
5455.8 µl L ⁻¹ O ₃ /15min	0.00	1.00	1.00	1.00	1.33	1.33	0.94
7274.4 µl L ⁻¹ O ₃ /20min	0.00	0.67	1.00	1.00	1.00	1.00	0.78
Indian grape guard	0.00	1.67	4.17	4.17*	4.17*	4.17*	3.06
African grape guard	0.00	1.00	3.53	3.53*	3.53*	3.53*	2.52
SMB powder 0.5g /500g grapes	0.00	1.33	3.33	3.33*	3.33*	3.33*	2.44
Inoculated Control	0.00	2.00	5.00	5.00*	5.00*	5.00*	3.67
uninoculated control	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean	0.00	1.04	2.38	2.42	2.50	2.50	
S.Em± CD @ 5 %	Treatments(T) Days of storage(D) Interaction (TxD) 0.13 0.11 0.32 0.36 0.31 0.89						

* Indicates same values of 5th day were typed for statistical purpose and observations were not taken further due to spoilage of fruit samples

Disease Severity (DS) scale: 0 → No disease, 1 → 5 % disease, 2 → >5- 15% disease, 3 → >15 - 30% disease, 4 → >30- 60% disease and 5 → >60% disease

Table 2: Effect of ozone and sodium metabisulfite (SMB) on firmness of table grapes inoculated with *Rhizopus stolonifer* under ambient condition (33±2°C, RH-37±5%)

Treatments	Firmness (N)						Mean
	Days of storage						
	Initial	3	5	7	9	12	
3637.2μl L ⁻¹ O ₃ /10min	96.10	91.12	81.17	77.73	73.70	56.09	79.32
5455.8μl L ⁻¹ O ₃ /15min	96.10	91.58	82.53	78.30	75.75	62.18	81.07
7274.4μl L ⁻¹ O ₃ /20min	96.10	92.11	84.13	78.40	78.15	66.18	82.51
Indian grape guard	96.10	87.65	66.73	66.73*	66.73*	66.73*	75.11
African grape guard	96.10	87.06	67.30	67.30*	67.30*	67.30*	75.39
SMB powder 0.5g /500g grapes	96.10	83.37	70.47	70.47*	70.47*	70.47*	76.89
Inoculated Control	96.10	86.62	64.10	64.10*	64.10*	64.10*	73.19
uninoculated control	96.10	90.56	79.47	76.60	74.44	57.97	79.19
Mean	96.10	88.76	74.49	72.45	71.33	63.88	
S.Em± CD @ 5 %	Treatments(T) Days of storage(D) Interaction (TxD) 0.49 0.43 1.20 1.38 1.20 3.38						

* Indicates same values of 5th day were typed for statistical purpose and observations were not taken further due to spoilage of fruit samples

Table 3: Effect of ozone and sodium metabisulfite (SMB) on physiological loss in weight (PLW %) in table grapes inoculated with *Rhizopus stolonifer* under ambient condition (33±2°C, RH-37±5%)

Treatments	PLW %						Mean
	Days of storage						
	Initial	3	5	7	9	12	
3637.2µl L ⁻¹ O ₃ /10min	0.00	13.35	16.56	18.32	21.43	23.91	15.59
5455.8µl L ⁻¹ O ₃ /15min	0.00	11.12	13.55	16.40	19.10	21.33	13.58
7274.4 µl L ⁻¹ O ₃ /20min	0.00	10.83	13.05	16.00	18.68	20.90	13.24
Indian grape guard	0.00	17.38	23.04	23.04*	23.04*	23.04*	18.26
African grape guard	0.00	15.84	22.08	22.08*	22.08*	22.08*	17.36
SMB powder 0.5g /500g grapes	0.00	15.32	19.78	19.78*	19.78*	19.78*	15.74
Inoculated Control	0.00	18.34	25.16	25.16*	25.16*	25.16*	19.83
uninoculated control	0.00	13.13	16.12	18.29	21.69	24.48	15.62
Mean	0.00	14.41	18.67	19.88	21.37	22.59	
S.Em± CD @ 5 %	Treatments(T) Days of storage(D) Interaction (TxD) 0.23 0.20 0.57 0.65 0.57 1.60						

* Indicates same values of 5th day were typed for statistical purpose and observations were not taken further due to spoilage of fruit samples

Table 4: Effect of ozone and sodium metabisulfite (SMB) on Total Soluble Solid (TSS °B) of table grapes inoculated with *Rhizopus stolonifer* under ambient condition (33±2°C, RH-37±5%)

