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Postharvest arginine spraying delayed ripening during storage of papaya

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ABSTRACT

Purpose: Climacteric metabolism makes papaya (*Carica papaya* L) a highly perishable fruit, especially under ambient conditions. Considering that few retail outlets (markets) have a cold chain for storage, it is necessary to evaluate technologies to extend the commercial shelf life of this fruit under ambient conditions. Thus, the objective of this research was to evaluate the effect of arginine application to delay ripening and preserve the physicochemical quality of papaya during storage under ambient conditions. **Research method:** Physiologically ripe 'Hawaii' papayas (stage 1) were harvested from a commercial orchard, selected (physiological injuries, pests and diseases), sanitized in a chloride solution and sprayed with solutions containing distilled water (control) and arginine (25 $mg.L^{-1}$) determined in preliminary tests. They were then placed on benches and kept under ambient conditions (28 ± 2 °C and 85 ± 5 % RH) for a period of 7 days with quality assessments performed daily. **Findings**: Spraying a solution containing arginine (25 $mg.L^{-1}$) significantly delayed the ripening of papayas, corroborated by reduced respiratory activity and ethylene production, the effects of which were observed in delayed chlorophyll loss in the peel, reduced mass loss, maintenance of firmness, reduction in total soluble solids accumulation and titratable acidity, in addition to reduced degradation of vitamin C and lycopene. **Research limitations**: Understanding the biochemical mechanism of arginine in the regulation of ripening. **Originality/Value**: The results of this study provide the producer/trader with a viable and easy-to- apply technology to ensure a product with a longer marketing period and quality for the final consumer.

INTRODUCTION

Papaya (*Carica papaya* L.) is one of the main economic crops in tropical and subtropical regions, characterized by its sweet and unique flavor, high nutritional value (vitamins A, C, and E), antioxidants (carotenoids), as well as proteins, amino acids, and minerals (Zheng et al., 2021; Mabunda et al., 2023). As a climacteric fruit, papaya ripens rapidly after harvest characterized by color change, pulp softening, and rotting during storage and transportation, resulting in short shelf life and high losses (Kahawattage et al., 2023; Zhou et al., 2024).

Fruit ripening is a genetically programmed process that leads to various physiological, biochemical, and metabolic changes which irreversibly alter its sensory characteristics (flavor, aroma, appearance) (Nasir et al., 2024). In the postharvest storage of climacteric fruits, such as papaya, this process is fully regulated by increased respiratory activity and ethylene production, which has a direct impact on the conservation potential since it favors the loss of quality and senescence (Corpas et al., 2023). In this sense, inhibition of ethylene biosynthesis or action is the most common strategy used in postharvest to prolong storage, maintain fruit quality, and extend the availability of fresh products (Wei et al., 2021). The findings of these studies contribute to the development of innovative approaches and technologies to ensure the delivery of high-quality fruits to consumers while minimizing waste.

During the ripening process, accumulation of primary metabolites, such as amino acids, plays a crucial role in forming aromatic compounds in fruits (Pott et al., 2020). Research has shown that amino acid metabolism is affected during postharvest storage and has a direct effect on the ripening and senescence of vegetables (Yuan et al., 2017; Tang et al., 2020). Among the amino acids, arginine is an important nitrogen-carbon (N/C) binding amino acid in plants (Shu et al., 2020) and acts as a biosynthetic precursor of important compounds such as polyamine (PA) and nitric oxide (NO) (Mirmiran et al., 2017), which are important messenger molecules in most physiological and biochemical processes, including the regulation of ethylene biosynthesis (Wei et al., 2021). Several researchers reported that the exogenous application of arginine (immersion) favored prolonged shelf life and improvement of the antioxidant system during ripening of fruits such as apples (Wills et al., 2016), plums (Mahmoudi et al., 2022), and persimmon (Nars et al., 2022).

At retail outlets (street markets), not all traders have facilities for cold storage, and considering the perishability of papaya, especially under ambient conditions, and the potential for exploitation of amino acids in the postharvest chain, this study aimed to evaluate the effect of arginine spraying as a strategy to control ripening and preserve the quality characteristics of papaya during storage at room temperature.

MATERIALS AND METHODS

Plant material

Papayas of the Solo group, cultivar ‘Hawaii’ were harvested physiologically ripe in a commercial orchard located in the city of Santa Izabel, Pará, Brazil. At harvest, fruits at maturity stage 1 were considered, that is, with the epicarp completely green, free of physiological defects or affected by pests and diseases. These were wrapped in bubble wrap to avoid mechanical damage during transportation and taken in plastic boxes to the Postharvest Quality Laboratory of the Socio-Environmental Institute of Science and Technology of the Amazon (ISACTA), Belém, Pará, Brazil.

Postharvest treatments and storage

In the laboratory, the fruits (200 units) were sanitized in a chloride solution (150 mg.L^{-1}) for 10 minutes, rinsed in running water, and allowed to dry under ambient conditions. Subsequently, they were randomly divided into two batches placed on masonry benches, and subjected to the following treatments. One batch of fruits (100 units) represented the control treatment and was sprayed with distilled water, the other batch (100 units) was sprayed with an arginine solution at 25 mg.L^{-1} (Sigma Aldrich) was selected after preliminary tests with concentrations ranging from 5 to 50 mg.L^{-1}, where concentrations lower than 25 mg.L-1 had no potential effect on ripening, and when higher than 35 mg.L^{-1}, they negatively affected the cell structure (softening). Both spray solutions (control and arginine) contained Tween 20 at 0.1 g.L^{-1} (Sigma Aldrich) as a wetting agent. After spraying, the fruits were kept under ambient conditions (28 ± 2 °C and 85 ± 5 % relative humidity) for 7 days.

Physicochemical quality assessment

The fruits of each treatment were assessed daily (in triplicate) for:

***Respiratory activity and ethylene production*:** For the determinations, 10 fruits with known mass (average 584.11 g) were individually placed in hermetic glass containers with a capacity of 2000 mL, previously exposed to the temperature and humidity conditions of the experiment. One hour after closing the vials, 1 mL gas samples were collected from the vials through a silicone septum, using a syringe suitable for chromatography (Hamilton, Gastight, Nevada, USA). The gas samples were analyzed in a Thermo Finnigan Trace 2000GC gas chromatograph. The chromatograph was equipped with a 2 m long Porapack N capillary column set at 80 °C, with hydrogen as carrier gas (40 mL min^{-1}). A methanator at 350 °C was used for respiration (CO_2) analyses. Gas samples were analyzed by a flame ionization detector at 250 °C. Respiration and ethylene production were determined by the difference between the initial (when the vials were closed) and final (after 1 h) gas concentrations, expressed in mL CO_2 kg^{-1} h^{-1} and µL C_2H_4 kg^{-1} h^{-1}, respectively.

***Fresh mass loss*:** Determined by weighing the fruits on an analytical scale (Mars, model AS 2000, São Paulo, Brazil) at the beginning of the experiment (initial weight) and again at each period of exposure to ethanol vaporization (final weight). The results were expressed as a percentage (percentage) based on the following equation (1):

FML (percentage) = Initial mass - Final mass / Initial mass × 100 (1)

***Firmness*:** It was determined with a digital penetrometer (53200 TR Turoni, Italy), taking 2 readings on opposite sides in the region of greatest diameter of the fruits. A thin layer of the skin was removed from the papayas, then it was placed firmly on the bench and the 8 mm tip was inserted with constant force until the fruit was pierced. The results were expressed in Newtons (N).

Peel and pulp color: Determined with the aid of a colorimeter (Minolta CR-300, Osaka, Japan), taking two readings per fruit in the region of greatest diameter of the fruits. The results were expressed in hue angle (h°).

***Total soluble solids*:** To determine total soluble solids (TSS), approximately three drops of fruit pulp juice were evaluated in a digital refractometer with automatic temperature correction (Atago PR-101, Atago Co Ltda., Tokyo, Japan). The results were expressed in ºBrix (AOAC, 2020).

Titratable acidity: To determine titratable acidity (TA), approximately 10 g of fruit pulp juice were taken and 90 mL of distilled water added. Titration was performed with 0.1 M sodium hydroxide (NaOH) solution to pH 8.1. The results were expressed in g of citric acid per 100 g of pulp (AOAC, 2020).

pH: Determined in 10 g of juice using a portable Gehaka potentiometer, model PG2000, and the results expressed in pH units.

TSS/TA ratio**:** Determined by the quotient between the TSS and the TA content.

Vitamin C content**:** To determine the ascorbic acid content, approximately 5 g of the pulp was homogenized with 25 mL of 1% oxalic acid, followed by centrifugation at 4,000 xg for 10 minutes. The collected extract (1 mL) was diluted in oxalic acid (4 mL) and titration was performed with a 2,6-dichlorophenol-indophenol solution (AOAC, 2020), and the results were expressed in mg of ascorbic acid per 100 g of pulp.

Lycopene content: Determined according to the methodology described by Sadler et al. (1990) with modifications. The sample (1.0 g) was homogenized with 50 mL of the hexane/acetone/ethanol solution (2:1:1, v/v/v) and left under stirring for 30 minutes. The homogenate was then transferred to a separatory funnel and 10 mL of distilled water was added. The solution was separated into a polar fraction (35 mL) and a nonpolar fraction (25 mL), the latter containing lycopene. The extract was collected and the absorbance of the solution in hexane was measured at 472 nm. The conversion of absorbance into lycopene concentration was based on the extinction coefficient of 3.450 specific for the pigment in hexane and the results were expressed in $\mu g.g^{-1}$ of sample.

Experimental design and statistical analysis

The experiment was conducted in a completely randomized design (CRD) under a 2×8 factorial arrangements, with two treatments (spraying with and without arginine) and eight evaluation times (0, 1, 2, 3, 4, 5, 6 and 7 days) with three replicates and the experimental plot composed of 3 fruits. The data were submitted to ANOVA and the means compared with each other by the Tukey test at the 5% probability level using the R software.

RESULTS

Treatment of fruits with arginine resulted in lower respiratory activity (CO_2) and ethylene production (C_2H_4) compared to untreated fruits (control). Considering the storage period, the peak production of CO_2 and C_2H_4 occurred at 4 days in the control fruits (61.1 mL CO_2 kg^{-1} h^{-1} and 6.93 µL C_2H_4 kg^{-1} h^{-1}) and at 6 days in those treated with the arginine (66.5 mL CO_2 kg^{-1} h^{-1} and 6.41 µL C_2H_4 kg^{-1} h^{-1}) (Fig. 1A and B), respectively.

The mass loss was 24.97% greater in the control fruits compared to those sprayed with arginine, especially after the 3rd and 5rd day of storage, respectively (Fig. 2A). Firmness reduced by 85.30% compared to the initial day, especially in the control fruits after 4 days of storage. In the papayas sprayed with arginine, the most significant reduction occurred after the 5rd day, but considering the entire storage period, they were 56.25% firmer than the control fruits (Fig. 2B).

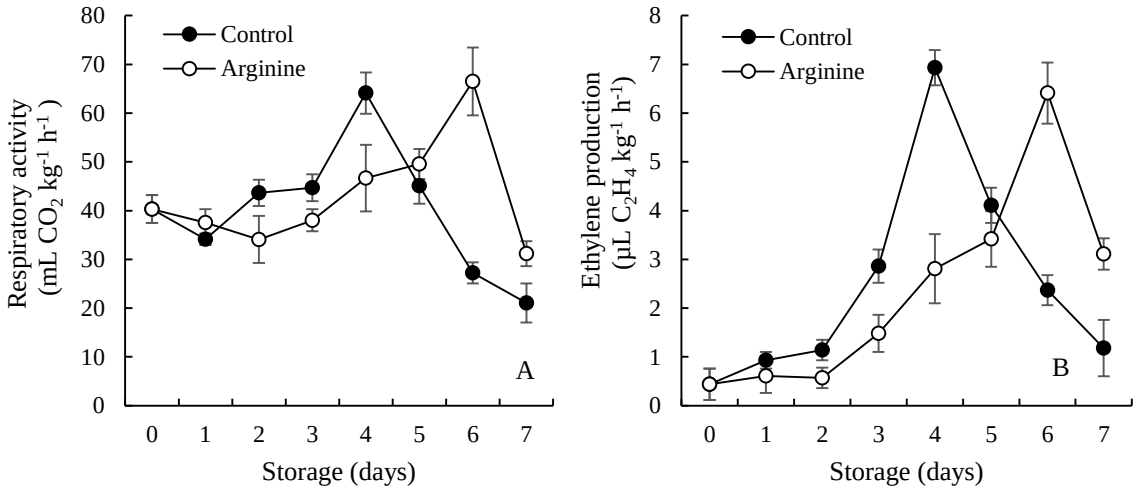

Fig. 1. Respiration and ethylene production in 'Hawaii' papayas sprayed or not with arginine and stored at room temperature (28 ±1 ºC) with 85-90% RH for 7 days.

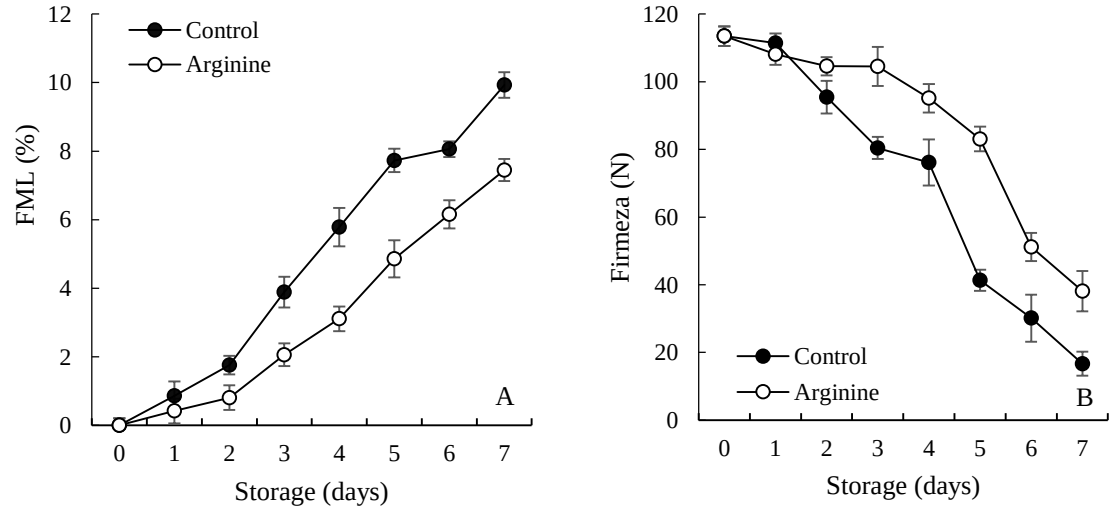

Fig. 2. Fresh mass loss (A) and firmness (B) in 'Hawaii' papayas sprayed or not with arginine and stored at room temperature (28 ±1 ºC) with 85-90% RH for 7 days.

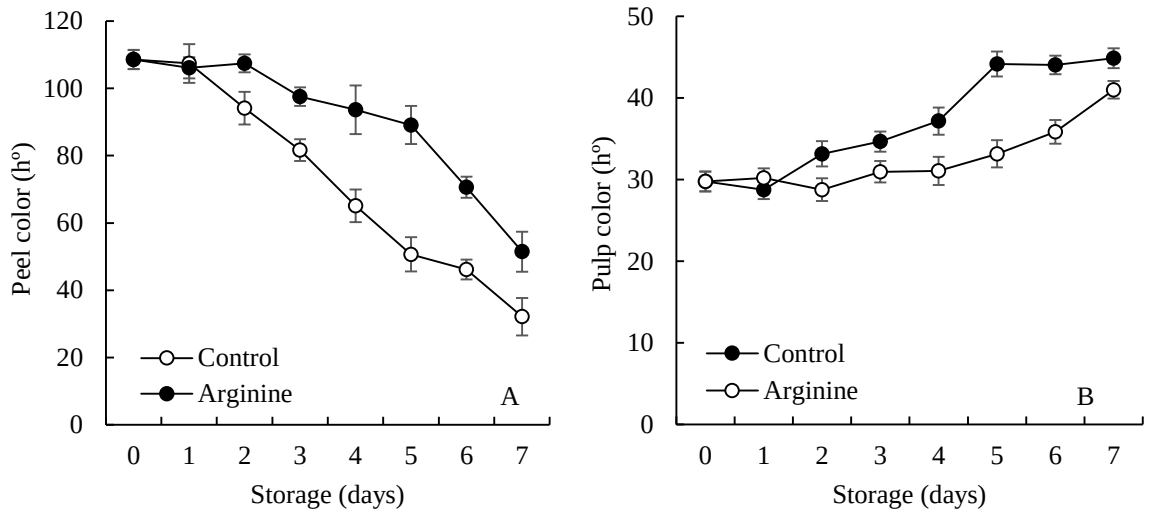

Fig. 3. Peel color (h°) (A), and pulp color (B) in 'Hawaii' papayas sprayed or not with arginine and stored at room temperature (28 ±1 ºC) with 85-90% RH) for 7 days.

Regarding skin color, untreated fruits showed color change at 2 days (yellow lines) with a change from green to yellow in the order of 13.36% in relation to the initial day, while for the same period, the change was only 1.12% in papayas sprayed with arginine, indicating greener fruits. At 6 days, the control fruits were completely ripe (yellow) with an average value of 46.13 hº, while the same value (51.47 hº) was only observed at 7 days in fruits sprayed with arginine (Fig. 3A, Fig. 7). In the pulp, there was an increase in average values with storage time; however, the control fruits showed higher values, mainly from the 4th day (44.16 hº), while in those treated with arginine, this increase was only significant after the 6th day (41.01 hº) (Fig. 3B, Fig. 7).

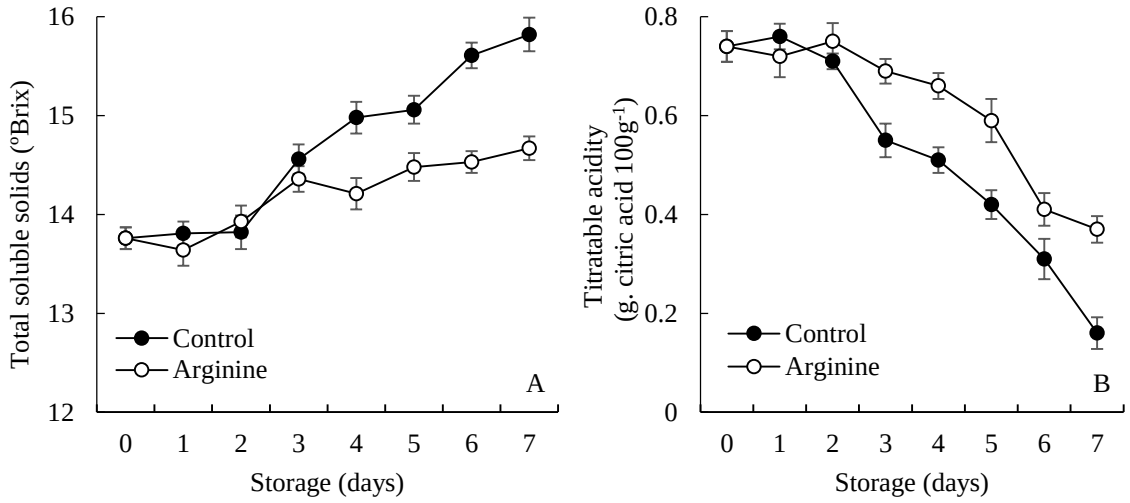

Fig. 4. Total soluble solids (A) and titratable acidity (B) in 'Hawaii' papayas sprayed or not with arginine and stored at room temperature (28 ±1 ºC) with 85-90% RH for 7 days.

There was little variation in the total soluble solids (TSS) content during storage, especially in papayas treated with arginine, whose increase was only 6.02% in relation to the initial day when compared to 13.21% in the control fruits (Fig. 4A). Regarding titratable acidity (TA), there were decreases in the average values with storage time, especially in untreated fruits immediately after the 2rd day of evaluation, with an average value of 0.16 g of citric acid 100 g-1 after 7 days. In papayas sprayed with arginine, the most significant reduction only occurred after 4 days of storage, and the average content was 56.75% higher in relation to the control fruits on the last day of evaluation (Fig. 4B).

The ratio (TSS/TA) increased considerably during storage, regardless of the treatment. In the control fruits, this increase was around 77.74% and was more pronounced from the 3rd day onwards, while in those sprayed with arginine, the increase was 70.84%, especially from the 5rd day of storage (Fig. 5A). The average pH values increased by 22.93% over the storage time. This increase was more evident in the control fruits after 4 days, reaching an average value of 6.23 after 7 days. On the other hand, in the papayas sprayed with arginine, stability was observed between day 0 (4.67) and the 4rd day of storage (4.81), followed by an increase and an average value of 5.89 after 7 days (Fig. 5B).

The vitamin C content increased by 21.85% during storage. In the control fruits, the maximum peak was obtained at 6 days (71.15 mg.100 g^{-1}) with subsequent stability. In the fruits sprayed with arginine, the highest content (64.43 mg.100 g^{-1}) was observed at 7 days, approximately 7.74% lower than in the untreated fruits (Fig. 6A). Regarding the lycopene content, an increase of 53.55% occurred in the control fruits between day 0 (11.56 µg.g^{-1}) and the fifth day of storage (24.89 µg.g^{-1}), followed by a decline. On the other hand, a gradual increase was observed until the 4rd day in the papayas sprayed with arginine (14.62%), followed by the rise and highest peak (25.17 µg.g^{-1}) at 7 days of evaluation (Fig. 6B).

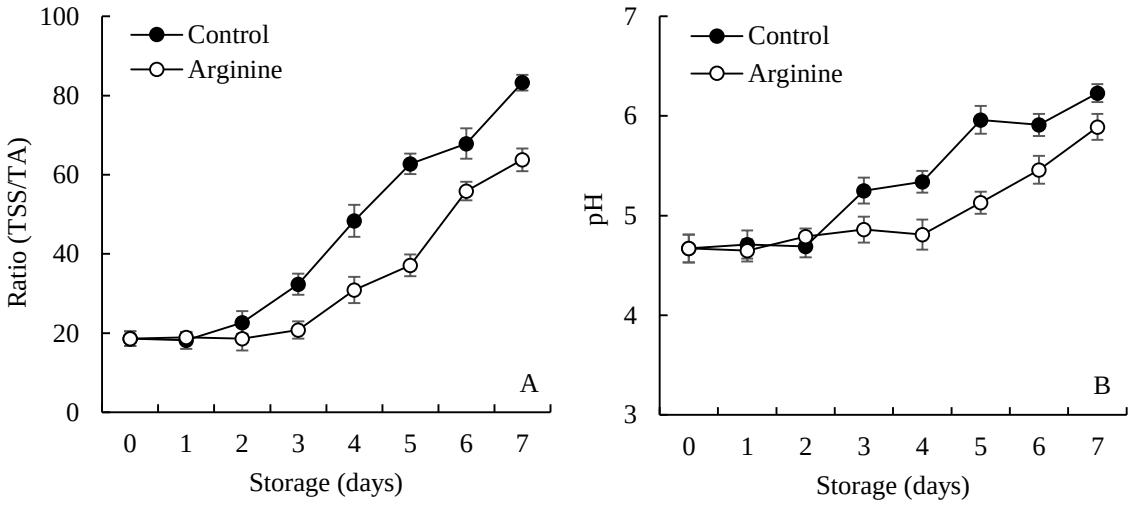

Fig. 5. TSS/TA ratio (A) and pH (B) in 'Hawaii' papayas sprayed or not with arginine and stored at room temperature (28 ±1 ºC) with 85-90% RH for 7 days.

