Research Article

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Bordeaux mixture accelerates ripening, delays senescence, and promotes metabolite accumulation in jujube fruit

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Abstract: The old, but eco-friendly pesticide, Bordeaux mixture (Bm), is widely used in agriculture. Here, the effects of Bm on preharvest fruit ripening, and postharvest senescence of fruit and the accumulation of antioxidants were studied in Ziziphus jujuba. A 200× dilution of Bm enhanced preharvest ripening and retarded jujube fruit postharvest senescence. Treatment with Bm increased the reddening index and promoted the accumulation of total phenolics (TP) and the total antioxidant capacity (TAC) in preharvest fruit. However, this Bm-accelerated ripening could be partly reversed using dimethylthiourea (DMTU), a specific scavenger of reactive oxygen species. Compared with preharvest fruit, Bm treatment decreased weight loss and decay and increased firmness in postharvest fruit. Similarly, Bm-delayed senescence was partly reversed using dimethylthiourea. Moreover, the decrease in TP and TAC during storage was partly impeded by Bm. A higher H2O2 content was detected in preharvest fruit than in postharvest fruit. Moreover, this Bm-induced H2O2 accumulation was significantly mitigated using dimethylthiourea. Interestingly, both preharvest and postharvest spraying with Bm significantly enhance copper and calcium accumulation in fruit. Thus, Bm can be widely used before or after harvest to improve fruit quality.

Keywords: Bordeaux mixture, hydrogen peroxide, jujube fruit, ripening, senescence

1 Introduction

Fresh jujube (the fruit of Ziziphus jujuba) is a nutritious climacteric fruit with beneficial medicinal properties that is appreciated greatly by consumers because it contains high levels of carbohydrates, organic acids, vitamins, phenolics, and trace minerals [1–5]. Phenolic compounds are important phytochemicals that are found ubiquitously in fruit, displaying high antioxidant ability, reducing highly oxidized reactive oxygen species (ROS), and inhibiting the enzymes catalyzing ROS production [6–8]. In addition, trace minerals, such as copper and calcium, are also required for human health [9,10]. However, jujube fruits are highly perishable during harvest and storage.

Fruit ripening and senescence profoundly affect nutrition quality [11]. However, accumulating evidence shows that postharvest treatment can significantly delay senescence and improve fruit quality during storage [12–15]. In addition, preharvest treatment can also effectively enhance postharvest fruit quality [16–18]. For example, preharvest application of methyl jasmonate improved fruit quality and the antioxidant content in plums during postharvest storage [16,19].

Most crop plants cannot attain their full genetic potential for growth and reproduction because of environmental conditions that limit their growth [20,21]. The use of pesticides...
has greatly increased crop yields and food quality for the growing world population [22,23]. However, many pesticides are toxic to humans and damage the environment.

Bordeaux mixture (Bm, a mixture of copper sulfate and lime) is widely used as an eco-friendly pesticide to improve crop yield and quality [24–27]. For example, the application of Bm could effectively control the rice blast disease [27]. Moreover, effects of this preventive pesticide on the metabolic response in crops and microbes were also investigated [28,29]. For example, treatment with Bm had no effect on the main sugar and organic acid content but reduced the free amino acid levels in ripe berries [28]. However, few studies have shown the effects of pesticides on preharvest fruit ripening and postharvest fruit senescence.

Both preharvest and postharvest fruit quality can be affected by environmental factors [30–32]. For example, the quality of preharvest mangos is affected by light, temperature, and carbon and water availability [32]. However, the effects of preharvest pesticide application on antioxidant accumulation in postharvest fruits have not been reported.

In this study, Bm was applied to preharvest jujube fruit during the maturity stage for several days, with the aim of investigating its possible roles in preharvest fruit ripening and postharvest fruit senescence. We further aimed to determine the effect of Bm on the preharvest and postharvest jujube fruit behavior and the accumulation of antioxidants and the possible underlying mechanisms.

2 Methods

The conducted research is not related to either human or animal use.

2.1 Plant materials and treatment

The study used Jujube (Ziziphus jujuba Mill., cv. ‘Dongzao’) fruit provided by the Jujube Research Center at Luoyang, Henan Province, China. The fruit was harvested by hand in the early morning and taken to our laboratory in under 2 h. Fruit with a uniform shape (diameter 2.5 ± 0.2 cm) and appearance (without visible defects during maturity) were chosen. This work comprised both preharvest and postharvest experiments.

