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## 1H-cyclopropabenzene and 1H-cyclopropa[b]naphthalene fumigation suppresses climacteric ethylene and respiration rates and modulates fruit quality in long-term controlled atmosphere-stored 'gold rush' pear fruit

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1 **1H-cyclopropabenzene and 1H-cyclopropa[b]naphthalene fumigation suppresses**  
2 **climacteric ethylene and respiration rates and modulates fruit quality in longterm**  
3 **controlled atmosphere stored ‘Gold Rush’ pear fruit**

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17 **Abstract**

18 ‘Gold Rush’ pear (*Pyrus communis* L.) is a russet-coloured fruit with soft buttery textured flesh and is gaining  
19 wide popularity in Australia and other countries along with other pear cultivars. The fruit are sensitive to ethylene,  
20 and exposure even at very low concentrations significantly reduce the storage duration as well as fruit quality  
21 during storage. The efficacy of two new ethylene antagonist compounds, namely 1H-cyclopropabenzene (BC) and  
22 1H-cyclopropa[b]naphthalene (NC), as well as 1-methylcyclopropene (1-MCP) in regulating ethylene production,  
23 respiration rates and maintaining the fruit quality of Gold Rush pear during 150 d and 200 d of controlled  
24 atmosphere (CA) storage ( $2.3 \pm 0.5$  % O<sub>2</sub> and  $0.4 \pm 0.15$  % CO<sub>2</sub> and  $0.50 \pm 0.71$  °C), was investigated. The pear  
25 fruit were fumigated with 1 μM BC ( $0.09 \mu\text{L.L}^{-1}$ ) or 1 μM NC ( $0.14 \mu\text{L.L}^{-1}$ ) or 18 μM ( $1 \mu\text{L.L}^{-1}$ ) 1-MCP for 18 h  
26 at room temperature and the untreated fruit were considered as the control. Following 150 d and 200 d CA storage,  
27 the fruit fumigated with BC and NC exhibited significantly reduced ethylene and respiratory climacteric peak  
28 rates and were lowest in the fruit treated with 1-MCP. The pear fruit fumigated with ethylene antagonists (BC, NC

29 and 1-MCP) exhibited lower physiological loss of weight (PLW) (up to 2.06 times) and higher fruit firmness (up  
30 to 1.07 times) throughout the CA storage period, compared to the control fruit. The fruit fumigated with BC and  
31 NC had lower levels of SSC, glucose and sorbitol compared to other treatments. There was no significant effect  
32 of ethylene antagonist treatments on levels of individual organic acids, total phenols, ascorbic acid and total  
33 antioxidant capacity of the fruit. Therefore, new ethylene antagonist compounds, BC and NC, exhibit the potential  
34 to act as ethylene antagonists in longterm CA stored 'Gold Rush' pears to retard the fruit ripening process, extend  
35 storage life and maintain the fruit quality. The effectiveness of the different concentrations of BC and NC in  
36 suppressing ethylene production in different cultivars of pears warrants further investigation.

37 **Keywords:**

38 Ethylene, ethylene antagonists, 1-methylcyclopropene, Gold Rush pear, climacteric fruit

39 **Abbreviations:** BC\_1*H*-cyclopropabenzene, NC\_1*H*-cyclopropa[*b*]naphthalene, 1-MCP\_1-methylcyclopropene

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41 not-for-profit sectors.

42 **1. Introduction**

43 'Gold Rush' cultivar of European pear (*Pyrus communis* L.) possess attractive russet-coloured pericarp with crisp  
44 and crunchy texture when not fully ripe. At the fully ripe stage, it turns to soft buttery textured flesh with high  
45 sugar content. The European pear fruit exhibit a steep rise in postharvest ethylene production and respiration rates,  
46 and therefore categorised as climacteric fruit (Biale and Young 1981). The climacteric fruits are sensitive to  
47 internal ethylene and exposure to external ethylene, even at a minute concentration promotes fruit ripening and  
48 also cause undesirable physical and physiological changes during storage (Iqbal et al. 2017). Ethylene accelerates  
49 the fruit softening, hydrolysis of cell wall materials, depletion of bioactive compounds and increases decay as well  
50 as the incidence of several postharvest physiological disorders (Tucker 2012; Iqbal et al. 2017). Several approaches  
51 have been investigated to downregulate the fruit ripening process such as manipulating the storage environment  
52 and regulating ethylene production and its action in the fruits (Gross et al. 2016). The controlled atmosphere (CA)  
53 storage comprised of higher levels of carbon dioxide and reduced levels of oxygen extend the storage of the fruit,  
54 due to reduced ethylene production as well as retarded respiration rate and the changes associated with the fruit  
55 ripening process (Keller et al. 2013; Saquet and Streif. 2017). However, Bai et al. (2009) reported a sudden increase