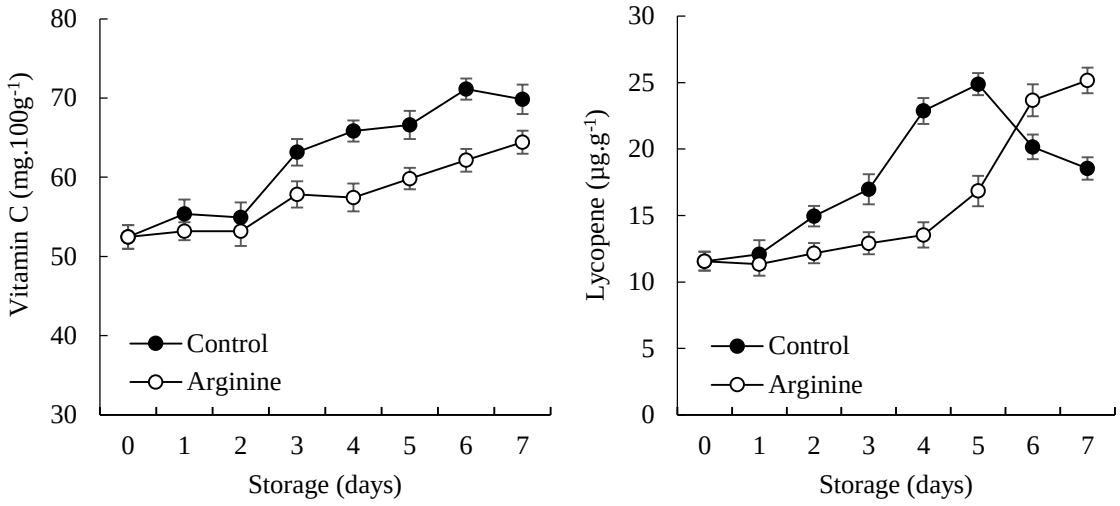

Fig. 6. Vitamin C (A) and lycopene (B) in 'Hawaii' papayas sprayed or not with arginine and stored at room temperature (28 ±1 ºC) with 85-90% RH for 7 days.

DISCUSSION

In postharvest, controlling respiratory activity is the main strategy to delay the physiological (transpiration) and biochemical (ethylene production) transformations that induce ripening. In this study, the delay in ripening characterized by lower respiratory activity and ethylene production in papayas sprayed with arginine (Fig. 1A and B) may be associated with both the role of this arginine in serving as a reserve source of nitrogen for plants, thereby regulating fruit senescence (Zhang et al., 2014), as well as with the biosynthetic capacity of molecules such as nitric oxide and polyamines that in their free forms may have anti-ripening and senescence properties (Gao et al., 2009).

Physiological water loss is one of the many postharvest disorders in the fresh fruit industry and is usually associated with transpiration and storage conditions. Generally, most vegetables lose quality when they reach a water loss greater than 5% of their initial weight, favoring processes such as wilting that leads to loss of commercial weight and fruit texture, induction of browning, reduction of flavor, acceleration of senescence and greater fluidity of the cell membrane that becomes more susceptible to attack by microorganisms and *chilling injury* (Lufu et al., 2020). In this study, this effect on quality loss could be observed in control fruits that presented mass loss greater than 5% after 4 days of storage, corroborated by the reduction in firmness. On the other hand, the delay in ripening caused by arginine treatment allowed a lower transpiration rate resulting in less mass (water) loss and consequent greater firmness during storage (Fig. 2A and B).

In climacteric fruits, the color change characterized by the loss of chlorophyll and synthesis of pigments (carotenoids, flavonoids) marks the beginning of ripening. In this study, there was a delay of 1 day for the onset of visual changes in the color of the peel and pulp and up to 2 days for the complete ripening of papayas sprayed with arginine compared to control fruits (Fig. 3A, B and C). Similarly, treatment by immersing tomatoes in an arginine solution significantly delayed the change from green to red during storage (Yu et al., 2024).

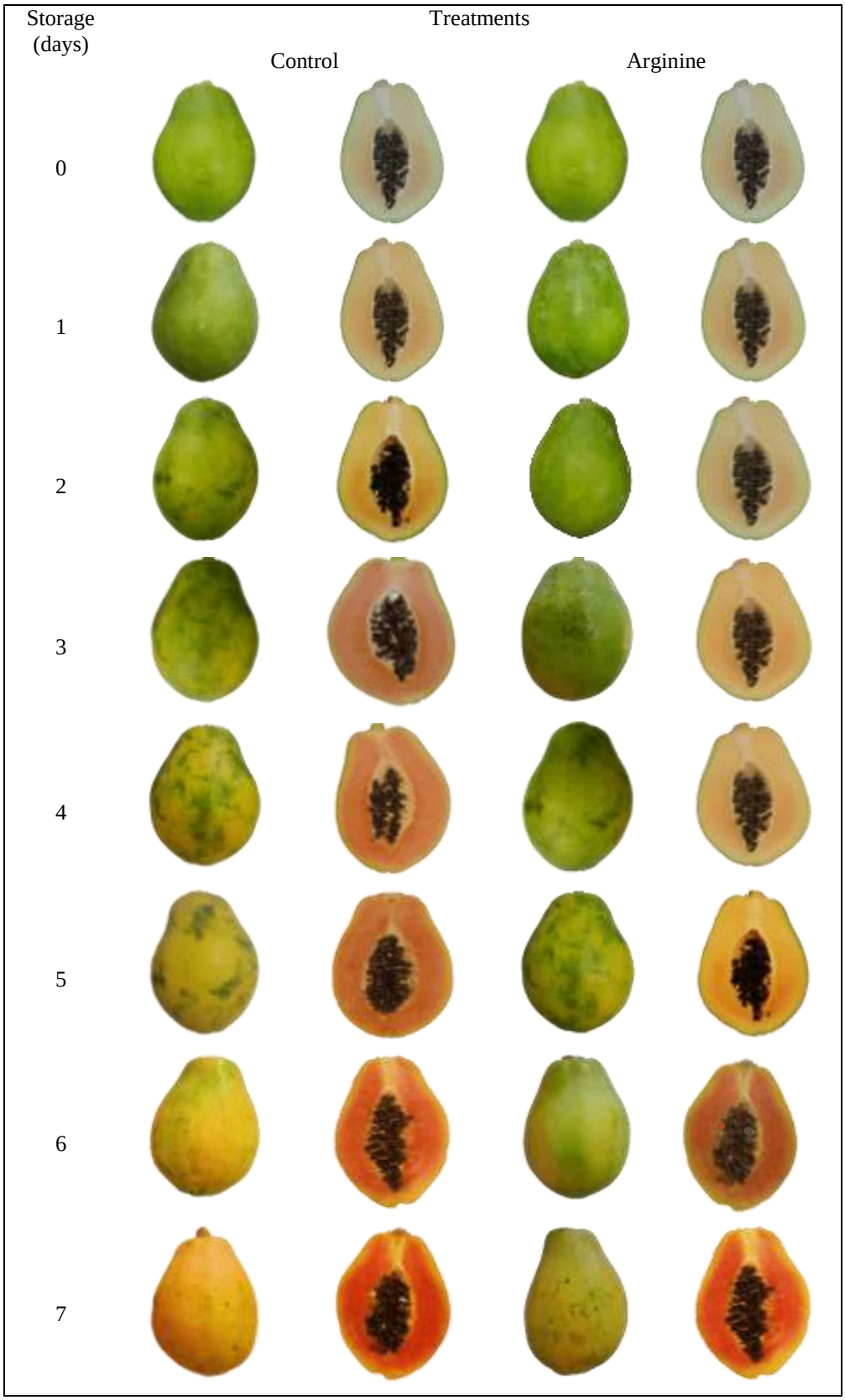

Fig. 7. Peel and pulp appearance quality of 'Hawaii' papayas sprayed or not (control) with arginine and stored at room temperature (28 ±1 ºC) with 85-90% RH for 7 days.

Ripening also promotes physicochemical changes, such as the synthesis and degradation of sugars and organic acids, which directly impact the flavor and aroma of the fruits. Normally, the accumulation of sugars (TSS) is linked to the degradation of some compounds that make up the cell wall (starch), for example, while the reduction of organic acids (TA) is explained by their use as substrates in respiratory metabolism and as a carbon source for the synthesis of new compounds, especially the volatile compounds responsible for aroma (Sanches et al., 2021). The balance of the TSS/TA ratio will define the flavor, since the higher the SS content and the lower the TA content, the better the flavor ratio. However, spraying papayas with arginine resulted in less TSS accumulation (Fig. 4A), in the degradation of organic acids (Fig. 4B), in the pH variation, and the flavor ratio of the fruits (Fig. 5A and B), respectively. These results corroborate the effect of this amino acid in delaying ripening, without compromising quality during storage. In broccoli (Sun et al., 2024) and tomatoes (Yu et al., 2024), treatment with arginine (5 mM) suppressed the activities of the main enzymes involved in ethylene biosynthesis, delaying ripening, TSS accumulation, and TA degradation, resulting in a longer shelf life. Furthermore, the continuous increase in TSS and reduction in TA and the better flavor balance (TSS/TA) indicate that arginine does not interfere with the sensory quality of papaya during storage.

The vitamin C (AsA) content of the fruit depends on many factors, including cultivar, ripening stage, orchard management, harvest time, and acidity. In addition, the duration and conditions of postharvest storage influence the AsA content even before processing (Arabia et al., 2024). Normally, the AsA content decreases during storage, since as an antioxidant molecule, L-ascorbic acid is used as a reducing agent to decrease the oxidative effect caused by reactive oxygen species (ROS) produced by respiratory metabolism. Thus, the higher the ripening stage, the lower the AsA content (Macedo et al., 2023). However, in this study, the increase in AsA content was proportional to the advancement in ripening (Fig. 6A), corroborating the literature reports that ascorbic acid biosynthesis occurs in papayas during storage (Selvaraj et al., 1982; Costa et al., 2010; Mendy et al., 2019). One of the AsA biosynthesis pathways occurs through the D-galacturonic acid route, an important compound that acts in the degradation of cell wall pectins. In this case, considering that ripening is a metabolic process that leads to senescence, the increase in AsA content in papayas may be the result of modification of the cell wall that would provide substrate for AsA biosynthesis, corroborated by the loss of fruit firmness (Fig. 2B) during storage. In this sense, the lower accumulation of AsA in fruits treated with arginine (Fig. 6A) is a reflection of the ripening control exerted by this molecule on fruit metabolism.

Lycopene is the most abundant carotenoid found in papaya pulp and, although it does not have vitamin A activity, it contributed to the red color of the fully ripe fruit, corroborated by the increase in concentration and color with storage time (Fig. 6B). In papayas sprayed with arginine, the lower accumulation of lycopene is related to the delay in ripening, that is, in the synthesis of the red color that was only achieved 2 days later in relation to the control fruits (Fig. 6B). In this study, the lycopene content (11.56 - 25.17 $\mu g.g^{-1}$) was close to those reported by Laurora et al. (2021) in 'Hawaii' papaya, whose levels ranged from 10.89 (green) to 27.70 $\mu g.g^{-1}$ (ripe), suggesting that arginine does not interfere in the biosynthesis of this pigment.

CONCLUSION

Arginine treatment via spraying is a promising, accessible and low-cost technology to delay the ripening of Hawaii papaya (2 days), considering the physicochemical quality aspects, such

as delay in the climacteric peak, less mass loss, greater firmness, less evolution of the yellow color, less accumulation of TSS, vitamin C and degradation of lycopene.

Conflict of interest

The authors have no conflict of interest to report.

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Recent advances in application of edible coatings for temperate fresh/fresh-cut fruits: a review

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ABSTRACT

Purpose: Temperate fruits not only provide essential nutrients but also contribute to the diversity and sustainability of horticultural production systems worldwide. The total production of fruits, increased during the past twenty years. However, postharvest losses of fruits due to spoilage, decay, and physiological deterioration pose a significant challenge to the global food supply chain, which leads to a decline in fruit quantity and quality after harvest. **Findings:** Edible coatings have emerged as a sustainable solution for extending the shelf life of fruits while reducing postharvest losses. The use of edible coatings is not only environmentally friendly but also addresses consumer demands for natural, safe, and healthy food products obtained through minimal processing. A wide array of edible coating materials is available, each possessing unique properties that influence their effectiveness in preserving fruits. The specific composition and application of edible coatings play a crucial role in their effectiveness in inhibiting microbial growth, reducing enzymatic browning, and maintaining the sensory quality of the fruits. **Limitations:** No limitations were found. **Directions for future research:** Future research should focus on exploring and developing new, sustainable, and biodegradable coating materials derived from renewable sources. Additionally, incorporating nanotechnology into edible coatings can enhance their properties, such as improved barrier properties, controlled release of active compounds, and enhanced antimicrobial activity. Continued research and innovation in this area hold significant promise for reducing postharvest losses, improving food security, and promoting sustainable agricultural practices. This review summarizes recent advances in different edible coating materials and their uses in prolonging shelf life and decreasing postharvest losses of important temperate fresh/fresh-cut fruits worldwide.

INTRODUCTION

Fruits are vital in human diet, rich in nutrients. Fruits play a crucial role in human health due to their rich content of essential nutrients and bioactive compounds. They are abundant sources of dietary fiber, antioxidants, phytochemicals, vitamins (such as vitamin C, thiamine, niacin, pyridoxine, folic acid), and minerals, all of which are vital for maintaining optimal health and preventing chronic diseases (Alemu, 2024). The consumption of fruits has been linked to various health benefits, including improved physical and mental health, preventing of non-communicable diseases like cardiovascular disease, diabetes mellitus, and certain cancers, and protection against neurological disorders (Jideani et al., 2021). The antioxidant components in fruits are typically credited for their protective impact.

The demand for fresh produce and fresh-cut fruits has significantly increased in recent years due to consumer preferences for convenient, healthy, and ready-to-eat products (Liyanapathiranage et al., 2023; Firdous et al., 2023). Consumers are attracted to fresh fruits for their freshness, nutritional value, safety, and overall eating experience, driving the growth in this market segment. Various emerging technologies such as active packaging, natural preservatives, and physical treatments have been developed to extend the shelf life of fresh/fresh-cut fruits while maintaining their quality and properties (Palumbo et al., 2022).

Global fruit production is increasing due to the demand of the world population, improving standards of living, and growing health consciousness in fruit consumption. Within a decade, the total production of temperate fruits, berries, citrus and tropical fruits significantly increased (Maringgal et al., 2020). However, postharvest losses of fruits due to spoilage, decay, and physiological deterioration pose a significant challenge to the global food supply chain, which leads to a decline in fruit quantity and quality after harvest. These losses result in economic losses for producers, retailers, and consumers; and contribute to food waste and environmental degradation. Losses can be caused by improper handling and storage, improper packaging, and microbial and fungal infections (Sanches & Repolho, 2022; Moradinezhad & Ranjbar, 2023). It is estimated that about 20-50% of fresh fruit produced losses at different postharvest stages (Nabi et al., 2017).

In recent years, a variety of strategies and methodologies have been established for the management of postharvest losses. One technique that shows potential in decreasing the respiration rate and prolonging the shelf life of fruits is modified atmosphere (Mitcham et al., 2023). The fundamental concept of this approach involves altering the gas composition surrounding the fruit through the manipulation or removal of gases essential for respiration (Moradinezhad et al., 2013). By creating an unsuitable environment, this method hinders the ripening process by modifying the internal gas configuration, decelerating the fruit's metabolic processes, thus retarding senescence and restraining microbial proliferation. Another practical and effective approach is the utilization of low temperatures for fruit preservation immediately postharvest and throughout storage. While maintaining fruits at reduced temperatures can uphold quality over extended periods, it also has the potential to induce chilling injury if storage conditions drop below recommended thresholds (Baloyi et al., 2023). Similar to modified atmosphere techniques, edible coatings serve as a protective layer on the product’s surface to impede gas exchange and halt the ripening process, thereby conserving the quality of the fruits. This methodology has demonstrated efficacy in numerous research investigations (Guimarães et al., 2021).

In this paper, a review of the advances made in the field of coating technology and its impact on the postharvest life of fruits is presented, highlighting the importance of preserving fruits and protecting them from spoilage by using novel techniques. The study will explore

different kinds of edible coating materials and their uses in prolonging shelf life and decreasing postharvest losses of important temperate fresh/fresh-cut fruits worldwide.

Edible coatings properties and applications

Edible coatings have emerged as a sustainable solution for extending the shelf life of fruits and vegetables while reducing food waste and postharvest losses. Edible coatings are thin layers of biodegradable natural polymers applied to food surfaces to enhance quality, shelf life, and reduce post-harvest losses (Perez-Vazquez et al., 2023). These coatings, made from polysaccharides (alginate, cellulose, chitosan, gums, pectin, and starch), proteins (gelatin, gluten, and zein), and lipids (fatty acids and waxes), act as a barrier against moisture loss, microbial growth, and oxidation, extending the shelf life of fresh-cut fruits and vegetables (Aaqil et al., 2024). By incorporating essential oils or nanoparticles, edible coatings can further improve physicochemical properties and provide antioxidant or antimicrobial effects (Kanwar et al., 2024). The use of edible coatings is not only environmentally friendly but also addresses consumer demands for natural, safe, and healthy food products obtained through minimal processing. Moreover, coatings present an environmentally sustainable substitute for conventional non-biodegradable packaging substances, thereby facilitating the mitigation of plastic waste and enhancing methods of food preservation (Pandya et al., 2023).

A wide array of edible coating materials is available, each possessing unique properties that influence their effectiveness in preserving fruits. This section provides an overview of various commonly employed edible coating materials, including their properties and applications in fruit preservation.

Chitosan

Chitosan, a biopolymer derived from chitin, is a versatile edible coating material with excellent film-forming and antimicrobial properties. Its cationic nature allows it to interact with the negatively charged cell walls of microorganisms, inhibiting their growth and preventing spoilage. Chitosan coatings also exhibit good barrier properties against gases and moisture, contributing to the preservation of fruit quality. Chitosan, exhibits unique properties such as biocompatibility, biodegradability, and non-toxicity, making it a promising material for various applications (El-Araby et al., 2024). The molecular weight and degree of deacetylation of chitosan play crucial roles in determining its physicochemical and biological characteristics. Chitosan can be sourced from different organisms like crustaceans, fungi, and insects, with commercial production mainly from crustaceans (Hemmamı et al., 2024). Its applications span industries, including healthcare, agriculture, cosmetics, and wastewater treatment, with recent advancements focusing on chitosan derivatives and nanocomposites to enhance its efficiency and broaden its utility (Román-Doval et al., 2023). In the biomedical field, chitosan has been extensively explored for drug delivery systems, tissue regeneration, gene delivery, and vaccination, showcasing its potential in various biomedical applications (El-Araby et al., 2024).

Carboxymethylcellulose (CMC)

Carboxymethylcellulose (CMC), a cellulose derivative, is a water-soluble polysaccharide widely used as a thickener and stabilizer in food applications. It exhibits excellent film-forming properties, forming strong and flexible coatings that can effectively reduce fruit moisture loss. CMC-based coatings also contribute to the preservation of fruit quality by retarding gas exchange and microbial growth. CMC plays a significant role in various food-related applications. Studies have shown that CMC is widely used in the food industry due to its safety approvals by regulatory bodies like the European Food Safety Authority (EFSA)

and the Food and Drug Administration (FDA) (Yildirim-Yalcin et al., 2022). CMC is utilized in edible film production, contributing to the recycling of food waste and promoting sustainability and biodegradability in food packaging (Costa et al., 2023). Furthermore, CMC has been incorporated into composite films with nanoparticles like TiO_2 to enhance antimicrobial properties, providing an alternative to traditional antibiotics in combating microbial resistance in food products (Elmehbad et al., 2024). Additionally, CMC has been explored for its potential in agriculture, offering solutions to challenges such as water absorption and fruit preservation postharvest (Yildirim-Yalcin et al., 2022). Overall, CMC's versatility and beneficial properties make it a valuable component in food technology, packaging, and agricultural practices.

Alginate

Alginate, a natural polysaccharide extracted from seaweed, is a widely used edible coating material. It forms strong and flexible films that can effectively reduce moisture loss and retard gas exchange in fruits (Luna-Zapién et al., 2023). Alginate coatings also exhibit good antimicrobial properties, inhibiting the growth of spoilage microorganisms and extending the shelf life of fruits. Alginate-based edible coatings have been extensively studied for their potential in enhancing post-harvest handling of various food commodities. These coatings can improve product quality by reducing mass loss and preserving color (De Simone et al., 2024). Incorporating probiotic *Lactiplantibacillus plantarum* strains into alginate coatings has shown promise in extending the shelf life of fruits like table grapes by controlling harmful microorganisms and reducing decay, thus enhancing safety and quality (De Simone et al., 2024). Additionally, alginate coatings combined with whey protein and curcumin have demonstrated excellent UV barrier properties, reduced water vapor transmission rates, and extended the shelf life of apples by suppressing respiration and moisture loss, ultimately reducing enzymatic browning and weight loss (Botalo et al., 2024). Overall, alginate-based edible coatings offer a versatile and effective solution for improving the post-harvest management and quality of various food products.

Zein

Zein, a protein extracted from corn, is another prominent edible coating material. Its hydrophobic nature makes it suitable for creating water-resistant coatings that can effectively prevent moisture loss from fruits. Zein coatings also exhibit good barrier properties against gases, further contributing to fruit preservation. Additionally, zein's ability to encapsulate bioactive compounds, such as antioxidants, enhances the nutritional value of coated fruits. Zein, has gained attention for its eco-friendly and versatile properties, making it an ideal candidate for edible coatings and films in the food industry (Egea et al., 2022). Studies have shown that zein-based coatings can effectively maintain the quality of fruits like "Granny Smith" apples by reducing weight loss and microbial contamination, thus extending their shelf life (Belay et al., 2023). Additionally, zein coatings have demonstrated good barrier properties against gases, light, and water, along with antimicrobial effects, which can further enhance the preservation of food products. Furthermore, the integration of essential oils with zein in coatings has demonstrated encouraging outcomes in the suppression of microbial proliferation on fruits such as dates, underscoring the potential of zein in food packaging (Salajegheh et al., 2020). Overall, zein-based edible coatings present a sustainable and secure solution for prolonging the shelf life of diverse food products while preserving their safety and quality.