For 7 days before harvest (for the preharvest study) or 0 days (for the postharvest study), the jujube fruit was sprayed separately with 200× diluted Bm, or distilled water (as a control). In the preharvest study, the fruit was collected to analyze the reddening index, H₂O₂ content, total phenolics (TP) content, and total antioxidant capacity (TAC) after treatment for 1, 4, and 7 days. For the postharvest study, the samples were further divided into two subgroups: the senescence group (group 1) and the antioxidant group (group 2). The samples were handled as follows: in group 1, the fruit samples were analyzed for their loss of weight, decay rate, and firmness following storage for 0, 1, 3, 5, and 7 days; in group 2, the fruit samples were prepared for analysis of their H₂O₂ content, TAC, and TP after storage for 1, 4, and 7 days.

For each parameter assay, three replications were performed for each treatment, with 10 fruit in each replicate. The storage temperature was 25 ± 0.5°C, and the relative humidity was approximately 70%.

2.2 Assessment of the reddening index

The extent of the red area on the fruit surface was used to assess fruit reddening:

0 = no redness; 1 = ≤33% red; 2 = 33–67% red; 3 = ≥67% red; and 4 = 100% red. The reddening index was calculated using the formula: \[ \text{Reddening index} = \frac{1 \times N_1 + 2 \times N_2 + 3 \times N_3 + 4 \times N_4}{4 \times N} \times 100 \], where \( N \) = total number of fruit assessed and \( N_1, N_2, N_3, \) and \( N_4 \) represent the numbers of fruit with reddening scale ratings of 1–4, respectively.

2.3 Measurements of TP and TAC

The TP was determined as described previously [33]. In brief, 1 g of fruit flesh was added with 0.02 L of 1% HCl-methanol and homogenized on ice. The homogenate was incubated in the dark for 20 min, followed by centrifugation at 4°C and 12,000×g for 10 min. The absorbance of the collected supernatant at 280 nm was measured, and the TP was expressed as gallic acid equivalents.

Fruit extracts were prepared to measure the TAC. The fruit was weighed and immediately flash-frozen in liquid nitrogen. Samples with a dry weight of ~1 g were ground in liquid nitrogen using a mortar and pestle to a powder, added to 0.1 L of 90% (w/v) methanol-water solution, and incubated in the dark for 25 h at room temperature. After filtration, the extracts from each replicate were pooled and placed in rotary evaporator to remove the solvent under a vacuum at 45°C. The resultant crude extracts were stored at 4°C in a desiccator until the TAC analysis.
reducing ability of plasma assay, with some modifications [34,35], was used to determine the TAC.

2.4 Determination of the loss of weight, decay rate, and firmness of fruit

Individual fruit was weighed before and after storage, and the loss of weight was calculated as the percentage of weight lost compared with the initial weight. The decay rate was calculated as the percentage of fruit displaying symptoms of decay relative to the total number of fruit in the treatment group. A pressure tester (GY-3, Aidehao Instrument Co., Ltd, Leqing, China) comprising a probe with a diameter of 8 mm was used to determine firmness, which was expressed as N.

2.5 Determination of copper and calcium

Collected fruit was washed, air-dried, and ground into a powder using a mortar and pestle. To extract copper and calcium, 1 g of the dried powdered sample was added to an acid-washed and dried digestion tube. Then, 10 mL of HNO₃, HClO₄, and H₂SO₄ (5:1:1) were added to the tube and incubated for 12 h. The tubes were placed in an 80°C digestion block and incubated for approximately 60 min. The temperature was then increased gradually to 120–130°C. At the end of digestion, the tubes were cooled, and the solutions were filtered and diluted to 100 mL using double-deionized water [2]. Copper (Cu) and calcium (Ca) levels in the filtrates were measured using an atomic absorption spectrometer (Analyst 700, Perkin Elmer, Waltham, MA, USA).

2.6 Detection of H₂O₂

The H₂O₂ contents of preharvest and postharvest jujube fruit (control, Bm, and Bm + dimethylthiourea [DMTU]) were determined using a previously published method [36]. H₂O₂ production in preharvest and postharvest fruit was assessed utilizing the oxidation of xylene orange assay, based on peroxide-mediated oxidation of Fe⁺⁺ ions. The resultant Fe³⁺ ions were reacted with the sodium salt of xylene orange, followed by colorimetric detection. The assay reagent (1 mL of 25 mM FeSO₄ and 25 mM (NH₄)₂SO₄, dissolved in 2.5 M H₂SO₄) was added to 100 mL of 100 mM sorbitol and 125 μM xylene orange. Collected tissue was ground and centrifuged for 5 min at 8,000×g. The supernatant (100 μL) was added to 1 mL of the prepared assay reagent. After incubation for 30 min, a spectrophotometer was used to record the absorbance of the Fe³⁺–xylene orange complex at 560 nm.