56 in ethylene production in the pear fruit after removing them from the CA storage. Previously, Watkins (2006) and  
57 Bai et al. (2009) reported that the pear fruit treated with ethylene antagonists such as 1-methylcyclopropene (1-  
58 MCP), reduced this sudden surge in rates of ethylene production. 1-MCP is a widely used ethylene antagonist and  
59 inhibits ethylene production and its action by irreversibly blocking the ethylene receptor sites in the fruit (Sisler  
60 2006). The efficacy of 1-MCP varied among genotypes, concentrations applied, storage temperature and treatment  
61 duration (Zhang et al. 2020). Moreover, it is difficult to handle 1-MCP, as its boiling point is very low and readily  
62 vaporises at room temperatures (Sisler et al. 2006). Several commercial products involving different delivery  
63 methods of 1-MCP as fumigation have been developed by different companies such as AgroFresh (SmartFresh™,  
64 ProTabs, SmartFresh™ InBox tablets, SmartFresh™ SmartTabs™), Hazel®, Logfresh® etc (AgroFresh 2021;  
65 Hazel Tech 2021; Logfresh 2021). Similarly, the liquid form of 1-MCP (Harvista™) has also been developed by  
66 AgroFresh whilst, Logfresh® has developed 1-MCP in liquid as well as in dustable powder form (Logfresh 2021).  
67 A pre-harvest spray application of Harvista™ has exhibited positive responses in maintaining fruit quality in  
68 different pear cultivars (Defilippi et al. 2010; Sakaldas et al. 2016; Villalobos-Acuna et al. 2017; Escribano et al.  
69 2017; Li et al. 2020). Singh et al. (2018) discovered the capacity of 1*H*-cyclopropabenzene (BC) and 1*H*-  
70 cyclopropa[*b*]naphthalene (NC) to antagonize the ethylene action in a similar mechanism to that of 1-MCP.  
71 Structurally these compounds are different from 1-MCP, making them more stable at room temperature than 1-  
72 MCP in natural form. Kyaw (2019) and Tokala et al. (2020) investigated different BC and NC formulations and  
73 reported that the fumigation treatments were relatively more effective in retarding the postharvest ethylene  
74 production and maintaining the postharvest quality of cold-stored fruit. Recently, Tokala et al. (2021 a, b) also  
75 reported the beneficial effects of BC and NC fumigation on postharvest fruit quality of ozonized cold stored  
76 ‘Cripps Pink’ and ‘Granny Smith’ apples as well as in controlled atmosphere storage. Our preliminary research  
77 findings claiming that BC and NC fumigation suppress climacteric ethylene production and maintain fruit quality  
78 of CA stored ‘Gold Rush’ pear have been presented at the 2019 ASHS Conference in Las Vegas, USA (Tokala et  
79 al. 2019). To the best of our knowledge, no detailed research work has been reported on the effects of BC, NC  
80 and 1-MCP on the rates of respiration and ethylene production as well as on the fruit quality of long term CA  
81 stored ‘Gold Rush’ pear. It was hypothesized that the fumigation treatment with ethylene antagonists (BC, NC and  
82 1-MCP) may effectively reduce the rates of climacteric ethylene and respiration while maintaining optimum fruit  
83 quality of ‘Gold Rush’ pears following 150 d and 200 d of CA storage. The objective of this study is to investigate  
84 the effects of BC and NC as well as 1-MCP in retarding the climacteric ethylene production, respiration rate and  
85 maintaining fruit quality of long-term CA stored ‘Gold Rush’ pear fruit.

## 86 2. Materials and methods

### 87 2.1. Plant materials

88 Mature pear (*Pyrus communis* L. cv. Gold Rush) fruit (fruit firmness  $82.03 \pm 4.14$  N; SSC  $11.43 \pm 0.05$  %; TA  
89  $0.09 \pm 0.01$  %) were harvested from the commercial orchard in Beedelup, Western Australia ( $34^{\circ}19' S$ ,  $116^{\circ}00'$   
90 E) on 20<sup>th</sup> March 2018. A negligible amount of ethylene, undetectable by gas chromatograph (GC), was produced  
91 by the fruit at this stage. The pear trees were 19 years old, grafted on *Pyrus calleryana* D6 (Callery Pear) rootstock  
92 and trained to modified the central leader system. The trees were planted with a spacing of  $5\text{ m} \times 1.5\text{ m}$  at North-  
93 South orientation and received uniform cultivation practices. The fruit were packed in corrugated cardboard boxes  
94 and immediately transported to Curtin Horticulture Research Laboratory, Perth using an air-conditioned vehicle.  
95 The pear fruit of relatively uniform size, free from mechanical injuries, bruises or any signs of pests or diseases,  
96 were used for the experiment.

### 97 2.2. Chemicals

98 The ethylene antagonist compounds (1-MCP, BC and NC) used in the experiment were synthesised at Chemistry  
99 Laboratory, Curtin University. The 1-MCP was synthesized following the procedure explained earlier by Fisher  
100 and Applequist (1965). The BC was synthesised from 1,3-cyclohexadiene and NC was synthesised from naphthene  
101 in anhydrous tetrahydrofuran, following the procedures explained previously by Davalian et al. (1980) and Billups  
102 and Chow (1973), respectively.

### 103 2.3. Fumigation treatments and CA storage conditions

104 The pear fruit were fumigated with  $1\text{ }\mu\text{M}$  BC ( $0.09\text{ }\mu\text{L.L}^{-1}$ ) or  $1\text{ }\mu\text{M}$  NC ( $0.14\text{ }\mu\text{L.L}^{-1}$ ) or  $18\text{ }\mu\text{M}$  ( $1\text{ }\mu\text{L.L}^{-1}$ ) 1-MCP  
105 for 18 h using 60 L plastic drums at room temperature ( $20 \pm 2\text{ }^{\circ}\text{C}$  and  $65 \pm 5\text{ \% RH}$ ). The fruit were arranged in  
106 plastic drums and calculated volumes of respective ethylene antagonist solution dissolved in ethanol were poured  
107 on to the filter paper in a Petri-plate. Granular soda lime (30 g) to absorb any excess carbon dioxide ( $\text{CO}_2$ ) and a  
108 battery-operated portable fan to uniformly distribute the ethylene antagonist vapours were placed inside the drum  
109 before hermetically sealing it. No fumigation treatment was given to control fruit but were placed in the same  
110 conditions as other treatments. The experiment was laid out by following a two-factor (ethylene antagonist  
111 treatments and CA storage times) factorial completely randomised design with four replications and fifteen fruit  
112 per replication. On completion of 18 h of fumigation treatment, the drums were unsealed in an open-air

113 environment and the fruit were immediately packed in corrugated cardboard boxes with softboard trays. All the  
114 boxes were labelled appropriately with respect to the treatment and transferred to CA storages at Carmel, Western  
115 Australia (32°00' S 116°06' E) and stored for 150 d and 200 d. The gas concentrations in the CA storages comprised  
116 of  $2.3 \pm 0.5$  % O<sub>2</sub> and  $0.4 \pm 0.15$  % CO<sub>2</sub> and  $0.50 \pm 0.71$  °C temperature. After completion of designated CA  
117 storage duration, the pear fruit were transferred to the laboratory, to determine the rates of ethylene production,  
118 respiration and fruit quality parameters analysis.