Pectin

Pectin, a naturally occurring polysaccharide extracted from fruits, is a widely used edible coating material. It exhibits excellent film-forming properties, owing to its ability to form gels in the presence of sugars and acids. Pectin-based coatings effectively reduce water loss and retard oxygen permeability, contributing to moisture retention and delayed ripening in fruits. Moreover, pectin's biodegradability and low toxicity make it an attractive choice for food packaging. Pectin-based edible coatings offer a promising solution for preserving food quality by enhancing shelf-life characteristics and providing a protective barrier against moisture loss and gas exchange (Freitas et al., 2021). These coatings, originating from natural polymers such as pectin, can be enhanced with bioactive components like essential oils to boost antioxidant attributes and structural qualities, thereby prolonging the shelf life of fruits and vegetables (Nastasi et al., 2022). By incorporating elements like protein hydrolyzate, pectin films can exhibit enhanced biodegradability, moisture resistance, and antioxidant capacity, making them ideal for food packaging applications (Freitas et al., 2021). Additionally, using pectin in edible coatings can lead to the development of composite materials with improved physicochemical properties, offering a natural and environmentally friendly alternative to traditional food preservation methods (Rohasmizah & Azizah, 2022).

Gums

Gums are complex carbohydrate molecules which have the ability to bind water and form gels at low concentration. These carbohydrates are often associated with proteins and minerals in their structure. Gums are of various types such as seed gums, exudate gums, microbial gums, mucilage gums, seaweeds gums, etc (Barak et al., 2020). Edible coatings made from natural gums like tragacanth, guar gum, almond gum, Arabic gum, and zedo gum have demonstrated encouraging outcomes in the conservation of the characteristics of different types of fruits and vegetables during storage (Zare-Bavani et al., 2024; Yazıcıoğlu, 2023; Rasool et al., 2023; Jahanshahi et al., 2023). These coatings have proven effective in mitigating weight loss, maintaining firmness, and improving color retention, and delay deterioration processes such as proteolysis and microbial growth, ultimately extending the shelf life of coated products. For example, coatings with almond gum have demonstrated superior physicochemical properties compared to synthetic gums, leading to better quality retention in pineapples (Rasool et al., 2023). Additionally, formulations incorporating aloe vera, starch, and Arabic gum have proven effective in prolonging the longevity of bananas by reducing the rate of chlorophyll breakdown and preserving their firmness (Tchinda et al., 2023). Overall, these natural gum-based edible coatings offer a sustainable and efficient solution for improving post-harvest quality and marketability of various perishable products.

Aloe vera gel

Aloe vera gel, extracted from the aloe vera plant, is a natural moisturizer with antimicrobial properties. It is often incorporated into edible coatings to enhance their moisturizing and antimicrobial effects. Aloe vera gel can improve the flexibility and barrier properties of coatings, contributing to better fruit preservation (Ahmed, 2024). Aloe vera gel has been extensively studied for its potential as an edible coating material to enhance food preservation and quality. Research has shown that incorporating aloe vera gel into edible coatings can effectively retard lipid oxidation, improve quality characteristics, and extend the shelf life of various food products, such as apples (Suhartatik & Karyantina, 2023), figs, and tomatoes (Tobing et al., 2024). The combination of aloe vera gel with other materials like chitosan has been found to enhance film-forming abilities, rheological properties, antioxidant effects, and microbial growth inhibition, making it a promising bio-based solution for food packaging and

preservation (Tobing et al., 2024). Additionally, using aloe vera gel has shown to increase public awareness and skills in utilizing natural coatings for fruits like strawberries (Álvarez-Barreto et al., 2023), contributing to sustainable food preservation practices.

Essential oils (EOs)

Essential oils (EOs) are volatile aromatic liquids extracted from various parts of aromatic plants, containing compounds like flavonoids, terpenoids, and phenylpropanoids, which contribute to their diverse medicinal properties such as antimicrobial, antioxidant, anti-inflammatory, and antiviral effects (Tiwari et al., 2023; Kahawattage et al., 2023). EOs have gained attention as green alternatives to synthetic chemicals due to their safety and effectiveness in inhibiting the growth of mycotoxigenic fungi and eliminating mycotoxins, making them valuable in food preservation (Tiwari et al., 2023). Essential oil edible coatings have shown significant potential in extending the storage life and enhancing the quality of different horticultural crops. Studies have highlighted the effectiveness of essential oil-based coatings in preserving fruits like mandarins (Liguori et al., 2024) and vegetables (Perez-Vazquez et al., 2023). These coatings, often incorporating oils like oregano, thyme, and cinnamon, exhibit antimicrobial properties that inhibit microbial growth, thereby extending the product's freshness. Essential oil concentrations and types play a crucial role in determining the physicochemical properties of the coatings, affecting factors such as viscosity, color, and transparency (Perez-Vazquez et al., 2023). Overall, essential oil edible coatings offer a natural and effective solution for enhancing food quality and safety.

Calcium chloride ($CaCl_2$)

Calcium chloride ($CaCl_2$), an inorganic salt, is often used in combination with other edible coating materials to enhance their film-forming properties and improve their barrier properties. It interacts with polysaccharides, such as pectin and alginate, to form stronger and more rigid coatings that can effectively reduce moisture loss and retard gas exchange. Calcium chloride plays a significant role in various edible coating applications as highlighted in the provided research contexts. Studies have shown that $CaCl_2$ can be utilized in edible coatings to enhance the quality and shelf life of fruits by inhibiting fungal attacks and maintaining membrane stability (Moradinezhad et al., 2019, 2021; Dorostkar et al., 2022a). Furthermore, the combination of $CaCl_2$ with other ingredients like starch, Arabic gum powder, and garlicin in edible coating agents has been demonstrated to prolong the fresh-keeping period of fruits like *Pyrus bretschneideri* Rehd., showcasing the versatility and effectiveness of $CaCl_2$ in food preservation applications (Araujo et al., 2021). These findings indicate the valuable role of $CaCl_2$ in edible coatings for enhancing food quality, extending shelf life, and improving consumer satisfaction.

Mechanisms of action of edible coatings

Edible coatings function as a protective barrier separating the fruit from its surrounding environment, significantly influencing various physiological and biochemical processes contributing to fruit preservation. These mechanisms are multifaceted and involve a complex interplay of factors that ultimately contribute to extended shelf life, reduced decay, and improved quality attributes.

Moisture retention

Edible coatings are crucial in minimizing moisture loss from fruits during storage. This is primarily achieved through the formation of a semi-permeable barrier that limits the rate of water vapor diffusion from the fruit to the surrounding atmosphere. The coating's ability to

retain moisture depends on its composition, thickness, and the relative humidity of the storage environment (Pham et al., 2023). For instance, coatings enriched with polysaccharides like pectin and alginate exhibit excellent moisture-retention properties due to their hydrophilic nature. They form a gel-like matrix that traps water molecules, effectively preventing dehydration. This moisture retention is essential for maintaining fruit turgor, texture, and overall appearance.

Gas exchange regulation

The composition and structure of edible coatings influence the permeability of gases like oxygen and carbon dioxide, which are crucial for respiration and ripening processes in fruits. Some coatings, particularly those based on hydrophobic polymers like zein, act as barriers to oxygen diffusion, slowing respiration rates and delaying ripening (Liyanapathiranage et al., 2023). This controlled gas exchange helps to maintain the desired level of oxygen for fruit respiration while minimizing the accumulation of ethylene, a ripening hormone that accelerates senescence (Sun et al., 2022).

Microbial growth inhibition

Edible coatings can also provide an effective barrier against microbial contamination, a major cause of fruit spoilage. The coating material itself may possess antimicrobial properties, such as chitosan, which exhibits antifungal activity. Moreover, the coating has the ability to establish a hostile environment for microbial growth by changing the pH, oxygen levels, and nutrient composition surrounding the surface of the fruit (Leite et al., 2023). This antimicrobial effect helps to suppress the growth of bacteria, yeasts, and molds, extending the shelf life of fruits and reducing spoilage rates.

Apart from the mechanisms as mentioned earlier, edible coatings can also influence fruit preservation in other ways, including:

Nutrient Enrichment: Certain coatings can be formulated to incorporate beneficial nutrients, such as vitamins or antioxidants, enhancing the nutritional value of the coated fruit.

Antioxidant Activity: Some coating materials, like polyphenols, possess antioxidant properties that protect fruits from oxidative damage, preserving their color, flavor, and nutritional quality.

Sensory Properties: Edible coatings can influence the sensory attributes of fruits, such as their appearance, texture, and flavor, enhancing consumer appeal.

Applications and effectiveness of edible coatings in fruit preservation

Edible coatings have found diverse applications in fruit preservation, targeting various challenges associated with postharvest handling and storage (Liyanapathiranage et al., 2023). Some key applications include:

Shelf life extension: Edible coatings act as a barrier against moisture loss, gas exchange, and microbial invasion, effectively extending the shelf life of fruits by slowing down respiration and delaying ripening. Studies have demonstrated that coatings can significantly reduce the decay rate and extend the storage life of fruits like strawberries, blueberries, apples, and mangoes.

Reduction of decay: Edible coatings can inhibit the growth of microorganisms that cause decay, thus preventing spoilage and preserving the fruit's quality. Coatings containing antimicrobial agents, such as chitosan or essential oils, have been shown to reduce the incidence of fungal infections and bacterial contamination effectively.

Maintenance of quality attributes: Edible coatings can help preserve the sensory quality of fruits by preventing loss of moisture, color, flavor, and texture. They can also reduce the incidence of enzymatic browning, a common problem in fruits like apples and bananas.

The effectiveness of edible coatings in fruit preservation is influenced by several factors, including the type of coating material, the fruit variety, the storage conditions, and the application method (Firdous et al., 2023). Different coating materials exhibit varying degrees of effectiveness depending on their properties. For example, pectin-based coatings are known for their moisture retention and gas barrier properties, while chitosan coatings offer antimicrobial activity (Liyanapathiranage et al., 2023). The effectiveness of coatings can vary depending on the fruit variety. Some fruits are more susceptible to decay or moisture loss than others, requiring specific coatings for optimal protection. Temperature, humidity, and gas composition of the storage environment significantly affect the effectiveness of coatings. Optimal storage conditions can enhance the protective effects of the coatings. The application method can influence the uniformity and thickness of the coating, impacting its effectiveness. Dip coating, spray coating, and brush coating are commonly used methods.

Edible coatings for temperate fruits

Temperate fruits encompass a variety of economically important crops adapted to middle-latitude climates, requiring cold periods for dormancy and exhibiting winter hardiness (Awasthi, 2023). Examples of important temperate fruits include apple, pear, apricot, peach, plum, grape, and strawberry, which are widely cultivated and consumed globally. These fruits not only provide essential nutrients but also contribute to the diversity and sustainability of horticultural production systems worldwide.

This section explores the application of edible coatings on important temperate fruits (pome fruits, stone fruits, and small fruits) especially focusing on the effect of various postharvest coatings on fresh-cut fruits, analyzing their efficacy in inhibiting microbial growth, slowing down deterioration and extending shelf life. The literature that was used in this review obtained from original research and review papers mainly published during 2019 to the Middle of 2024.

Edible coatings for pome fruits

Apple (*Malus domestica*)

Edible coatings are crucial in extending the shelf life and maintaining the quality of apples postharvest. Various studies have examined the effectiveness of different edible coatings on apples, such as CMC, sodium alginate (SA), citric acid (CA), oxalic acid (OA) (Magri et al., 2023), zein, and zein combined with nisin (Belay et al., 2023). Magri et al. (2023) investigated the impact of edible coatings and the combination treatments containing CMC (1%), sodium alginate (1%), citric acid (1%), and oxalic acid (0.5%) on fresh-cut apple. Findings indicated that all the combination treatments enhanced the shelf-life of fresh-cut apple by retarding the qualitative postharvest deterioration, total soluble solids, and titratable acidity. Also, antioxidant activities and bioactive compounds of coated fresh-cut apples significantly increased. These coatings have shown promising results in reducing weight loss, delaying microbial decay, enhancing antioxidant enzyme activities, and regulating respiration rates, thus improving the overall quality and shelf life of the fruit. Additionally, incorporating active oxygen scavengers like ascorbic acid in chitosan-based coatings has been found to delay quality deterioration further and maintain fruit firmness (Wang et al., 2023). Fresh-cut apples are particularly vulnerable to enzymatic browning and microbial deterioration. Edible coatings have proven to be effective in prolonging their shelf life. Research has shown that coatings made from chitosan, pectin, and alginate can significantly curb enzymatic browning

and microbial proliferation (Najafi Marghmaleki et al., 2021), resulting in a notable decrease in quality degradation. These coatings can also help retain moisture and maintain firmness, improving overall sensory attributes (Sanchís et al., 2017). Overall, edible coatings present a sustainable and effective method for preserving apples during storage and transportation.

Pear (*Pyrus communis*)

Various studies have investigated the application of edible coatings to enhance the quality and shelf life of pears. Research has shown that using composite coatings like sodium alginate with ginger oil can effectively control physiological and microbiological activities in fresh-cut pears, extending their shelf life significantly (Lamani & Ramaswamy, 2023). Lamani and Ramaswamy (2023) examined the impact of composite alginate and ginger essential oil-based edible coatings on the regulation of physiological and microbiological processes in fresh-cut pear during refrigerated storage. A solution consisting of 2% sodium alginate with 0.5% ginger oil as a natural antimicrobial agent was utilized as the coating material, while a 2% calcium chloride dip was employed for cross-linking and firming purposes. They found that both the coated fruits, with and without ginger oil, exhibited markedly superior preservation of product quality and absence of microbial spoilage for a duration of up to 15 days, in contrast to the control fruits, which experienced spoilage within a week. Additionally, the combination of edible coatings such as alginate or pectin with osmotic dehydration processes has been found to improve mass transfer kinetics, physicochemical parameters, and the retention of optical and mechanical properties in pear cubes (Campanone et al., 2024). In addition, the edible coating based on pectin combined with antibacterial and anti-browning agents effectively preserves fresh-cut pears, thereby improving quality characteristics and extending shelf life (Pleșoianu & Nour, 2022). They found that pectin coating delayed weight loss and improved the firmness of pears. Additionally, incorporating lemon peel essential oil into edible skin coatings made of chitosan and guar gum has been found to reduce weight loss, improve firmness, enhance antioxidant capacity, antibacterial efficiency, and overall acceptability of pears for up to 45 days at 4 ± 2°C (Iftikhar et al., 2022). Pears are known for their delicate texture and susceptibility to browning. Edible coatings, particularly those incorporating antioxidants like vitamin C and polyphenols, have been shown to control browning and microbial growth in fresh-cut pears effectively. Studies have reported that coatings based on chitosan, whey protein, and pectin can effectively extend the shelf life of pears, while maintaining their sensory quality (Mei et al., 2023). These findings highlight the effectiveness of edible coatings in preserving the quality and extending the shelf life of pear and its fresh-cut products.

Some examples regarding recent advances in the formulation and effects of edible coating for fresh/fresh-cut pome fruits are described in Table 1.

Table 1. Recent advances in application and effects of edible coating in temperate fresh/fresh-cut fruits (Pome fruits).

Fresh/fresh-cut fruit	Coating formulation	Outcomes	Reference
Apple	Carboxymethylcellulose, sodium alginate, citric acid, and oxalic acid	Combined coating inhibits flesh browning, maintains lower color changes, and improves antioxidant defense. Improved the shelf life of fresh-cut 'Annurca Rossa del Sud' apples.	(Magri et al., 2023)
Apple	Zein-nisin	Zein coating delayed weight loss on apples till day 21. Zein-nisin coating reduced yeast and mould.	(Belay et al., 2023)
Apple	Chitosan-based edible coating with ascorbic acid	Delayed deterioration, preserved firmness, moisture, and antioxidant enzyme activities in Custard apple.	(Wang et al., 2023)
Apple	Whey protein-based emulsion coating with transglutaminase and sunflower oil	Sunflower oil improves water resistance and mechanical properties. Whey protein-based emulsion coating treatment reduces weight loss and browning in fresh-cut apples.	(Xin et al., 2023)
Apple	Sodium alginate with Eugenia pyriformis leaf extract	Uvaia leaf extract reduced enzymatic browning in fresh-cut apples.	(Maldonado-Silva et al., 2020)
Apple	Nanochitosan	Reduced weight loss and moisture content. Preserved color, vitamin C, and antioxidants.	(Dasgupta & Mitra, 2024)
Pear	Edible coatings (alginate or pectin) combined with osmotic dehydration using glucose solution	Enhanced quality of pear cubes, improved weight reduction, water loss, and mechanical properties.	(Campanone et al., 2024)
Pear	Composite alginate-ginger oil	Extended the shelf-life of fresh-cut pears by controlling physiological and microbiological activities, enhanced product quality and preventing spoilage.	(Lamani & Ramaswamy, 2023)
Pear	Pectin-based edible coating combined with antimicrobials and antibrowning agents	Pectin coating delayed weight loss and improved firmness of fresh-cut pears.	(Plesoianu & Nour, 2022)
Pear	Guar gum and chitosan-based edible coatings enriched with lemon peel essential oil	Improved pear quality by reducing weight loss, enhancing firmness, antioxidant capacity, and antibacterial efficiency during storage.	(Iftikhar et al., 2022)
Pear	Whey protein-based edible coatings with lemon or lemongrass essential oils	Improved the quality of fresh-cut pears by preserving color, firmness, and sensory attributes during storage at 4°C.	(Galus et al., 2021)
Pear	Edible coating based on carboxymethylcellulose, sodium alginate, oxalic and citric acid	Preserved and improved the antioxidant content, delayed browning, and retarded the senescence.	(Magri et al., 2024)

Edible coatings for stone fruits

Plum (*Prunus domestica*)

Various studies have explored the use of different edible coatings on plum fruit such as chitosan grape-seed-oil nanoemulsion, arrowroot starch films, starch-based materials with whey protein, CMC and pectin-based coatings, and gum arabic coatings (Zsivanovits et al., 2023; Basiak et al., 2022; Panahirad et al., 2020a, b). Zsivanovits et al. (2023) investigated the effect of chitosan grape-seed-oil nanoemulsion on freshly cut plum fruits (var. Stanley). Various properties, including physical, physico-chemical, microbiological, and sensorial aspects, were assessed over a 9-day refrigeration period. By the 4th day, the control samples had already deteriorated in terms of safety and quality. In contrast, the coated samples maintained their quality and safety until the end of the storage duration. While the chitosan-coated fruits exhibited reduced microbiological contamination, those coated with chitosan grape-seed-oil displayed elevated values in terms of sensorial characteristics. Furthermore, the coated samples retained around 80% of their sensorial attributes by the end of the 9th day. These coatings have shown positively affected on parameters like microbiological contamination, sensorial properties, water vapor permeability, firmness, antioxidant capacity, weight loss, and shelf life extension. The coatings have demonstrated abilities to reduce mass loss, delay fruit ripening, preserve firmness, improve total phenolic content, and alleviate shrivel, making them valuable for postharvest maintenance and preservation of plums. Plums are prone to microbial spoilage, mainly due to the presence of yeast and mold. Coatings based on alginate, chitosan, and CMC have been shown to effectively inhibit microbial activity, while also maintaining the firmness and color of the plum fruit (Panahirad et al., 2020a; Riva et al., 2020). Panahirad et al. (2020a) investigated the impact of edible coatings based on CMC and pectin on various characteristics of plum fruit during cold storage, including titratable acidity, firmness, vitamin C, total soluble solids, pH, total phenolics, anthocyanin, flavonoid contents, total antioxidant capacity (DPPH)), peroxidase (POD), polyphenol oxidase (PPO), and polygalacturonase (PG) enzyme activities, as well as weight loss. The findings indicated that the coatings, whether used individually or in combination, had positive effects on most parameters measured, excluding weight loss. These coatings helped maintain firmness, enhance total phenols, anthocyanin, flavonoid contents, antioxidant capacity, and POD activity. Moreover, TSS levels decreased, pH values remained relatively stable, and the coatings slowed down TA and vitamin C losses while reducing enzymatic activities like PPO and PG. Notably, the optimal outcomes for the majority of parameters were observed with 1% CMC or 1.5% Pectin individually, and the combination of 0.5% pectin and 1.5% CMC proved to be the most effective.

Peach (*Prunus persica*)

Research has shown that applying edible coatings such as gum arabic (GA) and polyvinylpyrrolidone (PVP) with salicylic acid (SA) can significantly inhibit degrading enzyme activities, reduce browning symptoms, and minimize tissue breakdown in peaches, extending their shelf life (Taher et al., 2022). Taher et al. (2022) blended GA and PVP with varying concentrations of salicylic acid (SA) (0, 1, and 2 mM) and utilized as a coating on peach fruits to prolong their shelf life. The fruits were then coated and kept at room temperature (26 ± 1 °C) with relative humidity (51 ± 1%) for duration of 10 days. Peach fruit coated with GA/PVP-SA 2 mM exhibited a notable reduction in the activities of degrading enzymes like lipoxygenase (LOX), cellulase (CEL), and pectinase (PT) in comparison to both uncoated and coated fruits throughout the storage period. This led to the maintenance of cell wall compartments, consequently reducing browning symptoms by inhibiting the activities of polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL). Additionally, there was

a decrease in lipid peroxidation and ionic permeability. The findings indicate that the application of GA/PVP-SA 2 mM as an edible coating can help minimize fruit tissue breakdown, thereby extending the shelf life of peaches by up to 10 days without any signs of tissue breakdown.

Additionally, coatings based on aloe arborescens and 1-methylcyclopropene (1-MCP) have been effective in preserving the quality of white flesh peaches by slowing down maturation processes, limiting weight loss, and maintaining sensory characteristics (Sortino et al., 2020). Furthermore, a combination of chitosan and thymol is more effective in preserving peach quality, reducing weight loss, fungal decay, and maintaining firmness, anthocyanin, carotenoid content, and sensory attributes, thus extending the fruit's shelf life (Rahimi et al., 2019). Peaches are highly susceptible to enzymatic browning, which significantly affects their appearance and quality. Edible coatings containing antioxidants, such as vitamin C and polyphenols, effectively controlled browning in fresh-cut peaches. A recent review has also shown that application of different edible coatings can improve moisture retention and reduce microbial growth, contributing to a longer shelf life in fresh peaches (Aaqil et al., 2024). These studies collectively highlight the potential of edible coatings in enhancing the postharvest quality and prolonging the shelf life of peaches.