2.7 Data analysis

A completely randomized design was used to conduct the experiments, with each treatment having three replicates. Duncan’s multiple range test was used to analyze all the data (at p < 0.05 for significance) employing SPSS 13.0 software (IBM Corp., Armonk, NY, USA).

3 Results

3.1 Bm affected the reddening index, loss of weight, decay rate, and firmness

Preharvest, compared with that in the control group, the Bm-treated fruit rapidly became red, with a higher reddening index (Figure 1a). However, this Bm-enhanced reddening index was partially reversed using the specific ROS scavenger, DMTU (Figure 1a). For example, in preharvest fruit, the reddening index increased by 20% for the control, and by 87, and 68% in the Bm and Bm + DMTU treatment groups, respectively, after treatment for 7 days relative to the fruit treated for 1 day (Figure 1a; p < 0.05).

Over time, weight loss increased in all treatment groups; however, the Bm-treated fruit lost weight more slowly. Interestingly, this Bm-delayed weight loss was partially attenuated by DMTU treatment (Figure 1b). After 7 days of storage, weight loss in the jujube fruit was 43% (control), 13% (Bm), and 19% (Bm + DMTU) compared with the fruit at day 1 (Figure 1b; p < 0.05). In the control and treated fruit, decay was initially observed after 3 days of storage; thereafter, decay increased with prolonged storage time (Figure 1c). Bm treatments effectively suppressed the increase in decay. However, this Bm-inhibited decay could be significantly reversed using DMTU (Figure 1c). Compared with the control fruit, we observed decreases in the decay rate of 76 and 54%, respectively, for the Bm- and Bm + DMTU-treated fruit by the end of the postharvest storage period (Figure 1c; p < 0.05). The control and treated fruit showed decreased firmness over the 7 days of storage; however, this decrease was effectively inhibited by Bm treatment (Figure 1d). However, this Bm-enhanced firmness was significantly reversed by DMTU. By the end of the storage period, the firmness in the control group was 42 N, in
the Bm-treated group, it was 95 N, and in the Bm + DMTU-treated group, it was 79 N (Figure 1d; \( p < 0.05 \)).

### 3.2 Bm induced phenotype changes and \( \text{H}_2\text{O}_2 \) accumulation

The phenotype change (e.g., color and appearance) of jujube fruit can be used to preliminarily evaluate fruit ripening and senescence. We found that Bm treatment enhanced fruit ripening, and this effect could be partially alleviated using DMTU or strengthened by \( \text{H}_2\text{O}_2 \) treatment (Figure 2a). Compared with that in the preharvest fruit, Bm treatment-delayed postharvest fruit senescence could be partially reversed by DMTU or enhanced by \( \text{H}_2\text{O}_2 \) treatment (Figure 2b). In addition, the \( \text{H}_2\text{O}_2 \) content was measured after treatment for 1, 4, and 7 days (Figure 2c,d). Compared with the steady but slight increase in \( \text{H}_2\text{O}_2 \) content in the control, the Bm-treated fruit exhibited a pattern of rapid \( \text{H}_2\text{O}_2 \) increase, followed by a more gradual decrease in preharvest fruit (Figure 2c). For example, compared with that in the control, \( \text{H}_2\text{O}_2 \) was increased by 91, 41, and 23% in the Bm-treated fruit following treatment for 1, 4, and 7 days, respectively (Figure 2c).

Compared with those in preharvest fruit, contrasting change patterns were observed in postharvest fruit, regardless of Bm treatment (Figure 2d). Compared with the rapid and gradual increase in \( \text{H}_2\text{O}_2 \) content in the control fruit, the Bm-treated fruit exhibited a pattern of rapid \( \text{H}_2\text{O}_2 \) increase followed by a significant decrease in the postharvest fruit (Figure 2d). For example, in the Bm treatment group, the \( \text{H}_2\text{O}_2 \) contents were increased by 109, −18, and −44% after 1, 4, and 7 days, respectively, compared to the control (Figure 2b; \( p < 0.05 \)).