#### 119 *2.4. Determination of ethylene production and respiration rate*

120 Two pear fruit were randomly selected from each replication to determine the ethylene production and respiration  
121 rate. The chosen fruit were sealed in 1 L glass jars for 1 h and then the gas samples were drawn from the headspace,  
122 through a rubber septum at the top. The 1 mL gas sample was injected into a gas chromatograph (Model 6890N,  
123 Agilent Technology, CA, USA) to determine the ethylene production and 2 mL gas sample was injected into the  
124 infrared gas analyser (Servomex Gas Analyser, 1450 Food Package Analyser, Servomex Limited, UK) to estimate  
125 respiration rate, as the production of carbon dioxide. The complete details of the instruments and the procedure  
126 have been earlier explained by Tokala (2019). The ethylene production and respiration rate were estimated daily  
127 until a post climacteric stage. The ethylene production and respiration rate were calculated as  $\mu\text{mol.kg}^{-1}.\text{h}^{-1}$   
128 ethylene and  $\text{mmol.kg}^{-1}.\text{h}^{-1}$  CO<sub>2</sub>, respectively.

#### 129 *2.5. Physiological loss of weight (PLW)*

130 Fifteen fruit in each replication were weighed before transferring them into respective CA storage rooms and noted  
131 as initial weight. On completion of the respective storage periods, the final weight was then recorded. The PLW  
132 was calculated from initial and final weights using the following formula and expressed as %.

$$133 \quad \text{PLW (\%)} = \frac{\text{Initial weight (kg)} - \text{Final weight (kg)} \times 100}{\text{Initial weight (kg)}}$$

#### 134 *2.6. Fruit firmness*

135 The fruit firmness was determined from ten fruit per replication, by puncturing the peeled portion of pear fruit on  
136 opposite sites at the equatorial region. The Texture Analyser (TA Plus, Ametek Lloyd Instruments Limited, UK)  
137 fitted with an 8 mm (5/16") Magnus-Taylor probe was used to puncture the fruit at 7 mm sample depth with 100

138 mm s<sup>-1</sup> test speed and 5 N trigger force. The fruit firmness was calculated using Nexygen® v.4.6 software interface  
139 and expressed as newtons (N).

#### 140 *2.7. Soluble solids content (SSC), titratable acidity (TA) and SSC: TA ratio*

141 The pooled juice sample extracted from the slices cut from thirteen fruit per replication was used to determine  
142 SSC, TA and SSC: TA ratio. SSC was determined using an infrared digital refractometer (Atago – Palette PR 101,  
143 Atago Co., Tokyo, Japan) and expressed as %. The diluted fruit juice sample was titrated against the 0.01 N sodium  
144 hydroxide (NaOH) with 2-3 drops of phenolphthalein indicator till pale pink colour endpoint, to determine TA.  
145 The calculated TA was expressed as a percentage of malic acid. The SSC: TA ratio value was calculated by  
146 dividing SSC by TA values.

#### 147 *2.8. Individual sugars and organic acids*

148 The levels of individual sugars and organic acids in the fruit pulp samples, from thirteen fruit per replication, were  
149 determined using the reverse-phase high-performance liquid chromatography (RP-HPLC) system (Waters 1525,  
150 Milford Corporation, USA) following the method detailed earlier by Tokala (2019). The Dual  $\lambda$  UV absorbance  
151 detector (Water 2487, Milford Corporation, USA) at 214 nm was used to determine the individual organic acids  
152 (citric acid, tartaric acid, malic acid, succinic acid and fumaric acid). The Refractive Index (RI) detector (Water  
153 2414, Milford Corporation, USA) was used to estimate the levels of individual sugars (sucrose, glucose, fructose  
154 and sorbitol). The values of individual sugars and organic acids were calculated for the area of the chromatographic  
155 peaks using Breeze®2 software version 6.20 (Waters, Milford Corporation, USA) and are expressed as g.kg<sup>-1</sup> fresh  
156 weight basis.

#### 157 *2.9. Total phenols*

158 The levels of total phenols in the fruit pulp samples were determined using the Folin-Ciocalteu reagent method  
159 and a UV/VIS spectrophotometer (Jenway spectrophotometer Model 6405, UK) using the procedure explained  
160 earlier by Robles-Sánchez et al. (2009), with some modifications as detailed earlier by Tokala (2019). The levels  
161 of total phenols were calculated from the standard curve drawn using pure gallic acid and were expressed as g  
162 Gallic Acid Equivalent (GAE) kg<sup>-1</sup> fresh weight basis.

#### 163 *2.10. Ascorbic acid*

164 The ascorbic acid levels in the fruit pulp samples were determined using a UV/VIS spectrophotometer (Jenway  
165 spectrophotometer Model 6405, UK) following the procedure earlier detailed by Tokala (2019). The standard L-  
166 ascorbic acid curve was used to calculate levels of ascorbic acid and expressed as g.kg<sup>-1</sup> fresh weight basis.

#### 167 *2.11. Total antioxidant capacity*

168 The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay explained by Brand-Williams et al. (1995) was used to determine  
169 the total antioxidant capacity in the fruit pulp samples, following the procedure detailed by Tokala (2019). The  
170 absorbance of the samples prepared was recorded at 515 nm using a UV/VIS spectrophotometer (Jenway  
171 spectrophotometer Model 6405, UK). The levels of total antioxidant capacity were calculated using Trolox (6-  
172 hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) standard curve and expressed as μM kg<sup>-1</sup> Trolox fresh  
173 weight basis.

#### 174 *2.12. Statistical analysis*

175 The data were analysed using a two-way analysis of variance (ANOVA) to evaluate the effects of ethylene  
176 antagonist treatments, CA storage duration and their interaction. The *GenStat* software version 14.0 (Lawes  
177 Agricultural Trust, Rothamsted Experimental Station, UK) was used to analyse all the experimental data. The least  
178 significant difference (LSD) at 5 % error probability was calculated by F-test and treatment means were compared  
179 using Duncan multiple comparison tests. The results in the tables are presented as means ± standard errors (SE) of  
180 the means.