Sweet cherry (*Prunus avium*)

Recent studies have highlighted the benefits of different edible coatings on sweet cherries. For instance, natural edible coatings containing galbanum gum and cumin essential oil helped maintain phenolic compounds and antioxidants, enhancing the fruit's health-promoting phytochemicals and shelf life (Asghari et al., 2022). The application of edible chitosan coating was effective in maintaining total phenolics, and enhancing antioxidant enzymatic activities in sweet cherries (Hu & Feng, 2022). They examined the impact of applying edible chitosan coating (0.1, 0.3, 0.5, and 0.75% w/v) on the quality, respiration rate, total phenolic content, and anthocyanin changes in postharvest sweet cherry at 10 °C. Findings revealed that the use of chitosan edible coating effectively delayed the progression of postharvest ripening-related parameters, such as color, firmness, and respiration rate. It was recommended that optimal quality and improved antioxidant enzymatic activities in postharvest cherry fruits were achieved by applying an edible chitosan coating of 0.5% for up to 24 days at 10 °C. Using chitosan edible coating could be advantageous in prolonging shelf-life and preserving the quality of sweet cherries. Furthermore, carboxymethyl chitosan-gelatin-based coatings incorporating $CaCl_2$ and ascorbic acid preserved the quality and nutritional characteristics of sweet cherries, reducing decay and improving various parameters like firmness, acidity, and antioxidant capacity (Zhang et al., 2021). Also, in a study an edible nanoemulsion coating of alginate and soybean oil with $CaCl_2$ cross-linker utilized on sweet cherries (Gutiérrez-Jara et al., 2021). The results showed that nanoemulsion and $CaCl_2$ coating increased firmness and nutritional values and reduced fruit cracking by 53%. Edible coatings have been investigated to reduce microbial growth and extend the shelf life of cherries. A recent study has shown that coatings based on chitosan and aloe vera gel, combine with extractions of some medicinal plants can effectively inhibit microbial activity and maintain the nutritional quality of cherries during cold storage (Afonso et al., 2023).

Apricot (*Prunus armeniaca*)

Coatings containing chitosan nanoparticles (CHNPs) have been found to reduce weight loss, decay, and lipid peroxidation in stored apricots, ultimately extending their shelf life (Algarni et al., 2022). The sensory evaluation results showed that there was a significant difference in the overall acceptance scores between the CHNPs-treated samples and the other samples.

They indicated that chitosan nanoparticles treatment improved apricot quality, and extended shelf-life up to 30 days in cold storage and nine days at room temperature (Algarni et al., 2022). Furthermore, coatings with gum arabic have demonstrated effectiveness in preserving the physicochemical characteristics, texture, and antioxidant activity of fresh apricots during refrigerated storage for 12 days (Wani et al., 2019). Dorostkar et al. (2022b) investigated the effect of different postharvest calcium salt treatments on apricot fruit. They found that calcium chloride and calcium nitrate dipping treatments significantly preserved quality and reduced the decay of apricot fruit compared to control samples during cold storage. Also, soybean protein isolate-chitosan edible coating effectively reduces weight loss, maintains firmness, and inhibits pectin degradation in apricots, to enhance their shelf life and quality during storage at 2°C (Zhang et al., 2018). Apricots are susceptible to enzymatic browning and microbial spoilage, leading to a short shelf life for fresh-cut fruits. Edible coatings have been explored as a potential solution to address these issues. Coatings based on pectin, cellulose, bees wax, and alginate have shown promise in controlling microbial growth, extending the shelf life of apricots and maintaining their sensory quality (Kefayatullah & Wahab, 2023). However, among edible coating applied, bees wax 2% treatment had lower decay and chilling injury during 28 days of cold storage at 5 °C. These findings highlight the potential of edible coatings in enhancing the preservation and quality of apricots.

Some examples regarding recent advances in the formulation and effects of edible coating for fresh/fresh-cut stone fruits are described in Table 2.

Table 2. Recent advances in application and effects of edible coating in temperate fresh/fresh-cut fruits (Stone fruits).

Fresh/fresh-cut fruit	Coating formulation	Outcomes	Reference
Plum	Chitosan grape-seed-oil nanoemulsion	Coating enhanced postharvest quality of fresh-cut plums, preserving safety, quality, and sensorial parameters for up to 9 days during refrigerated storage.	(Zsivanovits et al., 2023)
Plum	Wheat starch and wheat starch-whey protein	Enhanced firmness and reduced weight loss.	(Basiak et al., 2022)
Plum	Polysaccharide-based edible coatings, like carboxymethylcellulose and pectin	Enhanceed postharvest quality of plums by preserving firmness, antioxidants, and enzyme activities.	(Panahirad et al., 2020a)
Plum	Pectin-based edible coating	Preserved antioxidative capacity, including ascorbic acid and phenolic compounds, enhanced fruit quality and shelf life.	(Panahirad et al., 2020b)
Plum	Carboxymethylcellulose-based edible coating	Improved plum fruit quality by maintaining firmness, acidity, antioxidants, and enzyme activities, enhancing shelf life and preserving qualitative properties.	(Panahirad et al., 2019)
Peach	Gum Arabic and Polyvinylpyrrolidone with Salicylic Acid	Reduced tissue breakdown, maintained cell wall integrity, and extended shelf life up to 10 days.	(Taher et al., 2022)
Peach	Aloe arborescens edible coating, alone or combined with 1-MCP	Extended the shelf life of white peach fruit by preserving quality attributes and sensory characteristics during storage.	(Sortino et al., 2020)
Peach	Chitosan and thymol essential oil	Maintained peach quality and extended shelf life, Combination had superior preservation effects compared to individual coatings.	(Rahimi et al., 2019)

Peach	Chitosan coatings incorporated with seabuckthorn leaf extract	Increased the antioxidant potential and controlled the browning up to 25 days under refrigerated temperature at 4 °C.	(Rather et al., 2024)
Sweet cherry	Galbanum gum and cumin essential oil	Effectively preserved sweet cherry quality, maintaining phytochemicals and antioxidants, enhancing shelf life and health benefits.	(Asghari et al., 2022)
Sweet cherry	Chitosan	Delayed ripening, maintained quality, and enhanced antioxidant enzymatic activities, extending shelf life.	(Hu & Feng, 2022)
Sweet cherry	Carboxymethyl chitosan-gelatin coating with $CaCl_2$ and ascorbic acid	Improved quality, reduced decay, and maintained nutritional properties of different sweet cherry cultivars during postharvest storage.	(Zhang et al., 2021)
Sweet cherry	Nanoemulsion of alginate and soybean oil with $CaCl_2$ cross-linker	Reduced cracking, enhanced firmness, and maintained quality parameters postharvest.	(Gutiérrez-Jara et al., 2021)
Sweet cherry	Nano-emulsion coatings containing hydroxypropyl methylcellulose (HPMC), beeswax (BW), and essential oils (thyme, cinnamon, clove, and peppermint)	Maintained quality attributes such as TSS, color, weight loss, respiration rate, firmness, total phenolic contents, and sensory evaluations.	(Iqbal et al., 2024)
Sweet cherry	Chitosan and aloe vera gel, combine with extractions of some medicinal plants	Inhibited microbial activity and maintained the nutritional quality of fruit	(Afonso et al., 2023)
Apricot	Chitosan nanoparticles	Improved apricot quality, reduced decay, and extended shelf-life up to 30 days in cold storage and 9 days at room temperature.	(Algarni et al., 2022)
Apricot	Edible coating with gum arabic	Enhanced apricot shelf life and improved physicochemical characteristics, texture, and antioxidant activity during refrigerated storage for 12 days.	(Wani et al., 2019)
Apricot	Soybean protein isolate-chitosan edible coating	Reduced weight loss, maintained firmness, and inhibited pectin degradation in apricots, enhanced shelf life during storage at 2°C.	(Zhang et al., 2018)
Apricot	Coatings based on pectin, cellulose, bees wax, and alginate	Controlled microbial growth, extended the shelf life of apricots and maintained sensory quality	(Kefayatullah & Wahab, 2023)

Edible coatings for small fruits

Strawberry (*Fragaria* × *ananassa*)

Research has shown that coatings such as chitosan, beeswax, moringa leaf extract, aloe vera gel, ascorbic acid, oxalic acid, Arrayan extract, essential oils, and Carnauba wax can effectively improve the quality and shelf life of strawberries by reducing weight loss, enhancing firmness, decreasing respiration rate and ethylene production, improving biochemical quality parameters, inhibiting microbial growth, and increasing antioxidant activity (Álvarez-Barreto et al., 2023; Shafique et al., 2023; Topno, 2024). Álvarez-Barreto et al. (2023) investigated the impact of different concentrations of Carnauba wax (0, 0.3, and 0.4% w/v) and aloe vera gel (0, 30, and 45% v/v) coatings on the quality and shelf life of strawberry fruit. They found that the treatment with the highest concentration of the two ingredients produced the lowest changes in weight loss, pH and ripeness index, as well as the lowest values of the *Botrytis cinerea* severity index. The coated fruit were not significantly different from uncoated samples and were well and scored acceptable by panelist from an organoleptic viewpoint. Zein nano-fiber film loaded with thyme essential oil significantly decreased weight loss and preserved the anthocyanin content, firmness and color of the strawberries, and reduced decay during cold storage (Ansarifar & Moradinezhad, 2022). Stored fruit in packages containing zein nanofiber significantly lowered microbial load, and maintained the total phenols and antioxidant activity of the strawberries during 15 days of storage at 4 °C (Ansarifar & Moradinezhad, 2021). The effect of essential oil concentrations from tangerine peel incorporated in sodium alginate-based edible coatings on physical properties was investigated (Utami et al., 2023). Sodium alginate-based edible coatings containing different concentrations of essential oil from tangerin peel significantly affected strawberry quality in all parameters including color, hardness, total dissolved solids content, and weight loss during storage at refrigerator temperature (Utami et al., 2023). These coatings have been found to maintain fruit quality, prevent decay and spoilage, and exhibit high consumer acceptance. Strawberries are highly perishable and prone to microbial spoilage and decay. Edible coatings have been widely investigated to extend the shelf life of fresh-cut strawberries. Recent reviews regarding edible coatings based on chitosan and pectin have shown effectiveness in inhibiting microbial growth, reducing moisture loss, extending the shelf life, and maintaining the firmness and color of the strawberries (Moghadas et al., 2024; de Albuquerque Sousa et al., 2024). Using these edible coatings at optimal concentrations, strawberries can be preserved for longer periods, ensuring that this nutrient-rich fruit remains fresh and appealing for consumption.

Grapes (*Vitis vinifera*)

Edible coatings for grapes have been extensively studied for their ability to extend shelf life and maintain quality. Various materials like propolis and aloe vera gel, grape seed tannins, hydroxypropylmethylcellulose, and kappa carrageenan, grape pomace extract combined with polyvinyl alcohol, and a mixture of alginate, galactomannans, cashew gum, and gelatin have been explored (Aljabary, 2024; de Souza et al., 2021; Lo'ay et al., 2021). In the research conducted by de Souza et al. (2021), the effect of edible coatings on the overall physicochemical makeup of phenolic content and antioxidant properties was assessed. The edible coatings, which included alginate (2%), galactomannans (0.5%), cashew gum (0.5%), and gelatin (2.0%), minimized weight loss in grapes while maintaining their hardness and color quality after nine days of storage in comparison to the control group. Furthermore, this formulation enhanced the levels of phenolic compounds, thereby boosting the significant antioxidant capacity of the coated grapes. Chitosan–zinc oxide nanoparticles and essential oil coatings improved grape quality by reducing microbial contamination, enhancing catalase

activity, and maintaining the freshness of grapes (Kadi, 2023). These coatings have shown promising results in reducing weight loss, delaying decay, enhancing firmness, and improving antioxidant properties, ultimately extending the storability of grapes. The use of edible coatings not only provides a protective barrier against microbial growth but also offers health benefits and preserves the sensory attributes of the fruit. Fresh grapes are susceptible to microbial growth and dehydration, leading to a short shelf life (Lo'ay et al., 2021). Edible coatings have been studied as a means to enhance the storage stability of grapes. Coatings based on chitosan, pectin, and alginate have shown promise in reducing microbial activity, inhibiting moisture loss, and maintaining the quality of grapes during storage (Moreira et al, 2023). Overall, edible coatings present a novel and effective approach to enhancing the postharvest quality and shelf life of grapes, potentially revolutionizing the fruit preservation industry.

Kiwi fruit (*Actinidia deliciosa*)

Recent studies have explored different edible coating materials and their effects on the quality and preservation of kiwifruit. For instance, CMC and aloe vera gel coatings have shown positive results in improving microbial properties and sensory attributes of fresh-cut kiwi fruit slices, enhancing their overall quality (Nikhil & Topno, 2023). Additionally, incorporating thymol-halloysite nanohybrids into chitosan biopolymer films has demonstrated enhanced antimicrobial and antioxidant activities, leading to prolonged preservation and shelf life of kiwi fruits (Salmas et al., 2022). Furthermore, coatings with mucilage from Opuntia ficus-indica or aloe arborescens have been found to reduce weight loss, microbial spoilage, and improve firmness in fresh-cut kiwifruits, highlighting their potential for shelf-life extension (Sortino et al., 2022). Moreover, nanoemulsion coatings with antioxidant and antimicrobial agents, including alginate, CMC, ascorbic acid, and vanillin, improved the shelf life of fresh cut kiwi slices by delaying decay, weight loss and microbial growth (Manzoor et al., 2021). Innovations like alginate coatings functionalized with hop extracts have also proven effective in preserving the quality and nutraceutical traits of fresh-cut kiwifruit during cold storage, further emphasizing the importance of edible coatings in maintaining fruit freshness and marketability (Carbone et al., 2021). However, Xanthan gum enhanced the shelf life and maintained the quality of fresh-cut kiwi slices compared to alginate- and chitosan-coated treatments (Guroo et al., 2021). Kiwis are known for their delicate texture and susceptibility to enzymatic browning. In general, edible coatings have demonstrated effectiveness as a potential solution to extend the shelf life of fresh-cut kiwis, inhibiting microbial growth and maintaining the quality of fruit.

Some examples regarding recent advances in the formulation and effects of edible coating for fresh/fresh-cut small fruits are described in Table 3.

Table 3. Recent advances in application and effects of edible coating in temperate fresh/fresh-cut fruits (Small fruits).

Fresh/fresh-cut fruit	Coating formulation	Outcomes	Reference
Strawberry	Chitosan and Beeswax	Reduced weight loss and maintained Vitamin C.	(Topno, 2024)
Strawberry	Moringa leaf extract, aloe vera gel, oxalic acid, and ascorbic acid	Improved strawberry quality, reduced weight loss, enhanced firmness, and increased antioxidant content during storage.	(Shafique et al., 2023)
Strawberry	Aloe Vera Gel and Carnauba Wax microparticles	Reduced weight loss, maintained ph, and inhibited *Botrytis cinerea* growth.	(Álvarez-Barreto et al., 2020)
Strawberry	Zein nano-fiber film loaded with thyme essential oil	Decreased weight loss and preserved the anthocyanin content, firmness and color, and reduced decay during cold storage	(Ansarifar & Moradinezhad, 2022)
Grape	Propolis and aloe vera gel	Preserved Thompson Seedless grapes' quality during cold storage, delayed deterioration and potentially extended storability up to 30 days at 5°C.	(Aljabary, 2024)
Grape	Alginate, galactomannans, cashew gum, and gelatin	Enhanced shelf-life of 'Italia' grapes by reduced weight loss, maintained firmness, color, and increased antioxidant properties.	(de Souza et al., 2021)
Grape	Composite coating of pectin, polyphenylene alcohol, and salicylic acid	Improved the quality and shelf life of 'Crimson Seedless' grapes by reducing weight loss, browning, and cell wall damage.	(Lo'ay et al., 2021)
Grape	Chitosan–zinc oxide nanoparticles and essential oil	Improved grape quality by reducing microbial contamination, enhancing catalase activity, and maintaining fruit freshness.	(Kadi, 2023)
Kiwi fruit	Carboxymethyl cellulose and aloe vera gel	Fresh-cut kiwi, with aloe vera gel 30% showing the best results in microbial analyses and sensory attributes.	(Nikhil & Topno, 2023)
Kiwi fruit	Thymol-halloysite nanostructures in chitosan/polyvinyl alcohol gels	Enhanced fruit preservation by improving antimicrobial, antioxidant, mechanical, and barrier properties, extending shelf life.	(Salmas et al., 2020)
Kiwi fruit	Opuntia ficus-indica and aloe arborescens mucilage	Enhanced kiwifruit shelf life by reducing weight loss and microbial spoilage, improving firmness, pectin content, and visual quality.	(Sortino et al., 2022)
Kiwi fruit	Nanoemulsion coatings with antioxidant and antimicrobial agents, including alginate, carboxymethylcellulose, Tween 80, ascorbic acid, and vanillin,	Improved the shelf life of fresh cut kiwi slices by delaying decay and microbial growth.	(Manzoor et al., 2021)
Kiwi fruit	Alginate coatings functionalized with hop extracts	Preserved the quality and nutraceutical traits of fresh-cut kiwifruit during cold storage.	(Carbone et al., 2021)

Future directions and challenges

Incorporating antimicrobial elements, antioxidants, or enzymes into active coatings could provide enhanced protection against microbial spoilage, oxidative decay, and physiological decline. This requires investigating natural and synthetic active components that can be smoothly integrated into the coating structure. It's crucial to ensure that consumers accept edible coatings for successful implementation. However, using edible coatings poses challenges in terms of production, storage, and large-scale usage while maintaining consumer acceptance, food safety, nutrition, and shelf life extension (Kumar et al., 2023). Edible films based on polysaccharides and proteins encounter difficulties with their poor water and gas barrier properties, requiring the addition of plasticizers, emulsifiers, and other components to enhance their mechanical and thermal resistance. Moreover, increased levels of biopolymers and active components like essential oils and plant extracts can negatively influence the flavor of the produce, directly impacting consumer satisfaction. This is also associated with the potential harm of these substances. Lastly, there are minimal safety and regulatory standards regarding the levels of active ingredients in edible coatings. Therefore, it is crucial to raise consumer awareness and establish regulations regarding the benefits of edible coating for both the environment and consumers to address consumer acceptance challenges on a large-scale commercial level.

CONCLUSION

Edible coatings have emerged as a promising strategy for extending the shelf life of temperate fresh/fresh-cut fruits, including apple, pear, plum, peach, cherry, apricot, strawberry, grape, and kiwi. The specific composition and application of these coatings play a crucial role in their effectiveness in inhibiting microbial growth, reducing enzymatic browning, and maintaining the sensory quality of the fruits. Further research is needed to optimize the use of edible coatings for specific fruit types and to develop innovative solutions that address the challenges associated with extending shelf life and maintaining freshness.

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A systematic review on plant-based edible coatings for quality improvement and extended postharvest life of fresh fruits and vegetables

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ABSTRACT

Purpose: Use plant-based edible coatings (PBECs) for maintaining quality of fresh fruits and vegetables (FFVs) are trending upward. Compared to modified atmosphere packaging they eliminate the use of non-biodegradable polyethylene films. Therefore, the present study aimed to bring a comprehensive systematic review of the published literature on plant- based edible coatings (PBECs) for quality maintenance and extension of postharvest life FFVs. **Findings:** The results revealed that PBECs are a better alternative to other protective films and packaging materials that utilize non-biodegradable polyethylene films or inorganic chemicals which pose negative impact on both consumers and environment. A wide range of ingredients including biopolymers, leaf extracts, plant waxes, essential oils, and plant byproducts have been intensively researched for their potential applications in the development of edible coatings. The coating treatments significantly retarded the rates of respiration and ethylene emission, activated antioxidative defense mechanisms, suppressed cell wall degrading enzymes, and retarded colour deterioration; all of which led to protecting the biochemical and organoleptic properties of FFVs. **Limitations:** Food items, when coated with some of the edible coatings, alterations of flavour and degradation of their properties upon exposure to light, oxygen & high temperature have been noted. Further, poor stability of the developed emulsion resulting inconsistencies in their effectiveness have been reported. **Conclusions:** In conclusion, PBECs could be considered as promising eco and consumer friendly strategy for maintaining and extending the postharvest life of FFVs. **Future trends:** It seems imperative to focus more on the development of composite coatings to enrich nutraceutical attributes of FFVs. Improving the efficacy of mode of action of the developed formulae alongside enhancing its stabilization and prevention of alterations in flavour when coated are critically important.

INTRODUCTION

Roughly one-third (30-40%) of global fresh fruits and vegetables (FFVs) are thrown away from production to consumption because their quality has dropped below an acceptance limit. On the other hand, nearly one billion people are chronically under-nourished and suffer from nutritional deficiencies (Porat et al., 2018). Today, food security is severely threatened due to climate change, lack of arable lands, and water scarcity. In this context, minimizing postharvest losses should be one of the leading strategies all around the world for ensuring food security of the burgeoning population. Minimizing postharvest losses is more sustainable and environmentally sounds than increasing production areas to compensate for these losses. While it may be impossible and uneconomical to eliminate postharvest losses totally, it is possible to reduce them at least by 50%. For that it is necessary to integrate correct postharvest management practices into supply chains and thereby curtail postharvest losses of FFVs.

There is an increasing trend to use plant-based edible coatings (PBECs) for maintaining quality of FFVs as they are both consumer and eco-friendly. Compared to modified atmosphere packaging they are relatively cheap, utilize the space in packing cartons & shipping containers effectively, and eliminate the use of non-biodegradable polyethylene plastic films (Firdous et al., 2023). Edible coatings are defined as thin layers (0.050 - 0.250 mm thickness) of edible material applied to the product surface in addition to or as a replacement for natural protective waxy coatings. They provide a barrier to moisture, oxygen, and solute movement for food (Dhall, 2013). As a result, they retard the rate of water loss, cellular respiration, thus hinders the rate of ripening and subsequent tissue senescence. Additionally, these coatings contribute to suppress the growth of pathogenic organisms and development of physiological disorders (Murmu & Mishra, 2017; Moradinezhad & Ranjbar, 2023), increase the aesthetic appearance of the produce by shining, hiding minor scars, and helping retain the commodity freshness. Moreover, they prevent the loss of volatile flavor compounds from the food while establishing mechanical protection.

Edible coatings are generally applied to the food material in liquid state. The major components of edible coating are polysaccharides, proteins and lipids. When developing new formulae currently, the researchers pay more attention to their consumer safety thus a diverse array of functional compounds are integrated. Figure 1 is a summary of compounds used to develop edible coating formulae.

The efficiency of the developed new formula depends on several factors namely;

- the nature of the coating ingredients and their concentrations
- the application method (dipping, spreading either manually or mechanically, spraying, etc)
- the uniformity of wetting and spreading on the surface and on the adhesion, cohesion, and durability
- The capability to act as barriers against water or oils permeation, and gas or vapor transmission.