Treatments	Total Soluble Solid (TSS °B)						Mean
	Days of storage						
	Initial	3	5	7	9	12	
3637.2µl L ⁻¹ O ₃ /10min	17.00	19.30	20.42	20.98	21.98	22.44	20.35
5455.8µl L ⁻¹ O ₃ /15min	17.00	18.60	19.47	20.33	20.83	21.21	19.57
7274.4µl L ⁻¹ O ₃ /20min	17.00	18.40	19.22	19.95	20.68	21.20	19.41
Indian grape guard	17.00	19.47	21.93	21.93*	21.93*	21.93*	20.70
African grape guard	17.00	19.55	22.10	22.10*	22.10*	22.10*	20.83
SMB powder 0.5g /500g grapes	17.00	19.30	21.77	21.77*	21.77*	21.77*	20.56
Inoculated Control	17.00	19.85	22.70	22.70*	22.70*	22.70*	21.28
uninoculated control	17.00	19.10	20.17	20.94	21.71	22.47	20.23
Mean	17.00	19.20	20.97	21.34	21.71	21.98	
S.Em± CD @ 5 %	Treatments(T) Days of storage(D) Interaction (TxD) 0.05 0.04 0.11 0.13 0.11 0.32						

* Indicates same values of 5th day were typed for statistical purpose and observations were not taken further due to spoilage of fruit samples

Table 5: Effect of ozone and sodium metabisulfite (SMB) on Titratable acidity (%) of table grapes inoculated with *Rhizopus stolonifer* under ambient condition (33±2°C, RH-37±5%)

Treatments	Titratable acidity (%)						Mean
	Days of storage						
	Initial	3	5	7	9	12	
3637.2µl L ⁻¹ O ₃ /10min	1.11	1.02	0.92	0.88	0.79	0.71	0.90
5455.8µl L ⁻¹ O ₃ /15min	1.11	1.04	0.99	0.96	0.91	0.85	0.98
7274.4µl L ⁻¹ O ₃ /20min	1.11	1.06	1.00	0.98	0.92	0.87	0.99
Indian grape guard	1.11	0.96	0.81	0.8*1	0.81*	0.81*	0.89
African grape guard	1.11	0.97	0.84	0.84*	0.84*	0.84*	0.90
SMB powder 0.5g /500g grapes	1.11	0.98	0.84	0.84*	0.84*	0.84*	0.91
Inoculated Control	1.11	0.95	0.79	0.79*	0.79*	0.79*	0.87
uninoculated control	1.11	1.03	0.95	0.91	0.80	0.69	0.92
Mean	1.11	1.00	0.89	0.88	0.84	0.80	
S.Em± CD @ 5 %	Treatments(T) Days of storage(D) Interaction (TxD) 0.01 0.00 0.01 0.01 0.01 0.03						

* Indicates same values of 5th day were typed for statistical purpose and observations were not taken further due to spoilage of fruit samples

Table 6: Effect of ozone and sodium metabisulfite (SMB) on TSS to acid ratio of table grapes inoculated with *Rhizopus stolonifer* under ambient condition (33±2°C, RH-37±5%)

Treatments	TSS to acid ratio						Mean
	Days of storage						
	Initial	3	5	7	9	12	
3637.2µl L ⁻¹ O ₃ /10min	15.32	18.51	21.16	23.02	27.27	32.43	22.95
5455.8µl L ⁻¹ O ₃ /15min	15.32	17.91	19.73	21.11	22.99	24.89	20.33
7274.4µl L ⁻¹ O ₃ /20min	15.32	17.47	19.27	20.44	22.43	24.33	19.88
Indian grape guard	15.32	20.36	29.70	29.08*	29.08*	29.08*	25.43
African grape guard	15.32	19.96	28.60	28.60*	28.60*	28.60*	24.95
SMB powder 0.5g /500g grapes	15.32	19.86	27.97	27.97*	27.97*	27.97*	24.51
Inoculated Control	15.32	20.90	30.29	30.29*	30.29*	30.29*	26.23
uninoculated control	15.32	19.05	22.28	23.94	27.95	31.80	23.39
Mean	15.32	19.25	24.88	25.56	27.07	28.67	
S.Em± CD @ 5 %	Treatments(T) Days of storage(D) Interaction (TxD) 0.19 0.16 0.45 0.52 0.45 1.27						

* Indicates same values of 5th day were typed for statistical purpose and observations were not taken further due to spoilage of fruit samples

4. Conclusion

Ozone (O₃) at the concentration of 7274.4µL L⁻¹ and 5455.8µL L⁻¹ recorded significantly least disease severity and higher retention of berry firmness, titratable acidity, least PLW, TSS and TSS/Acid Ratio compared to SMB treatments and inoculated control. In addition, SMB treatments and

inoculated control showed higher decay by *Rhizopus rot* in 5 days of storage during 12 days of storage. Therefore, we conclude that fumigation of grapes with Ozone at 7274.4µL L⁻¹ and 5455.8µL L⁻¹ controls *R. stolonifer* and could be better alternative to synthetic chemical fumigation, with ozone being approved as a Generally Recognized as Safe (GRAS), it can

be a good option for getting classified for “organic” tag, it can store grapes till 12 days in ambient storage without affecting quality and consumer acceptance. The other most important point of using ozone is that it, leaves no residue after treatment and reduces chemical residues which make it a consignment most suitable for export to other countries from India.

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