### 181 **3. Results**

#### 182 *3.1. Ethylene production and respiration rates*

183 The 'Gold Rush' pear fruit fumigated with BC, NC and 1-MCP exhibited reduced rates of ethylene climacteric  
184 peak by 28 %, 33 % and 99 % in 150 d CA stored and by 28 %, 17 % and 99 % in 200 d CA stored, respectively,  
185 when compared to the control fruit (Figure 1 and 2C). Similarly, when compared to control fruit, the rates of the  
186 respiratory climacteric peak were reduced by 20 %, 17 % and 43 % following 150 d and by 23 %, 13 % and 55 %  
187 following 200 d of CA storage in the fruit fumigated with BC, NC and 1-MCP, respectively (Figure 2D). When  
188 compared to the control fruit, the onset of ethylene climacteric peak was delayed by 1.25, 1.5 and 6.25 d following  
189 150 d and by 2.5, 0.5 and 5 d following 200 d of CA storage in the fruit fumigated with BC, NC and 1-MCP,

190 respectively (Figure 1 and 2A). However, the onset of the respiratory climacteric peak was not significantly  
191 affected by any of the ethylene antagonist treatments (Figure 2B).

### 192 3.2. PLW and fruit firmness

193 The PLW was significantly reduced by 34 %, 35 % and 52 % in the fruit fumigated with BC, NC and 1-MCP when  
194 compared to control fruit, respectively, irrespective of the CA storage period (Table 1). The firmness in the pear  
195 fruit fumigated with BC, NC and 1-MCP was maintained each 1.07 times higher than that of the firmness of control  
196 fruit, irrespective of the CA storage period (Table 1). There was no significant interaction effect between the  
197 ethylene antagonist treatment and the storage duration on the PLW and fruit firmness.

### 198 3.3. SSC, TA and SSC: TA ratio

199 The pear fruit fumigated with BC and NC exhibited significantly lower SSC values (11.61 % and 11.70 %,   
200 respectively) when compared to the fruit treated with 1-MCP and control fruit, irrespective of the CA storage  
201 period (Table 1). The SSC values increased by 1.04 folds with the extension of CA storage duration from 150 d to  
202 200 d (Table 1). The pear fruit fumigated with BC and stored for 150 d exhibited significantly lowest SSC values  
203 (11.35 %) when compared to all other treatments and control (Table 1). The values of TA as well as SSC: TA ratio  
204 were not significantly affected by any of the treatments (Supplementary, Appendix 1, Table 1).

### 205 3.4. Individual sugars and organic acids

206 Glucose, fructose, sucrose and sorbitol were determined from the treated and control fruit following 150 d and 200  
207 d CA storage, but fructose was the predominant sugar (Table 2). The pear fruit fumigated with 1-MCP exhibited  
208 significantly highest levels of glucose (5.18 g.kg<sup>-1</sup>) and sorbitol (11.86 g.kg<sup>-1</sup>) but lowest levels of sucrose (6.68  
209 g.kg<sup>-1</sup>), when compared to all other treatments and control (Table 2). The levels of sucrose were significantly  
210 higher (9.91 g.kg<sup>-1</sup>) in 150 d CA stored fruit than those stored for 200 d (7.86 g.kg<sup>-1</sup>). Whilst the levels of sorbitol  
211 were higher (11.37 g.kg<sup>-1</sup>) in the 200 d CA stored fruit than 150 d stored (10.27 g.kg<sup>-1</sup>) (Table 2). BC, NC and 1-  
212 MCP fumigation did not significantly affect the levels of fructose as compared to the control in 150 d and 200 d  
213 CA stored fruit. Malic acid, succinic acid and fumaric acid were quantified from the treated and control fruit  
214 following 150 d and 200 d CA storage, but succinic acid was the predominant organic acid (Table 2). The levels  
215 of malic acid, succinic acid and fumaric acid were also not significantly affected by BC, NC and 1-MCP fumigation  
216 treatments or CA storage duration (Supplementary, Appendix 1, Table 2).

217 *3.5. Total phenols, ascorbic acid and total antioxidant capacity*

218 BC, NC and 1-MCP fumigation treatments did not significantly influence the levels of total phenols, ascorbic acid  
219 and total antioxidant capacity as compared to the control in 150 and 200 d CA stored fruit. The levels of ascorbic  
220 acid and total antioxidant capacity were reduced by 15 % and 30 %, respectively, with an extension of CA storage  
221 duration from 150 d to 200 d. The interactions between BC, NC and 1-MCP fumigation treatments and CA storage  
222 periods were non-significant for total phenols, ascorbic acid and total antioxidant capacity (Supplementary,  
223 Appendix 1, Table 3).

224 **4. Discussion**

225 The efficacy of the two new ethylene antagonist compounds (BC and NC) and 1-MCP fumigation in  
226 downregulating the climacteric ethylene production, respiration rate and maintaining postharvest fruit quality of  
227 long-term CA stored ‘Gold Rush’ pear fruit has been investigated for the first time. BC, NC and 1-MCP fumigation  
228 treatments have effectively reduced the rates of the ethylene and respiratory climacteric peak in the ‘Gold Rush’  
229 pear fruits during CA storage (Figure 1 and 2). The 1-MCP inhibits the ethylene action in the fruit at the cellular  
230 level, by irreversibly blocking ethylene receptor sites and interfering with the expression of ethylene-responsive  
231 genes (Sisler et al. 2003; Apelbaum et al. 2008). Pirrung et al. (2008) proposed a cyclopropene ring-opening  
232 reaction mechanism forming a copper carbenoid intermediate to explain ethylene antagonistic action of 1-MCP.  
233 The intermediate formed blocks the ethylene action by irreversibly reacting with amino acids of the ethylene  
234 receptor protein domain. The BC and NC compounds also react with copper (I) cofactor situated with the ETR1  
235 ethylene receptor to antagonize the ethylene action in fruit and thereby retard ethylene production and respiration  
236 rates (Musa 2016; Singh et al. 2018; Tokala et al. 2020, 2021 a, b). BC and NC are structurally different from 1-  
237 MCP, but the proposed mode of antagonising ethylene action in the fruit is similar to 1-MCP (Musa 2016; Singh  
238 et al. 2018). The fruit fumigated with the 1-MCP exhibited very low levels of ethylene production ranging between  
239 0 to 0.02  $\mu\text{mol.kg}^{-1}.\text{h}^{-1}$  in both the storage durations studied. Xie et al. (2016) reported that European pear fruit  
240 fumigated with 1-MCP exhibited an inability to produce ethylene and ripen normally after extended low-  
241 temperature storage. There is a scope to investigate the necessity of post-storage ethylene application in “Gold  
242 Rush” pear fruit. Similar to ethylene production, when compared to control, the rates of the respiratory climacteric  
243 peak in 200 d of CA stored fruit was suppressed in the fruit fumigated with BC, NC and 1-MCP (Figure 2D). The  
244 ethylene antagonist action also inhibits or retard the respiration rates along with other ripening associated