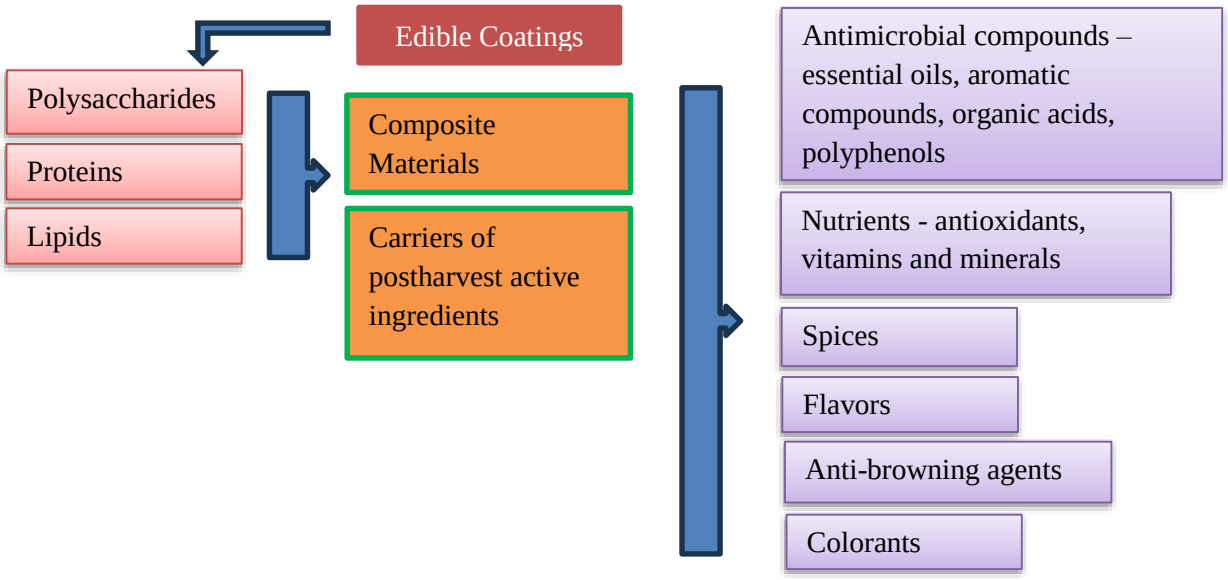

Fig. 1. Major components and additives used in edible coatings.

This study aimed to bring a comprehensive systematic review of the published literature from January 2013 to January 2024 on PBECs for quality improvement and extending the postharvest life of FFVs. Moreover, this review endeavored to depict the current key innovation areas in PBECs and to identify any knowledge gaps and inconsistencies in research that may necessitate further investigation.

MATERIALS AND METHODS

Eligibility criteria and sources of information

In this review we focused on the use of PBECs for improvements of postharvest quality and shelf-life of intact FFVs. Research articles published in Science Citation Indexed Journals during January 2013 to January 2024 were referred. They were retrieved from the Web of Science (https://clarivate.com/webofsciencegroup/solutions/web-of-science/) core collection. The major keywords used were “plant based edible coatings" "fruits” & “vegetables”, “by-product utilization”, "quality & shelf-life”. In addition, to seek innovative fields of studies in PBECs, the keywords “active and/or functional ingredients of plant origin" were used.

Study selection and data collection process

Articles available on application of PBECs published in English were included in this study. Coatings applied on animal products such as fish, meat and minimally processed or fresh cut products were excluded. Moreover, research papers that reflect the content of nanocomposite coatings, nanoemulsions or nanoencapsulation were excluded because of the emerging controversial issue on potential of inducing toxic effects at cellular level in the human body. For instance, according to Evans et al. (2017) exposure to nanomaterials can have unanticipated effects on the overall functionality of the cellular system, and the fidelity of cell division and DNA replication. DNA damage has been linked to cellular exposure to some nanomaterials, resulting in genome rearrangements, single and double-stranded breaks, as well as inter/intra-strand breaks (Onyeaka et al., 2022). Further, edible coatings developed purely by animal-based extractions and/or synthetic inorganic compounds were excluded too. Hence, edible coatings developed by plant extracts such as starches, proteins, gums, waxes, essential oils, and plant byproducts were included. Documents that report studies reflecting

improvements of film properties but not reporting their performance after evaluating the developed formula on shelf life of FFVs were excluded.

The retrieved documents were screened in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines, and they are comprised of following four stages i.e. (i) identifying articles to be reviewed (ii) screening the studies for review, (iii) figuring which studies are eligible, and (iv) selecting which studies to include in the systematic review. A total of 1893 (n= 1893) articles were identified. Articles published more than 10 years ago, studies that do not focus on plant-based compounds as the primary subject, studies that did not evaluate the developed coating formulation on intact FFVs for its postharvest performance were excluded. Further, book chapters, conference abstracts, proceedings, case studies and articles published in non-English were excluded. Following the application of exclusion criteria, 1788 (n= 1788) documents were removed. Subsequently, 105 (n=105) articles underwent further full-text screening. Utilizing the Mendeley reference manager to eliminate duplicates (n=21) and excluding irrelevant content (n=34), a total of 50 (n=50) documents were considered suitable for inclusion in this systematic review and the process followed is shown in Figure 2.

RESULTS AND DISCUSSION

Numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage are given via a flowchart in Figure 2.

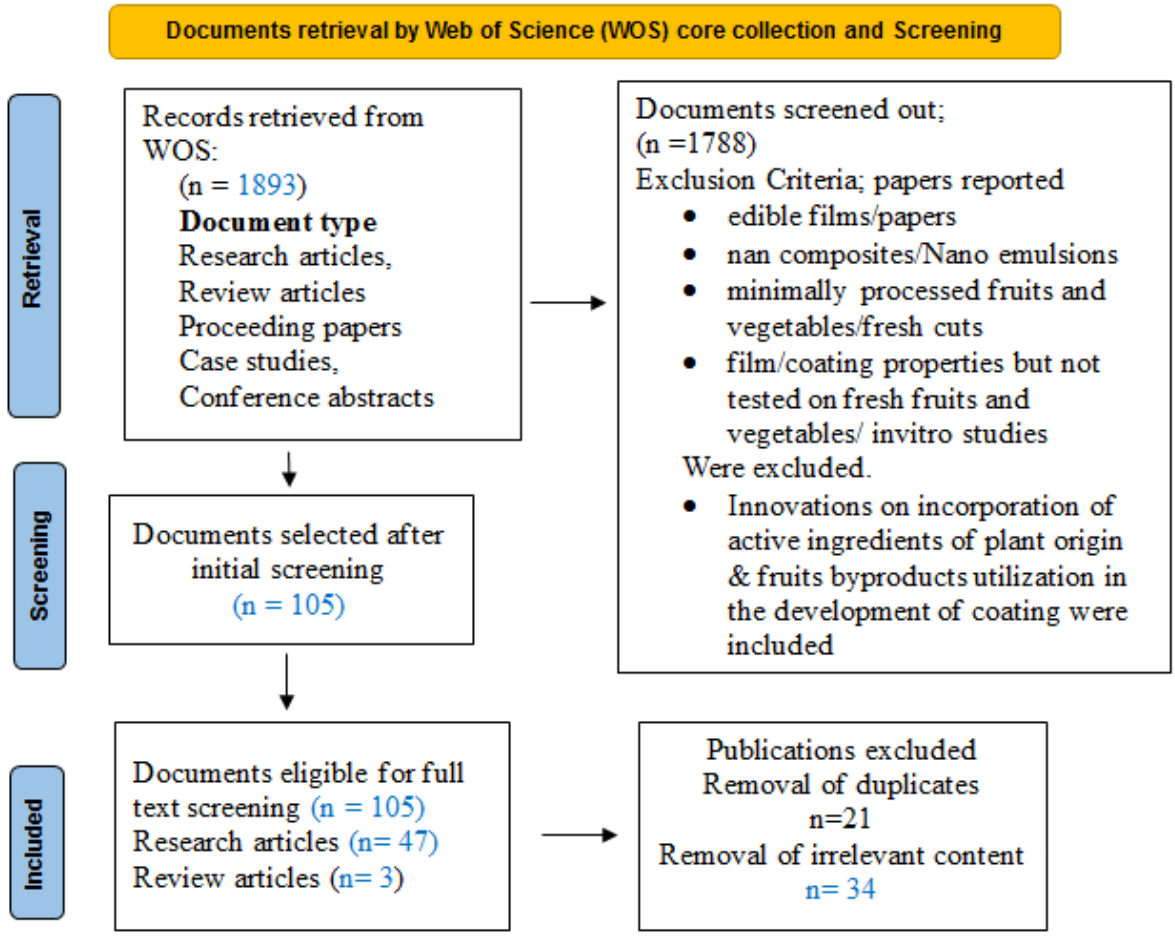

Fig. 2. Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) flowchart.

Polysaccharides and protein based edible coatings

Starches

Effect of application of edible coatings with 3% corn starch (CS), 1% gum Arabic (GA) and 1% lyophilized fish myofibrillar proteins (LMP) on postharvest quality of guava cv. Cortibel was investigated by Pereira et al. (2021). Three sprays were made on each fruit, with 8 mL of the coating solution at 40°C, with an interval of 1 min between them. After treatment fruits were stored at 24 ± 0.21°C, 64 ± 1.15% RH. The CS and LMP delayed the fruit ripening compared to the control and GA, examined by the color. After 7 days of storage, the coated fruits remained green, which was different from the control that lost this color by 3rd day of storage. A lower weight loss and a higher firmness were shown by fruit treated with LMP and CS during the storage which contributed positively to slowdown the rate of deterioration of guavas.

Thakur et al. (2018) studied the use of rice starch (RS), carrageenan (car) and fatty acid esters (FAEs) composite materials for fruit coating applications. Film solution (starch 3%, car 1.5% and FAEs 2%) was applied manually on plum (*Prunus salicina*) fruit and their impact on physiology and shelf life were evaluated during storage at 20 °C, 55 ± 5% RH for 3 weeks. RS composite coating was shown to be effective in reducing both weight loss and respiration rate and inhibiting the endogenous ethylene production compared to the uncoated control fruit.

Effect of flaxseed (*Linum usitatissimum* L.) and fenugreek (*Trigonella foenumgraecum* L.) polysaccharide-based edible coatings on postharvest quality of apple Cv. Kala Kulu has been studied by Rashid et al. (2020). Fruits were dipped for one min in respective coating solutions and stored at 20 ± 5 ºC, 80-85% RH. From the response optimization analysis, a combination of 2.5 g fenugreek and 1.5 g flaxseed polysaccharide-based coating was predicted to give desirable effects for all response variables. The findings proved that optimized fenugreek and flaxseed polysaccharide based edible coating could retain the quality of apple by minimizing weight loss, retarding the rate of reduction in firmness, TSS, TA, & pH through the mechanism of delaying respiration rate and ethylene production.

Khodaei et al. (2021) evaluated the effect of edible coatings of Persian gum (PG - 4% w/v), low methoxyl pectin (LMP - 2% w/v), carboxy methyl cellulose (CMC - 1% w/v), and tragacanth gum (TG - 0.6% w/v) on extending the shelf-life of fresh strawberries. Freshly harvested strawberries were dipped in respective coating solutions for 30 s which then packed in polyethylene terephthalate (PET) clamshell containers and stored in refrigerator (4 ˚C and 80% RH) for evaluation of their physicochemical properties for 16 days. The TG and CMC coated strawberries exhibited the lowest weight loss (3.65%) and decay (32.66%) on day 16 of storage, respectively. Edible coatings reduced the rate of deterioration in ascorbic acid, total phenolics, and anthocyanins in fruit over time. However, no significant difference observed between the coated and untreated strawberries in firmness and color characterization during storage. Coatings improved the sensorial attributes of the fruit during storage of which CMC coated strawberries exhibited the highest score.

Alginate

Alginate is a polysaccharide extracted from brown seaweeds of the Phaeophyceae class via alkaline extraction, followed by precipitation with either sodium or calcium chloride. It is composed of D-mannuronic acid and -L-guluronic acid and widely used in edible coatings. The U.S. Food and Drug Administration (FDA) classifies food-grade sodium alginate as generally regarded as safe (GRAS) and lists its usage as an emulsifier, stabilizer, thickener, and gelling agent. The European Commission (EC) lists alginic acid and its salts (E400–E404) as authorized food additives.

Three concentrations of freshly prepared coating solutions of sodium alginate (1, 2, and 3% w/v) have been evaluated on postharvest performance of mango cv. Mahali harvested at mature green stage (Rastegar et al., 2019). The fruits were immersed for 5 min in the respective coating formulations and placed as a single row in plastic crates which then stored at 15 ± 1°C, 85 ± 1% RH. Quality characteristics including acidity, ascorbic acid content and peel colour were not affected by the alginate treatments. In contrast, treatment with 3% alginate significantly reduced weight loss and maintained higher firmness (2-fold), total phenols (1.3-fold), and flavonoids content (1.7-fold), compared with the control. Higher antioxidant capacity was observed in 3% alginate treatments than the control.

Gum Arabic

Gum Arabic (GA), also known as Arabic gum is one of the biopolymers acquired from the branches and stems of Acacia trees (*Acacia spp*.). It is comprised of rhamnose, galactose, arabinose, and glucuronic acid with Ca, Mg, and K ions. It is commercially utilized as a food additive, because of its film shaping, emulsification, and encapsulation attributes. El-Gioushy et al. (2022) investigated the effects of edible coatings based on GA with cactus pear, moringa, and henna leaf extracts (10% GA; 10% GA + 10% moringa leaves extract; 10% GA + 10% cactus pear stems extract; and 10% GA + 3% henna leaves extract) on the storability and shelf life of guava (*Psidium guajava* cv. Maamoura). Fruit dipped for 2.5 min in respective coating solutions was stored in cold room (7±1 ^{o}C, 90 ± 5% RH) for 24 days. The combined treatments of 10% GA + 10% moringa leaf extract were the most effective coating for fresh guava which reduced weight loss, decay and *Rhizopus* rot infection (%), while also increasing marketable percentage, and delaying fruit softening. The treatment significantly retained total chlorophyll content, maintained vitamin C, acidity and TSS compared with untreated fruits during the cold-storage period.

In a study conducted by Tahir et al. (2018) revealed that GA (10 and 15% w/v) coatings maintained the total contents of phenolic, anthocyanin and TSS of strawberries (*Fragaria ananassa*) stored at 4±1^{o}C for 10 days. The treatment significantly ($p < .05$) retarded the increase in polyphenol oxidase (PPO) activity, reduced the weight loss, and completely inhibited the fungal infections. Out of the doses examined, 15% GA edible coating retained color, firmness, and increased antioxidant activity along with higher organoleptic quality compared to the control.

Shakir et al. (2022) investigated the effect of 10% GA, 0.5% CMC, and 10% GA+0.5% CMC coating formulations on postharvest quality attributes of tomato cv. Sahil F1 Hybrid harvested at turning stage of maturity. After the treatments, fruit were packed in clamshell PET boxes and stored for 20 days at 20 ◦C, 90±2% RH. Application of biocomposite hydrocolloid coating (10% GA+0.5% CMC) reduced the physiological weight loss, respiration rate, ethylene production, decay percentage and stress markers viz. malondialdehyde (MDA) and hydrogen peroxide (H_2O_2). It also inhibited the degradation of bioactive compounds (phenolics, ascorbic acid, and lycopene), retarded the rate of change of color, organic acids and soluble sugars. The composite treatment upregulated enzymatic reactive oxygen species (ROS) scavenging mechanism in tomato fruit more than GA or CMC alone coatings. Moreover, biocomposite coating delayed senescence by reducing activity of cell wall degrading enzymes and maintaining cell wall fractions.

Murmu and Mishra (2017) studied edible coating formulations based on GA (0 - 15 g/100 mL), sodium caseinate (SC) (0 - 2 g/ 100 mL) and Tulsi extract (TE) (0 - 5 mL/100 mL) on quality of guava stored at 28 ± 2 °C for 7 days. It was found that coating formulation containing GA concentration < 5 g/100 mL were too thin to slow down respiration, ripening, senescence, and mold growth whereas, those with GA concentration > 12 g/100 mL resulted

in too thick coating resulting in high rate of O_2 consumption, CO_2 evolution, water loss, higher firmness and adversely affected the overall acceptability of guava following 7 days of storage at 28 °C. The optimized coating formulation was 5 g/100 mL GA, 1 g/100 mL SC and 2.5 mL/100 mL Tulsi extract. Guava coated with the optimized formulation registered no mold growth and had a higher overall acceptability.

Khaliq et al. (2016) studied Mango (*Mangifera indica* L. cv. Choke Anan) dipped for 3 min in 10% GA and 3% calcium chloride (CA) alone or in combination was evaluated on physiological and biochemical quality attributes during storage at 6 °C, 90% RH for 28 days + 5 days at 25 °C. The combined treatment of 10% GA and 3% CA significantly alleviated chilling injury, reduced MDA content and electrolyte leakage compared to the control fruit. This treatment reduced the increase in H_2O_2 content, superoxide anion production rate and enhanced DPPH radical scavenging activity. Furthermore, 10% GA alone or in combination with 3% CA effectively inhibited the loss of total phenolic content and ascorbic acid. The result of transmission electron microscopy confirmed that treated fruit-maintained cell membrane integrity as a result of enhanced antioxidant defense system, thereby reducing oxidative damage and postharvest deterioration of treated mangoes.

Persian gum

Persian gum is an exudate polysaccharide from the trunk and branches of wild/mountain almond tree *(Prunus scoparia* Spach). This gum is a transparent, semi cloudy, odorless exudate and can be found in different shapes such as large granules, sugar crystals and powder with diverse colours varying from white to brownish red. It easily dissolves in water and has no odor. Khorram et al. (2017) studied a wide variety of coating formulations such as 1% Persian gum (PG), 5% gum Arabic (GA), 1% carboxymethyl cellulose (CMC), 0.5% beeswax (BW), 1% carnauba wax (CA) (w/v) to retain the fruit glossiness along with other postharvest quality attributes of Kinnow mandarins (*Citrus reticulata*). One of the reasons for fruit coating is increasing gloss to enhance the external appearance of them and 1% PG was the most effective treatment for increasing the glossiness of Kinnow mandarins.

Guar gum

Guar gum is a galactomannan rich flour, water soluble polysaccharide obtained from the leguminous Indian cluster bean *Cyamopsis tetragonoloba* (L.) Taub. The backbone of this hydrocolloid is a linear chain of D-mannopyranose units connected to each other by β-1,4-bonds linked to galactose residues by 1,6- bonds forming short side-branches. It is one of the most important thickeners and a versatile material used in the food industry. This galactomannan has similar properties as carrageenan, alginate, xanthan gum, and gum Arabic but guar gum has the advantage of being cheaper than the others. Saberi et al. (2018) studied edible composite coatings based on pea (*Pisum sativum*) starch and guar gum (PSGG), PSGG blended with lipid mixture containing the shellac wax (PSGG-Sh), and bilayer approach (PSGG as an internal layer and shellac as an external layer), on postharvest quality of 'Valencia' oranges stored at 5 °C for four weeks and at 20 °C for 7 days. The incorporation of lipid compounds into the PSGG coatings (PSGG-Sh) reduced fruit respiration rate, ethylene production, weight and firmness loss, peel pitting, and decay rate. Oranges coated with PSGG-Sh and a single layer PSGG coatings resulted in higher scores for overall flavor and freshness after four weeks at 5 °C followed by one week at 20 °C than uncoated fruit, as assessed by a sensory panel. However, although the bilayer coating reduced weight loss and respiration rate with improved firmness retention to a greater extent than the single layer PSGG coating, the bilayer coating resulted in higher levels of ethanol causing increased perception of off-flavors.

Ruelas-Chacon et al. (2017) studied the potential of guar gum edible coating on extension of shelf life and maintaining quality of tomato cv. Roma. Tomatoes harvested at light red stage of ripening were coated with 1.5% guar gum and stored for 20-days at 22 ± 2 °C, 40% RH. The treatment significantly enhanced fruit firmness while recording reduced weight loss, delayed changes on soluble-solids-content, retarded loss of total acidity, and decreased respiration rate compared to uncoated control.

Locust bean gum (LBG)

Locust bean gum is a polysaccharide belonging to the group of galactomannans, extracted from the seeds of carob tree (*Ceratonia siliqua*) and native to the Mediterranean region. Among a variety of biopolymers, LBG has been identified as an efficient protective coating component for FFVs preservation because of its selective permeability to O_2, excellent film forming properties, and its ability to act as an effective delivery matrix of different natural antimicrobial agents. Aloui et al. (2021) produced coatings based on three concentrations of LBG (1, 0.8 and 0.6%) by incorporating different concentrations of cutin monomers (0.2 and 0.4%) and/or cuticular wax (0.2 and 0.4%) extracted from tomato pomace. Their efficacy was evaluated in extending the shelf life of tomatoes (*Solanum lycopersicum*, cv. Cerasiforme) stored at 4 °C, 85–90% RH for 28 days. Coatings incorporating cutin monomers-based fraction were the most efficient in controlling weight loss, retaining firmness of coated tomatoes and reducing the growth of *Botrytis cinerea* by more than 55%.

Arabinoxylans

Arabinoxylans (AX) are important cereal non-starch polysaccharides constitute of a linear backbone of ß-(1-4)-linked D-xylopyrxylopyranosyl units to which α-L arabinofuranosyl substituents attached through O-2 and/or O-3. González-Estrada et al. (2017) studied incorporation of antagonistic yeasts *Debaryomyces hansenii* into AX based edible coating and evaluated its efficacy in preventing infection by *Penicillium italicum* that cause blue mold in Persian lime (*Citrus latifolia* Tanaka). Results revealed AX as a matrix compatible with *D. hansenii* maintained more than 97% viability of initial inoculum at temperatures (13 and 25 °C) examined. This may be due to polymeric matrix providing nutrients needed to sustain their survival. Preventive application of treatments was more effective than curative applications in controlling blue mold decay. This study demonstrates the potential application of bioactive AX coatings with *D. hansenii* as an alternative postharvest disease management tool. In addition, the applied coating was able to retain color and reduced the rate of weight loss.

Soy protein isolates

Soybean protein isolate (SPI) has excellent film-forming properties to prevent the migration of oxygen, carbon dioxide, and solute into the food matrix. SPI can be served as an ideal edible coating because of its low cost, availability and eco-friendliness. However, its hydrophilic property considerably leads to poor water-barrier performance (Li et al., 2019). Dave et al. (2017) studied the effect of optimized coatings of SPI, hydroxypropyl methylcellulose (HPMC), olive oil and potassium sorbate on quality parameters and shelf-life of pears (*Pyrus communis* L.) cv. Babughosha stored at ambient temperature (28 ± 5 °C , 60 ± 10% RH). The SPI based coatings retained the firmness of fruits, lowered the moisture loss, could also retain the levels of ascorbic acid, chlorophyll and sugars in the treated fruits. Activities of enzymes associated with fruit softening (β -galactosidase, polygalacturonase, pectin methyl esterase) showed delayed peaks. Amongst the treatments examined SPI 5.0%, HPMC 0.40%, olive oil 1%, potassium sorbate 0.22% (T1) and SPI 5.0%, HPMC 0.40%,

olive oil 0.98%, potassium sorbate 0.20% (T2) were found to have pronounced effect on retention of nutritional quality in pears. Observations of shelf-life extension established that T2 was successful in extending shelf-life up to 15 days, as compared to 8 days for untreated pear fruits.