### 3.3 Effects of Bm on TP and TAC

Figure 3 shows that exposure of fruit to Bm increased the TP (Figure 3a and b) and TAC compared with those in the water control (Figure 3c and d). Compared with the control, the increases in TP accumulation were approximately 4, 17, and 33%, respectively, in fruit treated with Bm for 1, 4, and 7 days (Figure 3a; \( p < 0.05 \)). Similarly, compared with the control, the increases in TP were 2, 11, and 24% following 1, 4, and 7 days storage, respectively (Figure 3b; \( p < 0.05 \)). Moreover, compared with the control, the TAC increased by approximately −5, 23, and 38% in the preharvest fruit.
Figure 2: Bordeaux mixture affects the fruit phenotype and H₂O₂ content. Effects of Bordeaux mixture (Bm) on ripening (a), senescence (b), and H₂O₂ accumulation (c and d) were measured in preharvest and postharvest jujube fruit following treatment for 1, 4, and 7 days. Bars indicate the standard deviations of the mean from three replicates, and means with the same letter did not show a significant difference (p < 0.05) according to Duncan’s multiple range test among treatments. DMTU, dimethylthiourea.

Figure 3: Effects of Bordeaux mixture on the antioxidant capacity in fruit. Effects of Bordeaux mixture (Bm) on TP content (a and b) and TAC (c and d) assessed in preharvest and postharvest jujube fruit following treatment for 1, 4, and 7 days. Bars indicate the standard deviations of the mean from three replicates, and means with the same letter did not show a significant difference (p < 0.05) according to Duncan’s multiple range test among treatments.
following Bm treatment for 1, 4, and 7 days, respectively (Figure 3c; p < 0.05). In for the postharvest fruit, compared with the control, the TAC increased by approximately -4, 35, and 171% in Bm-treated fruit following storage for 1, 4, and 7 days, respectively (Figure 3d; p < 0.05).

3.4 Copper and calcium content after Bm treatment

Bm significantly enhanced the Cu and Ca contents in preharvest and postharvest fruit (Table 1). Bm treatment for 7 days increased Cu levels by approximately 4.8- and 2.4-fold in preharvest or postharvest fruit compared with that in the control group, respectively (Table 1; p < 0.05). In addition, Bm treatment for 7 days increased the Ca content by 85.9 and 46.4% in preharvest or postharvest fruit compared with that in the control group, respectively (Table 1; p < 0.05).

4 Discussion

In this study, jujube fruit was sprayed with a 200× dilution of Bm, a dilution that is commonly used by farmers in China. Thus, using the pesticide at this concentration would not be expected to cause toxicological damage to crops or jujube trees. Herein, the effects of Bm application on preharvest ripening and postharvest senescence in jujube fruit were investigated.

Compared with the control, treatment with Bm significantly enhanced the reddening index, which is used to evaluate jujube fruit ripening [37]. Interestingly, this Bm-accelerated preharvest fruit ripening was partially reversed using the specific scavenger of ROS, DMTU, or further enhanced by H2O2. This suggested that Bm-induced ROS might contribute to preharvest fruit ripening. However, weight loss as well as the decay rate and firmness are used to evaluate postharvest fruit senescence [14]. The results showed that postharvest Bm treatment of jujube fruit significantly inhibited the loss in weight, decreased decay, and maintained their firmness. However, this Bm-delayed fruit senescence was partly abolished by DMTU or enhanced by H2O2. This suggested that Bm-mediated ROS production might also affect postharvest fruit senescence. ROS can promote postharvest fruit senescence [38] and stimulate fruit ripening [39,40]. Thus, the accumulation of H2O2 in preharvest and postharvest jujube fruit were also measured, which showed that Bm significantly enhanced the H2O2 content in preharvest fruit during the first 7 days. By contrast, in postharvest fruit, the H2O2 level increased at first and then decreased after Bm treatment during the first 7 days. This further confirmed that ROS are required for Bm to regulate preharvest ripening and postharvest senescence in jujube fruit.

In general, senescence is the subsequent result after plant ripening or maturation [41] and both are accompanied by increasing oxidative stress [42–44]. We then sought to determine the possible reasons for the observed phenomenon: the same concentration of Bm promoted preharvest fruit ripening but delayed postharvest senescence.

Published data show that ROS plays a dual role in regulating plant development and senescence [45,46]. For example, the antioxidant defense system (enzymatic/non-enzymatic antioxidants) is activated by low concentrations of H2O2, which can also enhance stress tolerance [47,48]. Herein, higher accumulation of H2O2 (2-fold increase) was observed in preharvest fruit compared with that in postharvest fruit without Bm treatment. Moreover, the H2O2 level was further upregulated by Bm for the long term; thus, oxidative stress would be expected, indicating accelerated preharvest fruit ripening [40]. Compared with preharvest fruit, the H2O2 levels rapidly decreased and maintained a lower level in postharvest fruit after treatment with Bm for 3 days. Thus, this low level of H2O2 might play a signaling role in deterring postharvest fruit senescence after treatment with Bm. However, why were lower levels of H2O2 measured in postharvest but not preharvest fruit? The mitochondrion is not only a respiration organelle but also is the main ROS generating site in plants [49]. Thus, the observed reduced ROS accumulation might be associated with the decline in metabolic activity (e.g., the respiration rate) in postharvest fruit compared with preharvest fruit [50]. Furthermore, data also indicated that low respiration rates help to delay postharvest fruit