245 physiological changes in climacteric fruits (Zhang et al. 2020). This reduction implies that the ethylene antagonist  
246 treatments effectively blocked the ethylene receptor sites and inhibited ethylene action in the fruit (Sisler 2006).

247 BC, NC and 1-MCP fumigation treatments have significantly reduced the PLW during CA storage (Table 1). The  
248 loss of weight in the fruit during storage is primarily due to water loss through continuous physiological processes  
249 such as respiration and transpiration (Becker and Fricke 1996). The rate of transpiration from the fruit surface  
250 during storage increases with an increase in the rate of respiration (Dhillon and Mahajan 2011). The reduction in  
251 PLW could be associated with decreasing trends of ethylene production and respiration in the fruit (Martínez-  
252 Romero et al. 2007). The maintenance of higher fruit firmness in BC, NC and 1-MCP fumigated pear fruit may be  
253 attributed to the downregulation of ethylene production and its action, which consequently reduced fruit softening  
254 and PLW (Giovannoni 2008). The phytohormone ethylene plays a key role in activating the cell wall hydrolysing  
255 enzymes during the fruit ripening process (Giovannoni 2008). The fruit firmness in pear fruit is closely related to  
256 the degree of ripeness, internal quality and possible shelf-life (Zhang et al. 2018).

257 The SSC values were maintained significantly lower in fruit fumigated with BC and NC, while the SSC was higher  
258 in the 1-MCP treated fruit. Inconsistencies of the SSC values in the fruit treated with ethylene antagonists have  
259 also been previously reported by Blankenship and Dole (2003). Fan et al. (1999) also indicated that the  
260 accumulation of sugars in the fruit during storage is not essentially associated with ethylene perception. The levels  
261 of individual sugars (glucose, fructose and sorbitol) in the fruit treated with 1-MCP were highest as compared to  
262 those fumigated with BC or NC and control fruit (Table 2). Similarly, Mahajan et al. (2010) also reported that the  
263 levels of sugars in 'Patharnakh' pear fruit treated with 1-MCP were higher than the control fruit. BC, NC and 1-  
264 MCP fumigation treatments did not significantly affect the levels of TA and individual organic acids in CA stored  
265 fruit. Similarly, 1-MCP fumigation did not significantly regulate the levels of TA in different cultivars of pear such  
266 as 'Blanquilla' (Larrigaudière et al. 2004), 'Red Clapp's (Calvo and Sozzi 2004), and 'Bartlett' (Trincherro et al.  
267 2004).

## 268 **5. Conclusions**

269 The fumigation treatment with novel ethylene antagonists (BC and NC) as well as 1-MCP were effective in  
270 downregulating ethylene production and respiration rate in the long-term CA stored 'Gold Rush' pear fruit but 1-  
271 MCP was more efficient. The BC and NC fumigation were at par with 1-MCP treatment in reducing PLW and  
272 loss of fruit firmness. Therefore, BC and NC possess the potential to be used as an ethylene antagonist in 'Gold

273 Rush' pear without causing any undesirable effects on the fruit quality during long-term CA storage. The effects  
274 of the different concentrations of these new ethylene antagonists in comparison with 1-MCP on suppressing  
275 ethylene production in different cultivars of Asian and European pears warrants further investigation.

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## 284 **Conflict of interest**

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

## 287 **Credit authorship contribution statement:**

288 **Vijay Yadav Tokala:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing -  
289 original draft.

290 **Zora Singh:** Conceptualization, Methodology, Supervision, Resources, Writing - review and editing.

291 **Poe Nandar Kyaw:** Investigation, Methodology, Writing - review and editing.