Coatings produced from plant leaf extracts

Aloe vera

Aloe vera (*Aloe barbadensis* Miller) is a succulent plant belonging to the family Asphodelaceae. *A. vera* leaves have been used for many centuries for their therapeutic properties, and over 75 active ingredients have been identified in its gel. An enormous number of research articles published in the last decade evident that *A. vera* gel coatings act as a partial barrier to moisture and O_2, reducing the respiration rate, thereby preventing anaerobic conditions and conserving fruit quality. Moreover, its odourless and colourless properties along with eco and consumer friendly properties make it highly researched plant leaf extract during the last decade. Guava fruits (cv. Thai) when treated with *A. vera* gel 25% + Chitosan 1% exhibited 13 days of postharvest life at 22±2 °C and 70-85% RH compared to the uncoated control samples where the postharvest life was 6 days (Supa et al., 2024). Similarly, Hassan et al. (2022) studied 20 and 40% *A. vera* gel alone or in combination with 1% lemongrass essential oil (EO) as an edible coating for strawberries stored at 5 ± 1 °C, 90–95% RH for up to 16 days. Treatment with *A. vera* gel 40% + lemongrass EO 1% led to the lowest weight loss, retained firmness and acidity, but increased the TSS and total anthocyanins compared to uncoated fruits during storage. The antioxidant activity was relatively stable of the fruits coated with *A. vera* gel combined with lemongrass EO up to 8 days under the said storage conditions.

A study was conducted by Khaliq et al. (2019) to find out the effect of *A. vera* gel (at 50 and 100%) alone or enriched with *Fagonia indica* plant extract at 1% on physiological and biochemical responses of sapodilla (*Manilkara zapota* L.) fruit stored at 20 °C for 12 days. Sapodilla fruit treated with *A. vera* 100% and *F. indica* 1% significantly reduced weight loss, decay incidence, TSS, and kept a high level of firmness and TA compared to untreated fruit. Both doses of *A. vera* (50% or 100%) when incorporated with 1% *F. indica* were effective in maintaining higher ascorbic acid, total flavonoids, total phenolics and radical scavenging activity of sapodilla fruit alongside optimal sensory quality attributes. Significant reduction in weight loss (by 30%) and better sensory attributes were shown when jujube (*Ziziphus jujuba* Mill.) fruit were coated by *A. vera* 33% (v/v) after 40 days of storage at 4 ± 1 °C, 80% RH compared to the untreated control (Moradinezhad et al., 2018).

Chrysargyris et al. (2016) examined 0, 5, 10, 15 and 20% *A. vera* gel coating on fruit quality maintenance of tomato up to 14 days at 11 °C, 90% RH. Results showed that 10 and 15% *A. vera* coating reduced the fruit ethylene production. The ripening index (TSS/TA) decreased after 7 days of storage in 10% *A. vera* gel coated fruit, maintaining the overall quality of treated tomatoes. Increased ascorbic acid content was reported in the tomatoes treated with 10% *A. vera* gel. In the tomatoes coated with 20% *A. vera* gel total phenolics and antioxidative status were increased. However, the authors reported that fruit firmness, TA, weight loss, respiration rate and fruit colour properties (L*, a*, b*) did not differ significantly among treatments.

Moringa

Moringa (*Moringa oleifera* Lam., Fam: Moringaceae) is one of the most useful trees in the tropics and subtropics of Asia and Africa. The leaf, bark, sap, root, flower and seed extracts of moringa plants possess antimicrobial and antioxidant activities, contributed by a high

concentration of phenolics, vitamins and carotenoids. Vast potential of moringa plant parts to be used as an ingredient in the development of different functional food products is well documented (Ngcobo et al., 2024).

(Tesfay & Magwaza, 2017) studied the efficacy of 2% moringa leaf extract coating along and in combination with two concentrations of chitosan (0.5 and 1%) and two concentrations of CMC (0.5 and 1%) on avocado (*Persea americana*, Mill. cv. Fuerte and Hass) fruit quality attributes during cold storage (5.5 °C, 95±2% RH) for 21 days. Fuerte fruit treated with combination of 2% moringa + 1%CMC showed significantly lower weight loss (1.78±0.08%), electrical conductivity (192.0±3.0 S/m) and respiration rate (167.4±40.8 mg/kg/h) compared to the untreated control with respective values of 4.7±0.7%, 290.0±5.0 S/m and 290.0±62.0 mg/kg/h. The same treatment maintained higher fruit firmness (50.0±4.25 N), lower polyphenol oxidase and lipid peroxidation activities in the cv. Fuerte. Similar results were shown for the cv. Hass too where a combination of 2% moringa leaf extract with 1% of CMC reduced mass loss almost by 50%, while mannoheptulose (a rare seven-carbon sugar which acts as an intracellular glycolytic inhibitor and its concentration is high in unripe avocados), was maintained by 8-folds.

Olive

Olive (*Olea europaea*, Fam: Oleaceae) leaf extract (OLE) is known to possess high antioxidant and antimicrobial activities, and it is reported as effective against several diseases such as coronary disease, diabetes, and some bacterial infections. Zam (2019) studied the addition of OLE (1%) in to chitosan (1%) and alginate (3%) based coatings on the quality of sweet cherries (*Prunus avium* L. Cv. Bigarreau Burlat) stored at 25 ± 5 °C, 65 ± 5% RH. The ripening process and increase in anthocyanins were found to be delayed with coating treatment. Chitosan and alginate coatings when enriched with OLE showed retarded rate of deterioration in ascorbic acid and total phenolic contents at the end of 20 days of storage.

Cashaw

Cashaw (Prosopis juliflora, Fam: Fabaceae) leaves have traditionally been used for curing mouth and throat infections, and many other diseases such as bronchitis, ulcers and skin parasitic infections. In vitro studies conducted by Saleh and Abu-Dieyeh (2022) has shown the effectiveness of *P. juliflora* water-soluble leaves ethanolic (PJ-WS-LE) extracts against pathogenic fungi and bacteria. the efficacy of this treatment has been investigated in-vivo by spraying 8 mg/mL of PJ-WS-LE extract on cucumbers (*Cucumis sativus* cv. Qatari). Samples sprayed with 8 mg/ml of PJ-WS-LE extract increased cucumber shelf life stored at 22 °C by 77%. At week 2, cucumber coated with PJ-WS-LE extract showed less than 10 CFU/g of mold as well as 76.9% and 85.8% reduction of total yeast and aerobic bacterial counts, respectively. PJ-WS-LE extract treated samples maintained the slightest change in TSS and 2,2-DPPH free radical scavenging activity.

Lotus

Lotus (*Nelumbo nucifera* Gaertn) is a widely cultivated aquatic perennial plant, and its leaf powder is traditionally used as herbal tea in China (Fan et al., 2019). Investigations indicated that lotus leaves have strong free radical scavenging and antioxidant activity, and these capacities are related to their flavonoid and phenolic compounds. Furthermore, lotus leaves have been confirmed for the effects of anti-oxidation, antimicrobial over the past years thus widely used as food and drugs. Fan et al. (2019) studied the effect of composite coating of lotus leaf extracts (LLE - 0.25, 0.5, 1.0, 2.0, 4.0 g/l) in combination with film formers sodium alginate, konjae glucomannan and starch (22% amylose content) on quality of fresh goji fruit

(*Lycium barbarum* L.) during storage at ambient temperature. The best coating formulation was 0.25% LLE+1% film former (the blend mass ratio 2:3:3 included sodium alginate, konjae glucomannan and starch) + 1% glycerin + 0.5% $CaCl_2$ which is defined based on the effect of the weight loss and decay ratio on goji berries. Goji fruit coated with LLE incorporated coating had significantly lower weight loss, decay rate and MDA content than the blank control (uncoated) and the positive control (fumigated with 1- methylcyclopropene) after 9 days of storage. Moreover, LLE incorporated coating was found to be effectively maintained ascorbic acid, TA, TSS and superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) activities at higher levels compared to other treatments. LLE incorporated coating could extend the shelf life of goji berries by about 4 days compared to the uncoated samples.

Betel

Among the natural polyphenols, *Piper betel* L. leaf extract (PBE) is rich in phenolic acid derivatives and flavonoids, which exhibit excellent antibacterial and antioxidant performance. PBE has also been found to be efficient in imparting antibacterial and antioxidant functions to chitosan, sago starch, and polyvinyl alcohol. Pham et al. (2023) focused on developing pectin/agarose-based edible coatings (PeA), which was functionalized with PBE (20, 30 and 40%) and its efficacy has been evaluated by applying it on Chiquita bananas stored at 20 °C, 64 %RH. Scanning electron microscope (SEM) analyses showed that PeA enriched with 30% PBE (PeA-PBE- 30) coating effectively sealed and uniformly dispersed on the fruit skin. Besides, PeA-PBE-30 coating significantly reduced the respiration rate of fresh bananas during the 8 days of storage period. Protective efficacy of PeA-PBE-30 coating was exhibited by having reduced rate of weight loss, retention of TSS, TA and juice pH of treated bananas compared to uncoated fruits and other treatments.

Lipid based coatings

Carnauba wax

Carnauba wax is derived from leaves of palm trees (*Copernica prunifera* Mill.) which are native to the tropical rainforest of Brazil. It is a GRAS compound and its use as a promising ingredient in plant-based edible coatings are discussed below. Carvalho do Lago et al. (2023) reported physicochemical quality and antioxidant capacity of Valencia Late and Natal IAC sweet oranges coated with carnauba wax/wood resin (also known as rosins that are residues left after distillation of the volatile fraction of pine oil and turpentine from the crude resin of the pine trees) stored in a cold chamber at 5 ± 1 °C, 60–70% RH for 60 days. Carnauba wax/wood resin coating treatment efficiently delayed fruit color development and prevented weight loss for both sweet oranges cultivars up to 60 days. Moreover, the physicochemical (firmness, TSS, TA and ripening index) and sensory quality of wax-coated fruits were preserved during cold storage. It has been observed that uncoated fruits showed a decrease in juice content compared to the fruit treated of both cultivars.

Indian jujube (*Ziziphus mauritiana* Lamk. cv. Cuimi) were immersed in canauba wax (CW) coating, CW containing glycerol monolaurate (CW-GML) coating, or distilled water (uncoated control) respectively for 60 s at 20 °C (Chen et al., 2019). Compared to the control, both CW and CW-GML coatings reduced jujube weight loss, respiration rate, and ethylene production, maintained lower activities of cell wall degrading enzymes namely polygalacturonase, pectin methylesterase and cellulase resulting in delayed flesh softening. The two coatings also delayed the change of skin color and retained higher contents of chlorophyll and ascorbic acid. The CW-GML coating significantly inhibited the decay of jujube fruit and retained better sensory quality. After 12 days of storage at 20 °C, 60–70%

RH, the decay index was lowest in CW-GML-coated jujube followed by CW-coated jujube while the highest was shown by the control fruit.
Nazoori et al. (2023) studied carnauba wax (0.5, 1, 1.5%) and CMC (0.5, 1, 1.5%) on postharvest quality, decay, physiological disorders, and antioxidant properties of pomegranate cv. Shirin Shahvar. The fruits were dipped for 5 min in the respective treatments and stored at 4 ± 0.5 °C, RH 85 ± 5% for 150 days. The carnauba wax treatment was effective in preserving the anthocyanin, flavonoid, and firmness of fruit while minimizing peroxidase and polyphenol oxidase activities.

Candelilla wax

Candelilla wax (CW) is derived from *Euphorbia antisyphilitica* Zucc., which is grown in the arid regions of northern Mexico and southern United States. Oregel-Zamudio et al. (2017) developed an edible film made of CW added with biocontrol bacteria to prolong the shelf life of strawberry. CW-based coatings were applied on fresh strawberries, inoculated with *Bacillus subtilis* HFC103, to evaluate their antifungal efficacy against *Rhizopus stolonifer*. Strawberries were immersed in the respective emulsion (without coating and non-inoculated), film (CW-based edible coating), bacteria (inoculated with *B. subtilis* HFC103) and film + bacteria (CW-based edible coating + *B. subtilis* HFC103) at 25 °C for one second and were allowed to dry at 25 °C for 15 min. The results showed that the combination of CW-based edible coating + biocontrol bacteria (*B. subtilis*) as a promising alternative for the reduction of postharvest decay caused by *Rhizopus stolonifer*.

Lacquer wax

Lacquer wax is an important fatty resource obtained from the mesocarp and seed of the berries of *Toxicodendron vernicifluum* which belongs to family Anacardiaceae. It contains hexadecanedioic acid, eicosanoic acid and dodecanedioic acid which contribute to its elasticity. Therefore, unlike general wax such as palm wax, insect wax and beeswax, lacquer wax is more elastic and softer in performance making a wide range of applications in different fields of the food, pharmaceutical and medical industries. Lacquer wax has also been applied in the field of high-end cosmetics due to its good moisture retention ability. In China, it has been used as edible vegetable oil for thousands of years. Hu et al. (2019) explored the effectiveness of lacquer wax coating (immersed in 1, 2, and 3% for 30 s) solutions in retarding the postharvest senescence of kiwifruit (*Actinidia deliciosa*) cv. Xuxiang. After the treatments fruits were stored at ambient temperature (22–26 °C, 45–60 % RH) and compared its effect with chitosan coatings. Results indicated that, apart from the effectiveness against the decrease of weight loss, coated kiwifruit exhibited slower ripening than uncoated samples, as indicated by inhibited loss of firmness, organic acids, and antioxidant activity, as well as decreased respiratory rate, and a delayed increase in the level of ethylene, MDA, and sugar. In addition, 2% lacquer wax exerted the same effect as 3% chitosan did in delaying kiwifruit senescence. These results suggest that lacquer wax coating is an effective alternative for prolonging the postharvest life of kiwifruit.

Rice bran wax

Rice bran wax (RBW) is a secondary by-product separated from the rice bran oil during the process of refining. It has good applicability in the formation of lipid-based edible coatings, after refining and removal of crude resinous matter. Being a hydrophobic substance, it avoids moisture loss from the product during storage and protects the commodity from microbial infestation. The RBW-based coating could prevent moisture loss from FFVs, retard the rate of metabolic activities thus delay the ripening process by modifying the immediate environment

of the product. Abhirami et al. (2020) examined the effect of RBW (5, 10, and 15% W/V) on shelf-life extension of tomato cv. Marutham CO3. Tomatoes of uniform colour (30-60% pink) and medium size were dipped 2-3 min in the respective coating solutions and stored at 32.7–34.4 °C and RH of 57.5- 88.3%. The results showed that 10% RBW emulsion effectively suppressed the rate of water loss, reduced the rate of deviation of TSS and fruit firmness from the initial values while reducing the rate of change of lycopene content compared to the uncoated fruit. RBW coating hindered the gas exchange recording a reduced rate of respiration compared to the uncoated tomatoes. Overall, the 10% RBW coated tomatoes had a shelf life of 27 days, compared to 18 days of the control samples.

Plant extracts, particularly essential oils offer great functionalities in biopolymer-based composites, for improving barrier properties as well as bioactivities such as antioxidation and antimicrobial properties. The following content describes the essential oils derived from wide range of plant materials and their efficacy in maintaining postharvest life of FFVs leading to loss reduction.

Olive oil

Dovale-Rosabal et al. (2015) evaluated the influence of chitosan-olive oil coatings (1% Ch+2% olive oil, 1% Ch+ 4% olive oil, 2% Ch+ 2% olive oil, 2% Ch+ 4% olive oil) on the quality of tomatoes cv. Charleston harvested at breaker stage. Coatings from chitosan-olive oil emulsion delayed the ripening and maintained the firmness of tomato cv. Charleston with respect to uncoated fruit, contributing to extend their shelf life during storage at ambient conditions (27±1 °C and 80% RH). However, the coating did not act as an effective barrier against weight loss as evidenced by higher weight losses of tomatoes coated with chitosan at 2% (w/v) with 2 and 4% (v/v) of olive oil.

Castro-Cegrí et al. (2023) studied dextrin (a natural polysaccharide obtained from potato starch) based edible coating enriched with oleuropein (major phenolic component in olive leaf extract) and olive oil on postharvest quality of Zucchini (*C. pepo* L. morphotype Zucchini). Freshly harvested fruits were dipped in dextrin (D), 1% (w/v) dextrin plus 0.3% (w/v) oleuropein (DO) and 1% (w/v) dextrin plus 0.2% (v/v) extra-virgin olive oil (DOO) for 5 min and then stored at 4 °C and 85–90% for 14 days. The application of dextrin coatings improved the storability of zucchini fruit at low temperature, maintaining fruit quality, increasing antioxidant defense, and diminishing oxidative stress. The addition of oleuropein and olive oil to the dextrin coating showed higher induction of antioxidant enzymes, and a greater accumulation of ascorbate and total phenolics.

Cinnamon essential oil

Cinnamon essential oil (CEO) is a yellow to reddish brown clear liquid with the unique pungent aroma of cinnamon. CEO has been proven to be an excellent antioxidant with broad-spectrum antimicrobial effect, with its main component being cinnamaldehyde. Use of CEO has been limited due to its instability and poor water solubility and prevalence of strong odours upon its application on FFVs.

CEO at concentrations of 1 and 2% and gum Arabic (GA) at concentrations of 5 and 10%, and the combination of GA and CEO for quality improvement of guava stored in cold storage (10 ± 1 °C, 90–95% RH) for 28 days were studied by Etemadipoor et al. (2019). The results showed that GA treatment enriched with CEO preserved color, firmness, chlorophylls, carotenoids and showed a slower rate of deviation of juice pH and soluble solids content. GA enriched with CEO maintained the qualitative characteristics of guava fruit by reducing the ripening rate. Ascorbic acid content decreased with a lower rate due to intensified antioxidant properties when enriched with CEO, which is rich in phenolic compounds such as trans-

cinnamaldehyde. They observed that 10% GA enriched with 1% CEO showed more water vapor barrier suppressing weight loss more than other treatments and maintained the quality and storability of guava fruit.

Spearmint essential oil

Spearmint (*Mentha spicata*, Fam: Lamiaceae) EO are widely used in the food, cosmetic, confectionary, chewing gum, toothpaste, and pharmaceutical industries. It has been reported to be an effective antibacterial and antioxidant agent that inhibits the growth of spoilage microorganisms contributing to extending the shelf life of different foods. Shahbazi (2018) examined the effects of antimicrobial active packaging based on 1% CMC and 1% chitosan coatings containing two different concentrations of *M. spicata* EO (MSO 0.1 and 0.2%) on physicochemical (weight loss, titratable acidity and pH); microbial (total viable count, psychrotrophic bacteria, yeasts and molds); sensory (appearance, color, texture and overall acceptability) properties; and respiration rate of strawberries during storage under refrigerated condition (4 ± 1 °C). The treatment of fruits with chitosan + MSO 0.2% and CMC + MSO 0.2% resulted in the best microbial, physicochemical and organoleptic properties after 12 days storage. The final population of *Listeria monocytogenes* in treated samples was decreased by 3.92–3.69 compared to control groups.

Pomegranate seed oil

Pomegranate seed oil (PSO) contains high levels of antioxidant activity and has been shown to have an antimicrobial effect on gram-negative and gram-positive bacteria. Melikoğlu et al. (2022) studied the effect of CMC enriched with different concentrations (0.1, 1, 2, and 3%) of PSO on postharvest quality attributes of strawberry cv. Emiralem. The fruits were dipped in respective coating solutions for 1 min and stored in the refrigerator (5 ± 1°C, 50 ± 5% RH) for 16 days. The addition of PSO contributed significantly to the improvement of moisture barrier properties of CMC films. The 3% PSO-enriched CMC coating showed higher efficacy in preserving weight loss, color, pH, and total phenolic content of strawberry cv. Emiralem during the storage period.

Tea seed oil

Tea seed oil (TSO) is a natural oil extracted from seeds of tea tree (*Camellia sinensis*, Family: Theaceae). Research evidenced that it contains 19.88% oil which is composed of unsaturated fatty acids, (70% oleic acid, 10% linoleic acid), and high levels of antioxidants such as phenolics along with many vitamins and minerals. It is used for many purposes such as cooking, antioxidant agents, medicine or biodiesel. Tran et al. (2021) studied different concentrations of TSO (0.05, 0.25, and 0.5% w/v) in combination with 1% chitosan (CH) on postharvest quality of pears cv. Kosui dipped for 1 min and stored at 25 °C,85 ± 5% RH for 21 days. CH incorporated with TSO showed a downward trend in respiration rate and showed significant differences in some biochemical properties such as TSS, pH, and color. Adding TSO enhanced the antifungal ability both in vitro and invivo during the storage of pears inoculated with a spore suspension of *Botrytis cinerea*.

Citrus fruit peel oil (Citral)

Citral is a main component of citrus fruit peel oil. It is a mixture of neral and geranial which are monoterpene aldehydes. Citral has been applied to food, cosmetics, and beverages as a natural ingredient for its passionate lemon aroma and flavor. EOs, which present citral have been demonstrated to show antimicrobial, antifungal, and antiparasitic characteristics, accomplishing citral a natural preservative and potential candidate in edible coatings.

Guerreiro et al. (2015) studied the different concentrations of alginate-based edible coatings enriched with citral (Cit) and eugenol (Eug) on fruit of the strawberry tree (*Arbutus unedo* L.) for enhancing the postharvest quality, safety and shelf-life extension. Arbutus berries were dipped in different concentrations of alginate (1%), Cit (0.15 and 0.3%) and Eug (0.1 and 0.2%) alone or combined solutions for 2 min. Results showed that combined treatment of alginate 1% + Cit 0.15% + Eug 0.10% preserved sensory and nutritional attributes and reduced microbial spoilage of arbutus berries compared to the other treatments and the control samples stored at 0.5 ^{o}C.

Plant byproduct incorporated edible coatings

Pomegranate peel extracts

Pomegranate peel is considered an important source of phenolic compounds namely gallic acid, ellagic acid, punicalagin A, punicalagin B and other tannins. In the domain of plant-derived compounds with antimicrobial potential, pomegranate peel extract (PPE) has been extensively investigated for its free radical scavenging effect and strong antioxidant capacity caused by the high concentration of biologically active components. Kumar et al. (2021) studied the effect of chitosan–pullulan (50:50) composite edible coating enriched with PPE on postharvest physicochemical characteristics of Safeda mango at ambient (23 °C, RH-45%) and cold (4 °C, RH-95%) storage conditions for up to 18 days. The chitosan–pullulan composite edible coating enriched with 5% PPE recorded significant retention of postharvest characteristics such as color, TSS and TA along with reduced physiological loss in weight compared to the control. Incorporating PPE in edible coating formulation enhanced the phenolic, flavonoid contents and antioxidant activity of coated mango and extended the postharvest life by 9 days compared to the control.

Use of edible coatings namely chitosan (CH) and locust bean gum (LBG) incorporated with chemically characterized water PPE (WPPE) or methanol PPE (MPPE) to control the growth of *Penicillium digitatum (green mold)* of orange has been reported by Kharchoufi et al. (2018). The results proved that the addition of 0.361 g dry WPPE/mL, both to CH and LBG coatings, significantly reduced disease incidence by 49 and 28% respectively, with respect to the controls.