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**Table 1: Effects of Bordeaux mixture on Cu and Ca assimilation in fruit**

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<th>Preharvest fruit</th>
<th>Postharvest fruit</th>
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<td></td>
<td>Control</td>
<td>Bm treatment</td>
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<td><strong>Copper (µg g⁻¹)</strong></td>
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<td></td>
<td>0.23 ± 0.04</td>
<td>1.34 ± 0.19</td>
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<tr>
<td><strong>Calcium (µg g⁻¹)</strong></td>
<td>122 ± 14</td>
<td>228 ± 21</td>
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Effects of Bordeaux mixture (Bm) treatment on Cu and Ca accumulation were detected in preharvest and postharvest jujube fruit after treatment for 7 days. All values are the mean ± standard deviation of triplicate samples. Values in the same row that are not followed by the same letter are significantly different (p < 0.05), as assessed using Duncan’s multiple range test.
senescence [38,51]. However, more experiments are required to identify the detailed molecular and genetic mechanisms that underlie these observations.

Oxidative stress has been shown to accelerate senescence and reduce postharvest fruit quality [38,52]. Previous reports have shown that antioxidant (e.g., phenolics) levels decline in fruit during ripening [53]. Here, we wanted to know how Bm affects TP accumulation and the TAC in preharvest and postharvest fruit. The accumulation of TP declined by different extents during ripening or storage. However, treatment with Bm significantly increased TP accumulation in the ripening stage but slowed down their degradation under storage conditions. The TAC showed similar alterations in preharvest and postharvest fruit. This suggested that preharvest application of Bm could significantly improve jujube fruit quality by increasing the TP and TAC. Previous reports have shown that short-term exposure to heat shock, UV-C, or H$_2$O$_2$ can delay postharvest fruit senescence by increasing the antioxidant capacity [54,55]. In addition, antioxidant compounds can be induced by certain abiotic stress treatments in freshly cut fruit [56,57]. Therefore, pesticide-improved fruit quality can be partly attributed to this chemical stress-induced antioxidant capacity.

Interestingly, higher accumulation of copper (5-fold increase) and calcium (2-fold increase) contents were observed in the Bm-treated jujube fruit (Table 1). This was partly in accordance with previous reports, which showed that copper levels in tissue extracts from surface-washed fruit increase up to 14-fold after treatment with Bm [28]. This suggested that Bm application on fruit can also benefit consumers in copper-deficient regions.

Consumers often prefer organic foods because they believe that organically grown foods are more nutritious. However, scientists cannot ascertain whether there are significant nutritional differences between organic and non-organic foods [58]. Interestingly, our work showed that Bm application before harvest could significantly improve antioxidant and mineral nutrient levels in jujube fruit. Bm is a less toxic pesticide, and its preharvest or even postharvest application appears to be beneficial to fruit. In addition, our work also partly explains why many fruit growers prefer to spray pesticides before harvest from a ripening and nutritional perspective.

5 Conclusions

Our study showed that treatment with Bm could significantly promote preharvest fruit ripening while also delaying postharvest fruit senescence. Interestingly, this pesticide-promoted preharvest fruit ripening and pesticide-delayed postharvest fruit senescence could be further enhanced by H$_2$O$_2$, or partially reversed using DMTU, a specific ROS scavenger. Compared with the controls, we measured higher levels of phenolics, TAC and higher accumulation of copper and calcium in Bm-treated jujube fruit. In addition, higher levels of H$_2$O$_2$ were found in the preharvest stage, but not the postharvest stage, and this background level of H$_2$O$_2$ could be further enhanced by pesticides in both preharvest and postharvest fruit in the first few days after treatment. Thus, the observed differences in ROS accumulation might determine the effects of the pesticide on jujube fruit: high ROS levels accelerate preharvest fruit ripening, while moderate ROS levels delay postharvest fruit senescence. In this study, we partly demonstrate the roles of Bm in promoting preharvest fruit ripening and delaying postharvest fruit senescence, implying that pesticide-mediated ROS production might be an indispensable factor that regulates the maturation and senescence of fruit. Finally, as far as we know, this is the first report showing that preharvest and postharvest application of Bm improves fruit quality from the perspective of ROS.

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Ethical approval: Ethic approval was not required for this research.

Data availability statement: All data analyzed during the current study are included in this published article. The detailed data can be provided on reasonable request.

References