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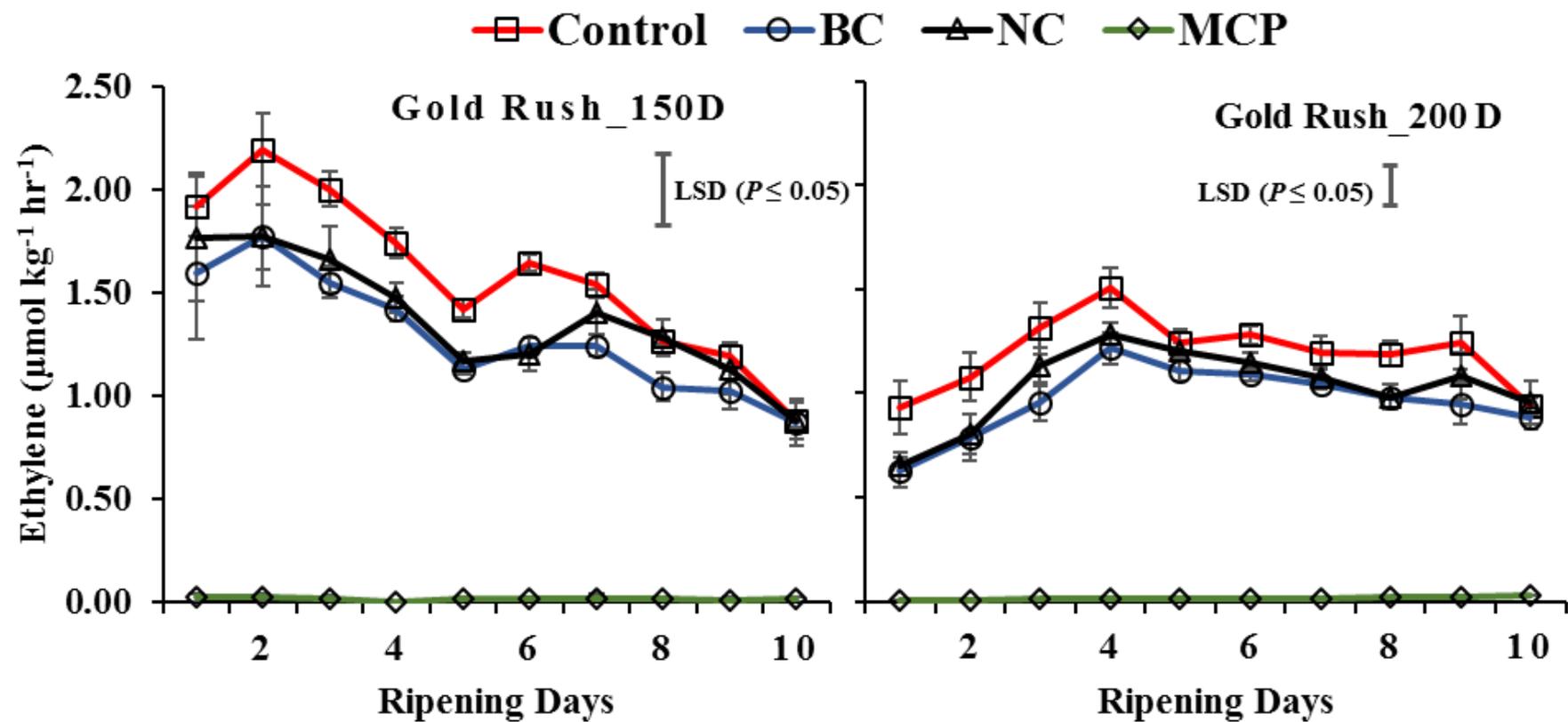


Figure 1. Effects of BC, NC and 1-MCP fumigation treatments (T) on ethylene production during ripening days (D) following 150 d and 200 d CA storage of ‘Gold Rush’ pear fruit. Vertical bars represent SE of mean values and are not visible when values are smaller than the symbol. n= 4 replicates (2 fruit per replication). LSD ( $P \leq 0.05$ ) T=0.11, D=0.17, TXD=0.35 for 150 d and T=0.06, D=0.10, TXD=0.19 for 200 d.