Apple peel polyphenols

Riaz et al. (2021) investigated the efficacy of chitosan (CH)-based apple peel polyphenols (APP) composite coatings to enhance the storage quality of strawberries (*Fragaria ananassa* cv Hongyan). Strawberries were coated with CH alone (0.0% APP), CH-APP1 (0.25% APP), CH-APP2 (0.50%), CH-APP3 (0.75%), CH-APP4 (1.0%). After dipping the strawberries in respective coating solutions for 30 s they were stored at 20°C, 35–40% RH. The results showed that CH-APP composite coatings inhibited the deterioration in total flavonoids and total anthocyanin while delayed fruit senescence. The weight loss and decay percentage were reduced to 10.91% and 19%, respectively, in the CH-APP4 treatment group. The highest value of TSS (6.8%) was observed in the CH-APP3 treatment group on the 6th day of storage. Coatings with CH-APP4 exhibited the maximum TA content (0.78 g/100 g) as compared to the CH only (0.68 g/100 g). The total phenol content was highest (1.3 mg/g) in the CH-APP2 treatment group at the end of storage.

Grape seed extracts

Grape seed is a byproduct of the juice or wine industry, and its extract has shown promising antioxidant and antimicrobial activities. It contains high amounts of polyphenols, quinones, flavonoids, catechin, epicatechin, gallic acid, proanthocyanidins, and alkaloids, which have

increased its antimicrobial properties against both gram-negative and gram-positive bacteria by membrane-disruptive effects. Emamifar et al. (2019) studied the effect of salep gum (*Orchis mascula*, obtained from dried and milled tuberous wild orchids) solution (SS) and grape seed extract (GSE) based edible coating on shelf life of strawberry cv. Parous harvested at maturity stage of 80% red colour. Fruits were dipped in coating solutions of 1.5% SS incorporated with three concentrations of GSE (0.5%, 1.5% and 3%) for 5 min. After that fruits were stored at 1 °C, 95% RH and evaluated for treatment efficacy at 4-day intervals up to 20 days. SS along with GSE showed synergistic effects on the quality of strawberries. The best coating formulation for retarding the microbial growth of fresh strawberries during cold storage were 1.5% SS + 3% GSE that kept the microbial load of fresh strawberries below the limit of microbial shelf life (5 log CFU/g) up to 20 days. It is also reported the lowest weight loss and TSS, and the highest firmness, TA and ascorbic acid content compared to uncoated fruit up to 20 days of cold storage. Furthermore, as the concentration of GSE in the coating formulation increased (0.5 to 3% as examined in the study), anthocyanin, total phenolic contents, antioxidant activity and SOD activity increased while POD activity decreased compared to uncoated strawberries. Moreover, the sensory evaluation showed that 1.5% SS + 3% GSE increased shelf life of fresh strawberries up to 20 days without any negative effects on their sensory attributes.

Edible coating formulae with grape juice (GJ) and cross-linked maize starch (CLMS) enriched with three different doses of GSE (0.5, 1 and 1.5%) were examined for the postharvest quality attributes of strawberries during storage at 4 °C for 12 days (Yıldırım-Yalçın et al., 2022). With increased amount of GSE in the coating formulation, it caused a significant decrease in microbial counts and retarded the rate of firmness loss of treated strawberries. Formula containing GJ (16.15:83.85 of GJ: water volume ratio) and 1.5% GSE had the lowest ($p \leq 0.05$) total mesophilic aerobic bacteria counts and yeast/mold counts respectively. GSE enriched coatings significantly suppressed the rate of deviation of initial color, preserved ascorbic acid, total anthocyanin, total phenolic content, and antioxidant capacity of strawberries during storage. However, they have reported that high amounts of GSE coating decreased the sensory attributes of strawberries.

REGULATORY ASPECTS OF EDIBLE COATINGS

Edible films are regarded as intrinsic components of the food they contain hence, every single component that is utilized for making edible films must follow food safety and quality laws and regulations. According to European Union Regulation No. 1935/2004, all food-contact ingredients and commodities must fulfil the four fundamental standards: (a) they must be made in accordance with excellent manufacturing practices; (b) they must not alter the food's color, odor, taste, or texture; (c) the material must not have an adverse effect on the composition of food, and (d) it must not endanger to human health (Armghan Khalid et al., 2022).

Each country has its own requirements regarding the permitted additives (ED, 1995, USDA—U. S. Food and Drug Administration, 2006). According to the US requirements, organic acids—acetic, lactic, citric, malic, propionic, tartaric, and their salts—are accepted as safe and applicable for general use. On the other hand, most of the essential oils used in the food, pharmaceutical, and cosmetic industries are also classified as safe and approved for use as food additives. In Sri Lanka, it's mandatory to comply with the Food Act No.26 of 1980 and should be adhered to the amendments made recently such as Food (Additives - General) Regulations 2019 and Food (Preservatives) Regulation, 2019.

CONCLUSION

The scanning of literature published during the last decade in the domain of PBECs showed that a wide range of compounds have been researched commencing from starches, proteins, lipids, gums & waxes, plant leaf extracts, essential oils, byproducts of plant processing industries. Use of PBECs in preserving quality and shelf life of harvested FFVs showed positive impact on retardation of rates of respiration, ethylene emission, physiological weight loss, colour degradation of which controlling the said properties are imperative in extending the shelf life of fresh produce. Further, due to high occurrence of antioxidant properties, PBECs have contributed to increased antioxidant defense system by upregulating enzymes (SOD, CAT, POD) involved in ROS scavenging mechanism resulting in reduced electrolyte leakage and MDA content and alleviated chilling injury. Plant based active ingredients suppressed the cell wall degrading enzymes such as PME, PGU and cellulase maintaining cellular membrane integrity. The influence on aforesaid properties resulted in maintaining fruit firmness for an extended period along with internal biochemical quality attributes and sensorial properties. Moreover, by reducing the rate of degradation of phenolic, flavonoids and ascorbic acid contents of treated FFVs PBECs have shown promising results in suppressing the growth of decay causing organisms of which one of the major causes of postharvest losses.

The key innovative areas in the field include (i) development of composite coatings to enrich functional properties to the FFVs for the benefit of consumers (ii) overcoming limitations such as altering flavor of the food items when coated, (iii) minimize susceptibility to degradation of their properties upon exposure to light, oxygen and high temperature, (iv) increase effectiveness with sustained release of active substances and (v) effective stabilization of the developed emulsion excluding hazardous emulsification agents which have been achieved by means of formulating them as hydrocolloid bio-composites. Moreover, preparation of edible coating formulations in powdered form instead of liquid form by freeze or spray drying techniques have been investigated which aid in preserving their properties for an extended period while providing a convenient way of use, ease of storage and transportation due to the weight and volume reduction.

FUTURE TRENDS

The key problem which requires future study is extending the storage life of FFVs without compromising their sensory and nutritional properties. Several researches including innovative and cost-effective food packaging for fresh produce are still at the early phase of study and at inquiry phase prior to large-scale industrial implementation. The formation of innovative edible coating materials is predicted to come from a detailed examination of biochemistry and its relationships with antimicrobial, physicochemical, and possible toxicity, as well as risk assessment. Primarily, more advancement is needed in terms of coating materials, particularly in terms of nutritional value, mineral migration and efficiency, ratio of cost-effectiveness, and current technological protocols to ensure and enhance storage life and preserve the quality of coated FFVs.

Conflict of interest

The authors have no conflict of interest in reporting.

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Low 3,6-dichloro-o-anisic acid concentration application inhibits calyx senescence and maintains Valencia Late oranges' postharvest quality during ambient storage in Ghana

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ABSTRACT

Purpose: This study determined whether postharvest application with low 3,6-dichloro-o-anisic acid concentration could inhibit calyx senescence and preserve internal and external qualities of Valencia Late oranges during extended storage. **Research method:** The experiments were conducted using a completely randomized design with three replicates of 50 fruits for each treatment. The oranges fruits were dipped in four treatment concentrations; control (0), 0.01, 0.03, or 0.05 mmol L^{-1} in experiment 1, and control (0), 0.003, and 0.01 mmol L^{-1} in experiment 2 for one minute. Post-treatment, the oranges were kept in solid cardboard boxes as individual treatment units (n = 50 fruits) with three units comprising a treatment and held at an ambient temperature (25 ± 2°C) and a 60%–65% RH. Oranges were evaluated every seven days for four weeks. **Findings:** The results showed that fruit dipped at 0.01 mmol L-1 for both experiments resulted in lower calyx browning and drop, weight loss, and fruit firmness compared to control and higher dicamba concentrations. Moreover, the treatment delayed the increase in total soluble solids and the decrease in titratable acidity, slowing the maturation rate. **Research limitation:** This study could not evaluate fruit carbon dioxide and ethylene production during storage to understand their impact on other quality changes due to lack of Gas Chromatography machines in the resident laboratory. **Originality/Value:** The results demonstrate the effects of dicamba treatments in delaying detrimental calyx changes and retaining fruit integrity during storage.

INTRODUCTION

Calyx browning and drops of citrus fruit are major external quality parameters that consumers often consider during the purchase. Consumers usually relate these external quality parameters to fruit's internal qualities, although these quality factors may not relate in some situations after harvest. This effect can significantly influence the commercialization of citrus fruit for the fresh market (Carvalho et al., 2008). Calyx senescence in citrus fruit mostly influences fungal attachment on the abscission zone of the citrus fruit which can result in fruit decay. Several plant growth regulators have been applied to citrus fruit to prevent calyx deterioration. The auxin, 2,4-Dichlorophenoxyacetic acid (2,4-D) is a synthetic plant growth regulator, which is often used in the citrus postharvest industry to prevent calyx senescence and maintain quality. However, the use of 2,4-D is now prohibited in the citrus industry in many countries for human safety and environmental concerns (Peterson et al., 2016). Therefore, there is a need to find an alternative to 2,4-D, to preserve the postharvest quality of citrus fruit calyxes.

Pre-harvest use of synthetic auxins, such as 3,5,6-trichloro-2-pyri-dyloxyacetic acid (3,5,6-TPA) increases fruit size, peel, pulp, juice, and acid contents and prevents fruit abscission (Agustí et al., 2002). Some researchers have found that 3,5,6-TPA and 2,4-dichlorophenxoyacetic acid isopropyl ester, reduce calyx changes of Clementine cultivars caused by ethylene degreening (Carvalho et al., 2008), but are less effective than 2,4-d. A previous study reports that fluroxypyr, which has lower toxicity than 2,4-d, significantly reduced calyx senescence rates in mandarins and orange fruits compared with control and 2,4-D treatment (Ma et al., 2015). The authors did not find a significant effect of the treatment on TA, TSS, and TSS/TA ratio in the juice of Satsuma mandarin, Newhall navel orange, and Olinda Valencia orange fruit.

A selective herbicide (3,6-dichloro-o-anisic acid) used to control a wide spectrum of broadleaf weeds and woody plants is registered for use in agriculture and other applications (EPA, 2009). In agricultural applications, dicamba is registered for use on rye, asparagus, barley, corn, oats, soybeans, sugarcane, and wheat. Dicamba is also registered for use on golf courses, residential lawns, and rights-of-way along utility lines, roadsides, and railways. At low doses, dicamba has similar hormonal properties to natural auxins (Kelley & Riechers, 2007). Reports have shown that high concentrations of dicamba in plant tissues induce abnormal and uncontrollable growth, disrupting normal plant functions, and resulting in death (Caux et al., 1993). This auxin is a class of phytohormones that are involved in plant developmental processes that occur at the cellular level, affecting cellular elongation and turgor, as well as cellular differentiation and division (Kelley & Riechers, 2007).

A plethora of studies have been conducted on the effectiveness of a range of compounds for their ability to retain the postharvest keeping quality of citrus fruits (Strano et al., 2022). Cronjé et al. (2005) investigated the effects of ethylene antagonists aminoethoxyvinylglycine (AVG) and 1-methylcyclopropene (1-MCP) (Shikwambana et al., 2023) and the synthetic auxin analog 1-naphthalene acetic acid (NAA) on citrus fruits and found that AVG maintained fruit firmness, however, did not have significant effect on calyx quality while NAA application led to an increased in citrus calyx abscission. However, a lower 1-MCP concentration (100 ppb) maintained calyx retention but had a desiccating effect on the calyx whereas higher concentrations (500 ppb) resulted in higher calyx senescence (Ali et al., 2016; Cronjé et al., 2005). In other investigations, Carvalho et al. (2008) applied 3,5,6-TPA on a range of Clementine mandarin cultivars and found a significant reduction in calyx senescence without detrimental effects on internal fruit quality. Sdiri et al. (2013) compared the efficiency of 2,4-D to S-ethyl-4-chloro-O-tolyloxythioacetate (MCPA) and 2-(4-amino-3,5-

dichloro-6-flfluoropyridin-2yl) oxyacetic acid to maintain citrus fruit quality and reported that these treatments did not exceed the performance of 2,4- D, which is consistent with the finding of Alhassan et al. (2020) where 3,56-TPA delayed calyx senescence of citrus. Currently, there is no reported study of dicamba on citrus calyx senescence and internal quality during ambient storage (25 ± 2 °C) in any country, thus knowledge of its efficacy in maintaining fruit quality could garner support for its approval for postharvest application. Therefore, the objective of this study was to investigate the effects of the postharvest application of dicamba on calyx senescence and internal quality factors of Valencia Late oranges during ambient storage.

MATERIALS AND METHODS

Two experiments were conducted to accomplish the objective of this study. The same experimental design was applied for both experiments with the same replications in each case. The treatments were four (4) and three (3) concentrations for the first and second experiments, respectively. The same parameters were experimented on in each case of the two experiments.

Experiment 1

Experimental design, replications, and treatments

The experiment was conducted using a completely randomized design (CRD) applying four (4) treatment concentrations and three (3) replicates of 50 fruits for each treatment. The dicamba concentrations applied were 0 (control), 0.01, 0.03, and 0.05 mmol L^{-1} and the fruits were dipped in the solutions for 1 min each. The zero concentration was only water which was considered a control. The fruits were air-dried and kept in solid cardboard boxes as replicates (n = 50 fruits) with three replicates comprising a treatment. The cardboard boxes were placed on benches in the postharvest laboratory at ambient temperature (25 ± 2 °C) with relative humidity (RH) of 60-65% to stimulate shelf-life. Both external and internal fruit quality was determined on initial fruit condition and every 7 days for 4 weeks.

Source of experimental plant materials

Mature Valencia Late oranges (*Citrus sinensis,* L. Osbeck) were obtained at the commercial maturity stage; when the TSS and TA levels of fruits are greater than 8.5% and 0.4% citric acid respectively (Ritenour, 2015), with calyxes from a farmer from Obuasi in the Ashanti region of Ghana. The fruits were packaged in corrugated cardboard boxes and transported to Dr. Hilla Limann Technical University Postharvest Laboratory in Wa in the Upper Region of Ghana for investigation. Immediately after arriving the fruits were sanitized with commercial sodium hypochlorite solution (25 ppm) then cleaned, sorted, and sized.

Sampling of experimental plant materials

Healthy orange fruit samples (Citrus *sinensis* L., Valencia variety) were used for the experiments. In total, one thousand four hundred (1400) fruits free of undesirable characteristics such as physical damage, mould, and polluting components were used for the experiments to avoid contamination and physiological changes (Tiencheu et al., 2021). The oranges were transported in plastic crates at an ambient temperature (26 ± 1 °C) to the experimental location for the physiochemical analyses and shelf-life studies. The samples were sorted into two groups; intact and defective fruits. Fruit with their calyxes intact were used for the study. The sampling procedure was according to a study with similar purposes on Navel oranges (Alhassan et al., 2022).

Evaluation of calyx changes

One hundred and fifty fruits were visually examined for calyx senescence (browning and abscission) with different treatment concentrations. The Browning index of citrus calyx was scored according to the method of Li et al. (2018) and Alhassan et al. (2022), where; 1, no browning; 2, browning less than 1/4 of the total area of the calyx; 3, browning less than 1/2 of the total area of the calyx; 4, browning less than 3/4 of the total area of the calyx; and 5, browning more than 3/4 of the total area of the calyx. Calyx browning was calculated using the following formula (1); browning level of the calyxes in each replicate divided by the total number of calyxes evaluated. Calyxes that had dropped were counted during each assessment day and were classified as abscised calyx (Sdiri et al., 2013). Calyx abscission was expressed as a percentage.

$$\text{Calyx abscission} = \left(\frac{\text{The number of calyx abscissed}}{\text{Total number of fruits in the sample}}\right) \times 100\% \quad (1)$$

Determination of fruit weight loss

Weight loss of fruits was measured according to the previous report by Chaudhary et al. (2012). The weight of each sample was recorded on initial weight using an electronic analytic weighing scale (Model Kean &Sohn GmbH, D-72336, Germany) every seven (7) days for four (4) weeks. Weight loss during storage was determined by weighing three groups of the samples in each treatment. The percentage weight loss of the fruits was calculated as (2):

$$\text{Weight loss (WL)} = \left(\frac{\text{Initial weight} - \text{Final weight}}{\text{Final weight}}\right) \times 100\% \quad (2)$$

Fruit firmness determination

A sample of twenty-five (25) fruits from each replicate of every treatment was determined on two opposite sides of the equatorial zone of each fruit with a Digital Fruit Firmness Tester (Bareiss-HPE III Fff, ABQ Industrial, The Woodlands TX, USA) (Dasgan et al., 2024). The evaluation was performed by depressing a test anvil against the skin of the orange fruits to measure the resistance against controlled spring pressure (Singh & Reddy, 2006). The data were expressed as the average firmness of 75 fruits for each treatment and expressed in Newton (N), according to Alhassan et al. (2024).

Measurement of fruits' internal qualities

The TSS of the oranges was determined using a handheld digital refractometer (Atago Co. Ltd., Tokyo Atago, Japan) as described by Alhassan et al. (2024). Six fruits from each treatment (three replicates) were juiced and filtered through two layers of gauze to retain the solid particles (Tiencheu et al., 2021). The juice samples were put in the prism to the refractometer and readings were taken. Data were expressed as average percentage °brix. The titratable acidity level of the oranges was measured according to the method described by Ercisli and Orhan (2007). Five (5) mL of the juice sample was measured into a 10 mL graduated cylinder and was transferred into a clean Erlenmeyer flask—approximately 100 mL of distilled water. Five (5) drops of phenolphthalein were added, followed by titration with 0.1N sodium hydroxide (NaOH) solution until a light pink colour was obtained. The data were expressed as citric acid. The fruit maturity index (MI) was calculated using the TSS/TA ratio (Alhassan et al., 2022).

Experiment 2

To find an optimal treatment concentration to maintain the citrus fruit quality, in experiment 2, the orange fruit was obtained from the same source and a similar was followed as in experiment 1, however, 0.003 mmolL^{-1} was compared with 0.01 mmol L^{-1} dicamba concentration. This lower concentration (0.003 mmol L^{-1}) was applied because in experiment one 0.01 mmol L^{-1} maintained the fruit's internal and external quality, hence it is thought that 0.01 mmol L^{-1} should be compared with a further lower concentration (0.003 mmol L^{-1}). Storage temperature (25 ± 2 °C) and relative humidity (60-65%) were monitored using TinyTag data loggers (TinyTag View 2, Gemini Data Loggers, UK) throughout storage. Both exterior quality (calyx browning and calyx abscission) and internal quality factors of fruit were evaluated on the initial day, then subsequently every seven days' intervals for 4 weeks, as done in experiment one.

Statistical analysis

The data of this experiment were statistically processed using the SPSS version 24.0 software package (SPSS, Chicago, IL, USA). The two-way analysis of variance (ANOVA) was performed, and Fisher's test was used in the analyses to determine the Least Significant Differences (LSD) among treatment means at a significance level of $p \leq 0.05$. Separate analyses were carried out with the data for each of experiments one and two. The errors for these ANOVAS were tested for homogeneity of variances (Snedecor & Cochran, 1980) and found to be statistically ($p > 0.05$) not different, so the results for the different experiments were pooled for analysis.

RESULTS AND DISCUSSION

Fruit calyx browning

Evaluation of Valencia Late oranges calyx browning was based on subjective score criteria. There was a significant effect of the treatment and storage time on the calyx browning of the fruits ($p < 0.05$), with a significant interaction of these factors during storage. Low dicamba concentrations significantly delayed fruit calyx browning compared to control and higher dicamba concentrations. Increasing the concentration of the auxin treatment increased calyx browning of the fruit, but still had a lower rate of browning of fruit compared to control after the twenty-eight (28) days of storage. Orange fruit dipped in 0.01 mmol L^{-1} dicamba concentration was the most effective in reducing calyx senescence to 1.8 compared to the other concentrations. As indicated in Figure 1, almost all calyxes of the fruits under the control treatment were brown (3.6 out of a best score of 1) at the end of storage, and signals of rot around the calyx stalk ends of some fruits. Calyxes of fruits treated with dicamba were still green and fresh, particularly, fruit treated with 0.01 mmol L^{-1}, but there was no significant difference between this concentration and 0.03 mmol L^{-1} which had a score of 2.0 at the end of storage.

In experiment 2, dicamba treatment had a significant effect on Valencia Late oranges calyx browning compared with untreated fruit. Pre-storage dipping (dipping in solution before storage) with 0.01 mmol L^{-1} dicamba treatment was more efficacious in delaying calyx browning score of 2.5 relative to the effect of 0.003 mmol L^{-1} dicamba concentration of 2.1. The results demonstrate that 0.01 mmol L^{-1} dicamba concentration reduced calyx browning better than the control fruit (3.7 score), suggesting that this concentration may be optimal for delaying calyx browning of Valencia Late oranges (Table 1). Previous studies indicate that at low doses, dicamba has similar hormonal properties to natural auxins (Kelley & Riechers, 2007). Other researchers demonstrated that higher dicamba concentrations in plant tissues

induce abnormal and uncontrollable growth, disrupting normal plant functions, and resulting in death (Caux et al., 1993). However, orange fruit dipped at 0.05 mmol L^{-1} concentration before storage slightly increased calyx browning but still had better quality than control fruit although not significantly different. This meant that higher dicamba concentration may not have a beneficial effect of inhibiting calyx browning and retaining quality, as this concentration could be more than the amount of auxin the fruit can metabolize during storage. Dipping Valencia Late oranges with lower dicamba concentration (0.01 mmol L^{-1}) before storage inhibited calyx browning and maintained internal quality parameters could be due to low metabolic activities (Alhassan et al., 2022), which is consistent with a report that lower ethylene and respiration reduced calyx senescence of 'Afourer' Mandarins held at 5 and 20 °C for 8 weeks (Li et al., 2018).