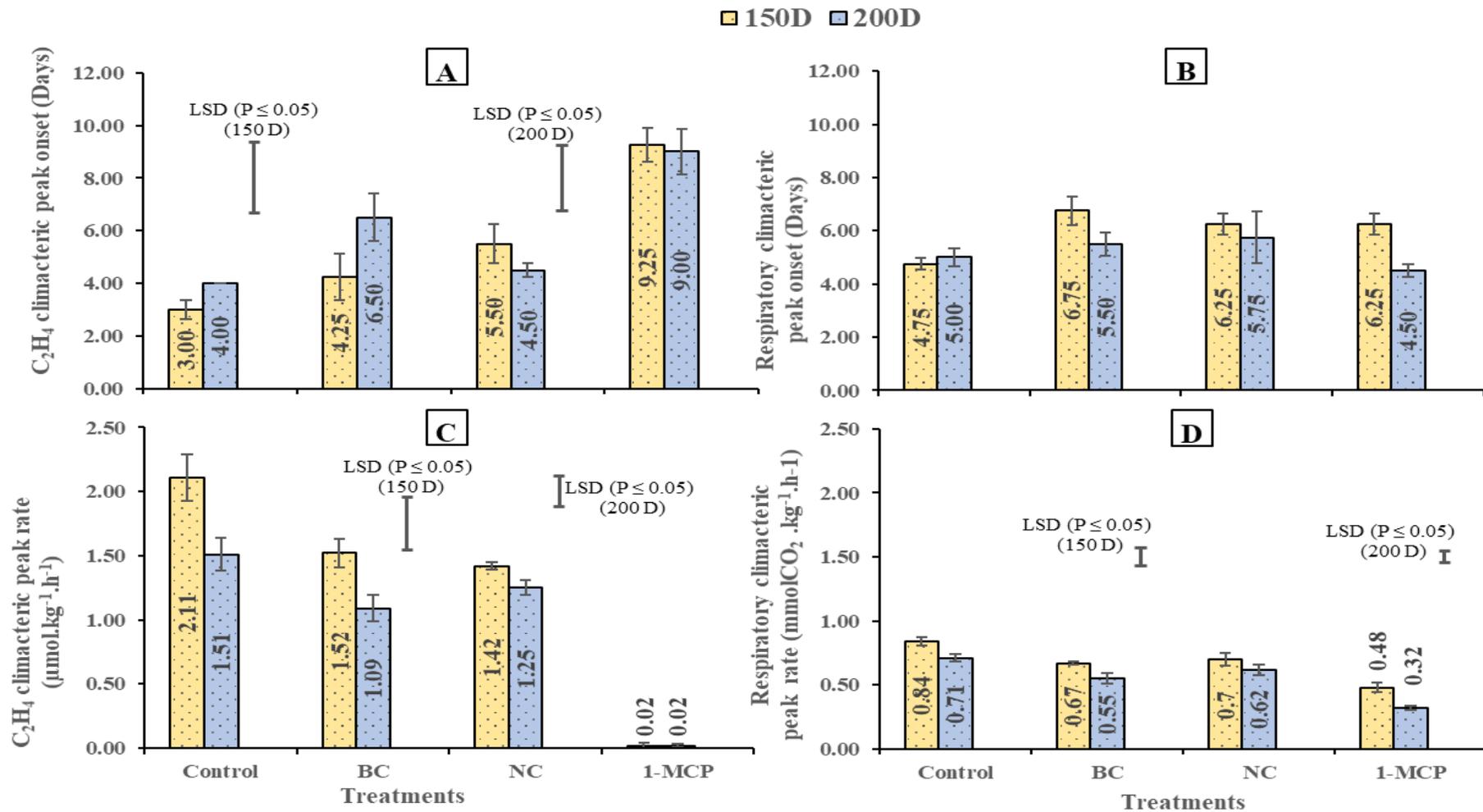


Figure 2. Effects of BC, NC and 1-MCP fumigation treatments on the climacteric peak onset (days) (A); peak rates of ethylene ( $\mu\text{mol.kg}^{-1}.\text{h}^{-1}$ ); (B) a peak rates ethylene ( $\mu\text{mol.kg}^{-1}.\text{h}^{-1}$ ) (C); climacteric respiration peak onset (days) (D) a peak rate ( $\text{mmolCO}_2.\text{kg}^{-1}.\text{h}^{-1}$ ) in 150 and 200 d CA stored Gold Rush pear fruit. Vertical bars represent SE of mean values.  $n=4$  replicates (2 fruit per replication). LSD ( $P \leq 0.05$ ): (A) 2.69 for 150 d and 2.47 for 200 d (B) non-significant for 150 d and 200 d (C) 0.41 for 150 d and 0.23 for 200 d (D) 0.14 for 150 d and 0.09 for 200 d

Table 1. Effects of BC, NC and 1-MCP fumigation on the physiological loss of weight (PLW) (%), fruit firmness (N) and soluble solids concentration (SSC) (%) of the ‘Gold Rush’ pear fruit following 150 and 200 d CA storage.

Treatment	CA storage period (days)		Mean (T)
	150	200	
	PLW (%)		
Control	2.15±0.31	2.43±0.21	2.29 <sup>B</sup>
BC	1.34±0.19	1.71±0.08	1.52 <sup>A</sup>
NC	1.32±0.40	1.67±0.20	1.49 <sup>A</sup>
1-MCP	1.11±0.38	1.11±0.30	1.11 <sup>A</sup>
Mean (D)	1.48	1.73	
LSD ( $P \leq 0.05$ )	T = 0.63	D = ns	TXD = ns
	Fruit firmness (N)		
Control	70.34±0.54	69.76±1.65	70.05 <sup>A</sup>
BC	77.70±2.14	72.17±1.24	74.94 <sup>B</sup>
NC	76.69±1.81	72.81±0.80	74.75 <sup>B</sup>
1-MCP	77.61±0.83	72.90±1.19	75.25 <sup>B</sup>
Mean (D)	75.58 <sup>B</sup>	71.91 <sup>A</sup>	
LSD ( $P \leq 0.05$ )	T = 3.32	D = 2.35	TXD = ns
	SSC (%)		
Control	11.63±0.02 <sup>b</sup>	12.05±0.02 <sup>d</sup>	11.84 <sup>B</sup>
BC	11.35±0.02 <sup>a</sup>	11.88±0.02 <sup>c</sup>	11.61 <sup>A</sup>
NC	11.58±0.02 <sup>b</sup>	11.83±0.02 <sup>c</sup>	11.70 <sup>A</sup>
1-MCP	11.60±0.09 <sup>b</sup>	12.05±0.02 <sup>d</sup>	11.83 <sup>B</sup>
Mean (D)	11.54 <sup>A</sup>	11.95 <sup>B</sup>	
LSD ( $P \leq 0.05$ )	T = 0.10	D = 0.07	TXD = 0.14

ns = non-significant, T = treatments, D = CA storage period, n = 4 replicates (15 fruit (PLW), 10 fruit (fruit firmness), 13 fruit (SSC) per replication), mean ± SE. Mean separation for significant analysis of variance within the columns and rows were tested by Duncan’s multiple range tests at ( $P \leq 0.05$ ). Mean values followed by a similar letter are not significantly different within the columns or rows. The lower case was used for mean interactions and the upper case was used for treatment and storage period means. Mean values without letters within columns or rows are non-significant.

Table 2. Effects of BC, NC and 1-MCP fumigation on the levels of individual sugars (g kg<sup>-1</sup>) in the pulp of 150 and 200 d CA stored 'Gold Rush' pear fruit.

Treatment	CA storage period (days)		Mean (T)
	150	200	
Glucose (g.kg <sup>-1</sup> )			
Control	4.55±0.19 <sup>b</sup>	4.99±0.18 <sup>bc</sup>	4.77 <sup>B</sup>
BC	4.50±0.06 <sup>b</sup>	4.53±0.11 <sup>b</sup>	4.54 <sup>AB</sup>
NC	4.80±0.07 <sup>b</sup>	3.96±0.15 <sup>a</sup>	4.38 <sup>A</sup>
1-MCP	5.05±0.19 <sup>bc</sup>	5.32±0.01 <sup>c</sup>	5.18 <sup>C</sup>
Mean (D)	4.73	4.70	
LSD ( $P \leq 0.05$ )	T = 0.34	D = ns	TXD = 0.48
Fructose (g.kg <sup>-1</sup> )			
Control	30.53±0.40	30.87±0.16	30.70
BC	31.39±0.21	30.20±0.49	30.80
NC	30.15±0.49	30.87±0.43	30.51
1-MCP	31.24±1.23	31.63±0.73	31.43
Mean (D)	30.83	30.89	
LSD ( $P \leq 0.05$ )	T = ns	D = ns	TXD = ns
Sucrose (g.kg <sup>-1</sup> )			
Control	10.42±0.6.67	8.71±0.69	9.56 <sup>B</sup>
BC	11.22±0.5.75	8.76±0.24	9.99 <sup>B</sup>
NC	9.95±0.1.76	8.66±0.24	9.31 <sup>B</sup>
1-MCP	8.04±0.7.24	5.31±0.20	6.68 <sup>A</sup>
Mean (D)	9.91 <sup>B</sup>	7.86 <sup>A</sup>	
LSD ( $P \leq 0.05$ )	T = 1.2.04	D = 0.8.51	TXD = ns
Sorbitol (g.kg <sup>-1</sup> )			
Control	9.90±0.26	11.13±0.21	10.51 <sup>A</sup>
BC	9.84±0.12	10.94±0.21	10.39 <sup>A</sup>
NC	9.57±0.26	11.44±0.12	10.50 <sup>A</sup>
1-MCP	11.76±0.53	11.96±0.50	11.86 <sup>B</sup>
Mean (D)	10.27 <sup>A</sup>	11.37 <sup>B</sup>	
LSD ( $P \leq 0.05$ )	T = 0.78	D = 0.55	TXD = ns

ns = non-significant, T = treatments, D = CA storage period, n = 4 replicates (13 fruit per replication), mean ± SE. The Duncan's multiple range tests at ( $P \leq 0.05$ ) was used the test the mean separation for significant analysis of variance within the columns and rows. Mean values followed by a similar letter are not significantly different within the columns or rows. The lower case was used for mean interactions and the upper case was used for treatment and storage period means. Mean values without letters within columns or rows are non-significant.

Supplementary Table 1. Effects of BC, NC and 1-MCP fumigation on the changes in the titratable acidity (TA) (%) and SSC: TA ratio in the juice of 150 and 200 d CA stored 'Gold Rush' pear fruit.

Treatment	CA storage period (days)		Mean (T)
	150	200	
Titratable acidity (TA) (%)			
Control	0.06±0.01	0.07±0.01	0.07
BC	0.07±0.01	0.09±0.01	0.08
NC	0.08±0.00	0.09±0.01	0.09
1-MCP	0.08±0.01	0.09±0.01	0.08
Mean (D)	0.07	0.08	
LSD ( $P \leq 0.05$ )	T=ns	D=ns	TXD=ns
SSC: TA			
Control	217.04±36.38	174.85±12.50	195.95
BC	176.31±30.25	132.96±7.53	154.63
NC	143.97±0.27	132.40±7.50	138.18
1-MCP	161.55±17.48	142.41±6.65	151.98
Mean (D)	174.71	145.66	
LSD ( $P \leq 0.05$ )	T=ns	D=ns	TXD=ns

ns = non-significant, T = treatments, D = CA storage period, n = 4 replicates (13 fruit per replication), mean ± SE. The Duncan's multiple range tests at ( $P \leq 0.05$ ) was used the test the mean separation for significant analysis of variance within the columns and rows. Mean values without letters within columns or rows are non-significant.

Supplementary Table 2 Effects of BC, NC and 1-MCP fumigation on the levels of individual organic acids (g.kg<sup>-1</sup>) in the pulp of 150 and 200 d CA stored 'Gold Rush' pear fruit.

Treatment	CA storage period (days)		Mean (T)
	150	200	
Malic acid (g.kg <sup>-1</sup> )			
Control	1.25±0.13	0.98±0.06	1.11
BC	1.22±0.08	1.25±0.14	1.23
NC	1.31±0.30	1.35±0.06	1.33
1-MCP	1.36±0.22	1.22±0.38	1.29
Mean (D)	1.28	1.20	
LSD ( <i>P</i> ≤ 0.05)	T=ns	D=ns	TXD=ns
Succinic acid (g.kg <sup>-1</sup> )			
Control	4.30±0.20	2.66±0.60	3.48
BC	4.52±0.26	3.55±0.32	4.03
NC	4.13±0.52	2.83±0.33	3.48
1-MCP	3.33±0.55	3.93±0.26	3.63
Mean (D)	4.07B	3.24A	
LSD ( <i>P</i> ≤ 0.05)	T=ns	D=0.73	TXD=ns
Fumaric acid (g.kg <sup>-1</sup> )			
Control	0.29±0.04	0.28±0.05	0.29
BC	0.19±0.00	0.21±0.02	0.20
NC	0.24±0.01	0.26±0.03	0.25
1-MCP	0.20±0.01	0.23±0.04	0.22
Mean (D)	0.23	0.25	
LSD ( <i>P</i> ≤ 0.05)	T=ns	D=ns	TXD=ns

ns = non-significant, T = treatments, D = CA storage period, n = 4 replicates (13 fruit per replication), mean ± SE. The Duncan's multiple range tests at (*P* ≤ 0.05) was used the test the mean separation for significant analysis of variance within the columns and rows. Mean values without letters within columns or rows are non-significant.

Supplementary Table 3. Effects of BC, NC and 1-MCP on the levels of total phenols, ascorbic acid and total antioxidant capacity in the pulp of 150 and 200 d CA stored ‘Gold Rush’ pear fruit.

Treatment	CA storage period (days)		Mean (T)
	150	200	
Total phenols (g.GAEkg <sup>-1</sup> )			
Control	11.41±0.73	12.16±1.23	11.79
BC	12.16±0.82	12.82±2.58	12.49
NC	11.04±0.49	10.20±0.87	10.62
1-MCP	10.29±0.82	10.29±0.50	10.29
Mean (D)	11.23	11.37	
LSD ( $P \leq 0.05$ )	T=ns	D=ns	TXD=ns
Ascorbic acid (g.kg <sup>-1</sup> )			
Control	5.26±0.18	4.56±0.44	4.91
BC	5.12±0.29	4.88±0.34	5.00
NC	4.81±0.24	4.14±0.05	4.48
1-MCP	5.47±0.19	3.97±0.25	4.72
Mean (D)	5.17B	4.39A	
LSD ( $P \leq 0.05$ )	T=ns	D=0.44	TXD=ns
Total antioxidant capacity (µM.kg <sup>-1</sup> Trolox)			
Control	5.31±0.46	3.95±0.19	4.63
BC	5.72±0.33	3.76±0.22	4.74
NC	4.68±0.22	3.60±0.13	4.14
1-MCP	5.50±0.28	3.65±0.10	4.57
Mean (D)	5.31B	3.74A	
LSD ( $P \leq 0.05$ )	T=ns	D=0.45	TXD=ns

ns = non-significant, T = treatments, D = CA storage period, n = 4 replicates (13 fruit per replication), mean ± SE. The Duncan’s multiple range tests at ( $P \leq 0.05$ ) was used the test the mean separation for significant analysis of variance within the columns and rows. Mean values without letters within columns or rows are non-significant.