Fruit calyx drop

The presence of calyx on citrus fruit is a show of good quality for many consumers to purchase and hence needs to be attached (Alhassan et al., 2020). There was a significant effect of dicamba treatment and storage time on calyx drop citrus varieties ($p < 0.05$) with significant interactions during storage. The results showed that lower dicamba concentrations significantly reduced the rates of calyx drop compared to untreated fruit. Calyx drop increased with an increased browning index in the different auxin treatment concentrations during storage. Treatment with 0.01 mmol L^{-1} dicamba concentration greatly reduced calyx drops to 13.3% compared to control with 55.6% and 0.05 mmol L^{-1} dicamba concentration with 23.3% as shown in Figure 2. There was a significant effect of dicamba treatment on Valencia Late oranges calyx drop compared to control fruit in experiment 2. This study observed that 0.01 mmol L^{-1} dicamba treatment had a 15.7% calyx drop during storage when compared to 0.003 mmol L^{-1} dicamba concentration with 16.2%. Although data between the two concentrations was statistically insignificant ($p > 0.05$), the lower concentration showed more promise in inhibiting calyx in the experiments of this study. The results also indicate 0.01 mmol L^{-1} dicamba treatment delayed calyx drop more relative to control. This difference in efficacy suggests that 0.01 mmol L^{-1} could be the optimal dicamba concentration to delay calyx abscission oranges (Table 1). Ma et al. (2015) in their study inhibited citrus calyx abscission in four varieties with low fluroxypyry treatment concentrations during extended storage. Oranges dipped with 0.05 mmol L^{-1} concentration marginally increased calyx drop but yet gave better calyx retention compared to untreated fruit, which suggests higher concentration may not have a beneficial effect on reducing calyx abscission as this concentration could have provided increased hormonal properties than the amount citrus fruits can metabolize. This effect is consistent with the findings by Kelley and Riechers (2007) that at low doses, dicamba has similar hormonal properties as natural plant auxins. It has been reported that the inductions of abnormal and uncontrollable growth, disrupted normal plant functions, resulting in death due to increased dicamba concentrations (Caux et al., 1993).

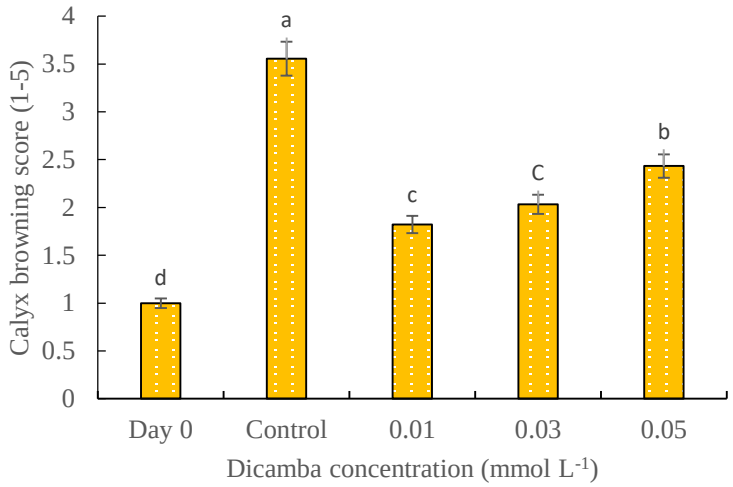

Fig. 1. Effect of postharvest dicamba treatment on Valencia Late orange fruit calyx browning stored at ambient temperature (25 ± 2 ºC) and RH of 60-65% for 4 weeks. Treatments applied: control (no dicamba), 0.01, 0.03 and 0.05 mmol L^{-1} dicamba concentration. Means with different letters are significantly different according to Fisher's LSD test ($p \leq 0.05$).

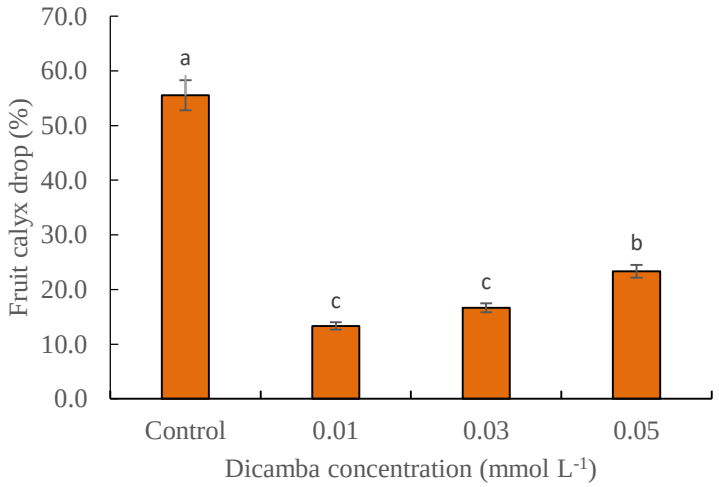

Fig. 2. Effect of postharvest dicamba treatment on Valencia Late oranges fruit calyx drop during storage stored at ambient temperature (25 ± 2 ºC) and RH of 60-65% for 4 weeks. Treatments applied: control (no dicamba), 0.01, 0.03 and 0.05 mmol L^{-1} dicamba concentration. Means with different letters are significantly different according to Fisher's LSD test ($p \leq 0.05$).

Fruit weight loss

There was a significant effect of dicamba treatment on weight loss of Valencia Late oranges ($p < 0.05$) during storage, however, there was also a significant effect of storage time on weight loss of the fruit ($p < 0.05$) but there was no significant ($p > 0.05$) interactions between these factors. As shown in Figure 3, the percentage weight loss of fruit at the end of storage was significantly reduced ($p < 0.05$) to 1.26% and 1.64% by 0.01 and 0.03 mmol L^{-1} dicamba treatments respectively compared to untreated fruit with 32.5% weight loss during storage period. Fruits dipped at 0.05 mmol L^{-1} dicamba concentration had little increase in weight loss but was lower than that of control. Similarly, in experiment 2, low dicamba treatment concentration positively impacted Valencia Late oranges' weight loss during the storage regime. The results demonstrated that 0.01 mmol L^{-1} dicamba application reduced the weight

loss of the oranges to 10.6% relative to 0.003 mmol L^{-1} with 11.4% although data for the two treatments was statistically insignificant ($p > 0.05$). However, both treatment concentrations inhibited water loss better than the control fruit with 23.1% as demonstrated in Table 1. Citrus fruit weight loss is an important parameter during the postharvest life of citrus fruit since this can either help maintain quality or adversely affect other quality factors. In the present study, an increase in percentage weight loss of fruit observed altered the commercial quality of the non-treated fruit in Valencia Late oranges during storage, as the majority of fruit that had their calyxes senesced corresponded with an increase in weight loss. Lower dicamba treatment (0.01 mmol L^{-1}) in experiments 1 and 2 significantly reduced weight loss and maintained citrus quality factors. The beneficial role of 0.01 mmol L^{-1} dicamba treatment in reducing weight loss and maintaining other quality factors of Valencia Late oranges, indicating that auxins could reduce metabolism and extend storage life. This result is consistent with Alhassan et al. (2022) where pre-storage dipping of Valencia oranges with a range of plant growth regulators reduced senescence and maintained quality. Although no study has shown the effect of postharvest application of dicamba in minimising weight loss of Valencia Late oranges, a study has demonstrated that dicamba fed to adult rats for 90 days with approximately 500 mg/kg/day showed no effects on weight loss, however, at doses up to 1000 mg/kg/day reduced body weight gain, and changes in the liver's weight, colour, and size (EPA, 2005).

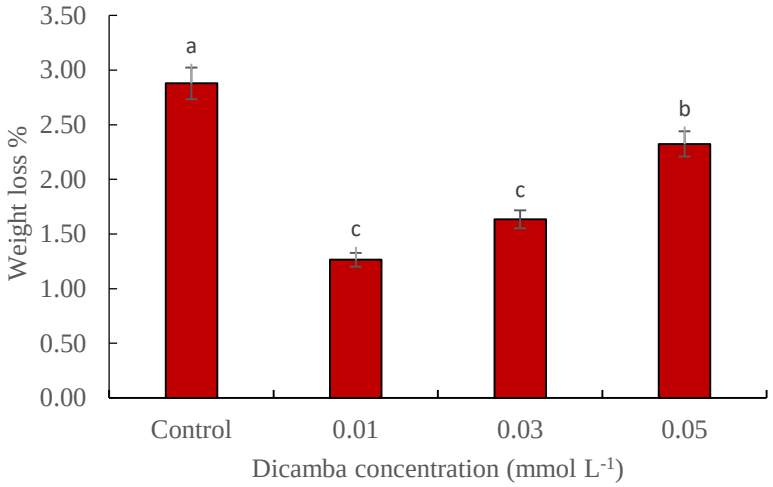

Fig. 3. Effect of postharvest dicamba treatment on Valencia late orange fruits' weight loss stored at ambient temperature (25 ± 2 °C), and RH of 60-65% for 4 weeks. Treatments applied: control (no dicamba), 0.01, 0.03 and 0.05 mmol L^{-1} dicamba concentration. Means with different letters are significantly different according to Fisher's LSD test ($p \leq 0.05$).

Fruit firmness level

There was a significant effect of dicamba treatment on orange fruit firmness during storage ($p < 0.05$). Pre-storage dipping (dipping before storage) of the orange fruit with 0.01 mmol L^{-1} retained the highest firmness level (38.4N) during storage compared to control fruit (31.8N) and the increased dicamba treatment concentrations. Increasing dicamba treatment concentrations (0.03 or 0.05 mmol L^{-1}) slightly reduces the retention of fruit firmness from 35.4N to 33.6N respectively. However, dicamba-treated fruits showed higher firmness levels than control fruit though control fruit firmness was not significantly different ($p > 0.05$) relative to 0.05 mmol L^{-1} dicamba at the end of storage as indicated in Figure 4. The results in experiment 2, showed dicamba treatment on Valencia Late oranges greatly delayed loss of fruit firmness compared to untreated samples. As expected, pre-storage dipping with 0.01 mmol L^{-1} dicamba inhibited firmness loss (31.2N) of more than no dicamba-treated fruit (24.7N) and 0.003 mmol L^{-1} (30.5N) but there was no significant difference ($p > 0.05$) between oranges treated with 0.003 and 0.01 mmol L^{-1} dicamba concentrations (Table 1). Low dicamba concentration decreased fruit weight loss and delayed firmness loss during the storage regime. As demonstrated in this study, treatment with 0.01 mmol L^{-1} significantly reduced weight loss, and therefore maintained fruit firmness during storage. However, increased auxin concentrations slightly reduced fruit firmness but retained higher firmness than untreated fruit. The result of this study disagrees with the previous findings of Ma et al. (2015), who observed no significant effects of similar auxin such as fluroxypyr treatment on firmness of four citrus varieties during storage at 5 and 20 °C for 12 weeks.

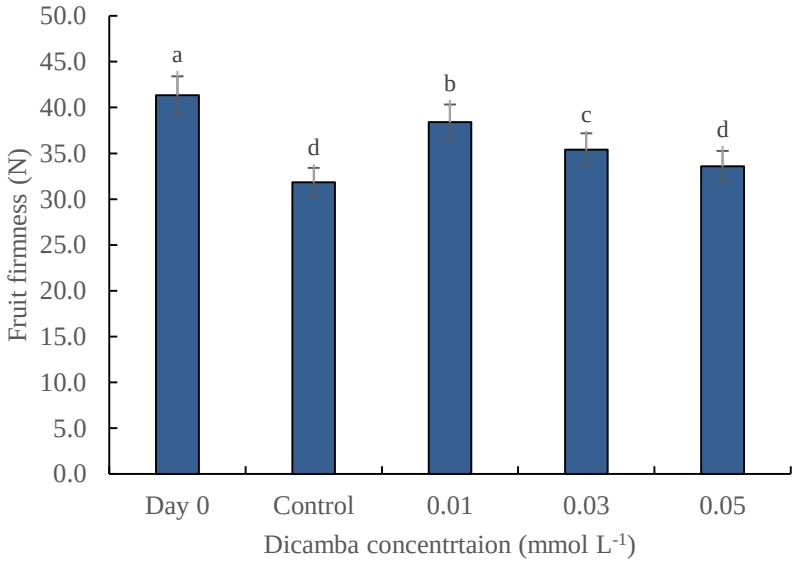

Fig. 4. Effect of postharvest dicamba treatment on Valencia Late oranges' firmness during ambient storage (25 ± 2 °C), and RH of 60-65% for 4 weeks. Treatments applied: control (no dicamba), 0.01, 0.03 and 0.05 mmol L^{-1} dicamba concentration. Means with different letters are significantly different according to Fisher's LSD test ($p \leq 0.05$).

Effect of dicamba application on internal fruit qualities

There was a significant effect of dicamba treatment and storage time on the total soluble solids (TSS) of Valencia late oranges ($p < 0.05$), however, there was no significant ($p > 0.05$) interaction between the auxin treatments and storage time. Results from this study indicate that Valencia late oranges can be treated with 0.01, or 0.03 mmol L^{-1} dicamba treatments at 25 ± 2 °C and RH of 60-65% for up to 28 days without detrimental effect on their internal quality as expressed by the fruit TSS level (11.2 to 11.5 °brix). The TSS of oranges slightly increased with an increased dicamba treatment concentration but increased at a decreased rate (11.1 to 11.2 °brix) at a low dicamba concentration (0.01 mmol L^{-1}) compared to the other dicamba concentrations of this investigation. However, the TSS content of the control fruit was the highest (11.1 and 10.4 °brix for the two experiments) among all the treatments, which showed a loss of fruit quality. When the experiment was repeated with control, 0.003 and 0.01 mmol L^{-1} as treatment concentrations, 0.01 mmol L^{-1} showed 11.3 °brix compared to 0.003 mmol L^{-1} with 11.7 °brix at the end of storage. Despite this, the two treatments were statistically insignificant ($p > 0.05$), but there was a significant difference ($p < 0.05$) between fruit treated with dicamba and untreated fruit (Table 1). Control fruit showed an increase in TSS from 10.4 to 14.6 °brix at the end of storage, which led to a rise in maturity index and reduced the fruit's internal quality and external appearance (Fig. 5). The result of this study disagrees with the finding of Ma et al. (2015), who reported that auxins such as fluroxypyr have no significant effect on TSS of Satsuma mandarin and Olinda Valencia oranges stored at 5 and 20 °C for 12 weeks.

There was also a significant effect of dicamba treatment and storage time on titratable acidity (TA) of the citrus fruit ($p < 0.05$), however, there was also an interaction between dicamba treatment and storage time on TA of Valencia Late oranges dipped at low dicamba treatment concentrations. As expected, there was a general decline in the TA level across all fruits during storage under the different treatments. The TA of fruit dipped in 0.01 mmol L^{-1} dicamba before storage was significantly higher (1.30 citric acid) than control fruit with 1.02 citric acid, 0.03 with 1.17 citric acid and 0.05 mmol L^{-1} with 1.11 citric acid (Fig. 6). When the experiment was repeated using control (0), 0.003, and 0.01 mmol L^{-1} dicamba concentrations the 0.01 mmol L^{-1} treatment showed similar trends to experiment 1 with TA of 6.1 citric acid at the end of storage compared to control fruit with 4.3 citric acid and 0.003 mmol L^{-1} with 5.9 citric acid. This shows the TA decline in untreated oranges was more pronounced during storage than in fruits dipped with dicamba prior to storage. This effect contributes to an increase in the TSS content. In a similar study, fluroxypyr was found to have low toxicity similar to dicamba, however, it did not have a significant effect on the TA level of fruit during storage (Ma et al., 2015). However, there was a significant effect of dicamba treatment and storage time on the maturity index of the oranges ($p < 0.05$) at the end of storage.

The TSS/TA ratio of citrus fruit is an important quality factor affecting sensory qualities (Barros et al., 2012). Comparing the treatment concentrations in this study, there was a gradual increase in maturity index due to the decrease in the juice acidity with a corresponding TSS increase of the Valencia Late fruit during ambient storage. The effects of pre-storage dipping of dicamba auxin at different concentrations on TSS, TA, and their ratio consistently relate to corresponding increases or decreases in calyx senescence and the other quality parameters of the orange fruit held for 4 weeks during ambient storage in the present investigation.

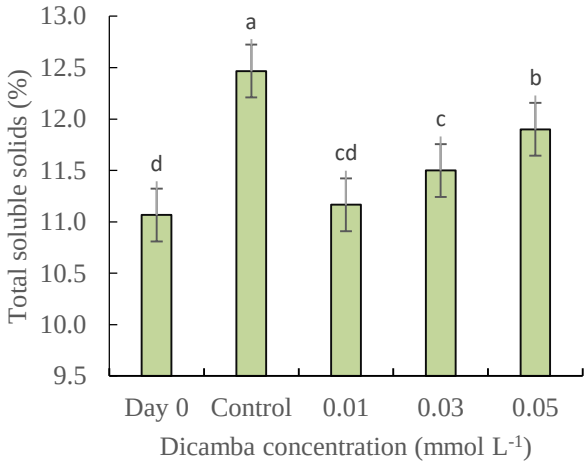

Fig. 5. Effect of postharvest dicamba treatment on Valencia Late oranges TSS content during ambient storage (25 ± 2 ºC), and RH of 60-65% for 4 weeks. Treatments applied: control (no dicamba), 0.01, 0.03 and 0.05 mmol L^{-1} dicamba concentration. Means with different letters are significantly different according to Fisher's LSD test ($p \leq 0.05$).

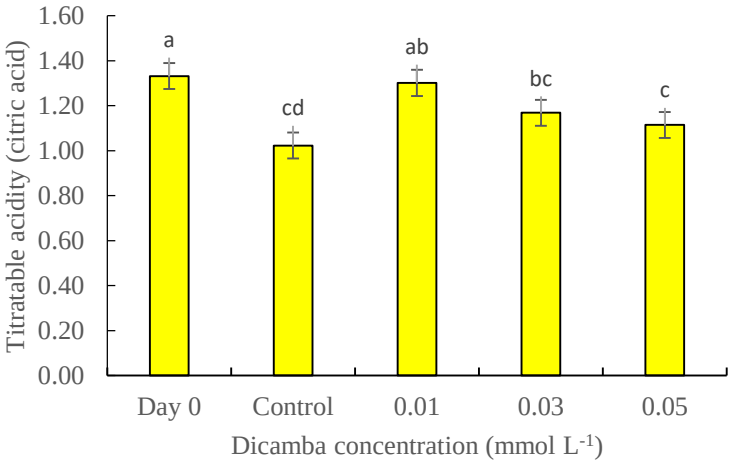

Fig. 6. Effect of postharvest dicamba treatment on Valencia Late oranges TA stored at ambient temperature (25 ± 2 ºC) and RH of 60-65% for 4 weeks. Treatments applied: control (no dicamba), 0.01, 0.03 and 0.05 mmol L^{-1} dicamba concentration. Means with different letters are significantly different according to Fisher's LSD test ($p \leq 0.05$).

Table 1: Effect of postharvest dicamba treatment on quality factors of Valencia Late oranges during ambient storage.

Dicamba Conc. (mmol L^{-1})	External and internal quality factors evaluated						
	Calyx browning (score)	Calyx drop (%)	Weight loss (%)	Fruit Firmness (N)	TSS (%)	TA (Citric acid)	TSS: TA
Day 0	1.0^{c}	0.0^{c}	0.0^{c}	35.4^{c}	10.4^{c}	8.1^{c}	1.3^{c}
Control	3.7^{a}	32.5^{a}	23.1^{a}	24.7^{b}	14.6^{a}	4.3^{b}	3.4^{a}
0.003	2.5^{b}	16.2^{b}	11.4^{b}	30.5^{a}	11.7^{b}	5.9^{a}	2.0^{b}
0.01	2.1^{b}	15.7^{b}	10.6^{b}	31.2^{a}	11.3^{b}	6.1^{a}	1.9^{b}
P < 0.05							

Internal and external quality assessment under control (no dicamba) and dicamba concentrations (0.003 and 0.01 mmol L^{-1}) at a temperature of 25 ± 2 ℃ and 60-65% RH.

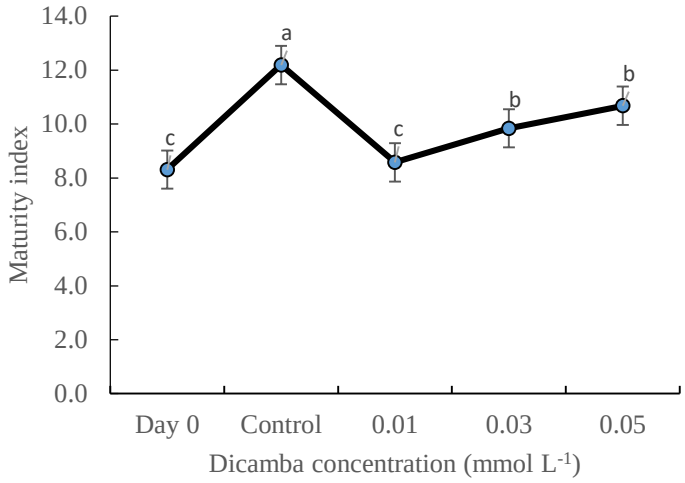

Fig. 7. Effect of postharvest dicamba treatment on Valencia Late oranges maturity index stored at ambient temperature (25 ± 2 °C), and RH of 60-65% for 4 weeks. Treatments applied: control (no dicamba), 0.01, 0.03, and 0.05 mmol L^{-1} dicamba concentration. Means with different letters are significantly different according to Fisher's LSD test ($p \leq 0.05$

CONCLUSION

This study investigated the effects of the postharvest application of dicamba on calyx senescence and internal quality factors of Valencia Late oranges during ambient storage. Low dicamba concentration applications were more effective in retaining calyx quality and ameliorating adverse changes in fruit internal quality parameters. The auxins reduced weight loss, and delayed firmness loss. Pre-storage dipping with low dicamba treatment delayed TSS increase and inhibited the TA decline and fruit maturity; extending the Valencia Late oranges' postharvest storage. This beneficial effect is associated with a decrease in fruit senescence. The results demonstrate the impact of dicamba treatments in delaying detrimental calyx changes and retaining fruit integrity during storage. Dicamba application effectively delays orange fruit calyx disorders and maintains good internal quality parameters. This positive result could contribute to shaping the decision to approve dicamba for postharvest utilisation in the citrus industry. However, further study is required to confirm the impact of dicamba treatment on a broader range of citrus types and storage conditions during extended storage.

Conflict of interest

The author declares no conflict of interest regarding the publication of this work.

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Photosynthetic efficiency and chlorophyll fluorescence responses of *Viola ignobilis* Rupr. subjected to different biostimulants and two light intensities

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ABSTRACT

Purpose: *Viola ignobilis* Rupr. is one of the important medicinal species which is in danger of extinction due to the over harvesting from the main habitats. Nowadays, domestication of medicinal plants in accordance with sustainable agricultural methods is a new important challenge. In this research, we used biostimulants and optimization of light intensity as eco-friendly approaches to improve yield and photosynthetic efficiency of *V. ignobilis* Rupr. **Research Method:** The experiment was set up in a split plot arranged in a randomized complete block design with 3 replications. The main factor was two light levels (50% and 100% of full sunlight) and as sub factors plants were treated with animal derived protein hydrolysate (A.PH), vegetal derived protein hydrolysate (V.PH), seaweed extract (SE), the combination of A.PH + SE and V.PH + SE and also, water served as a control. **Findings:** Light intensities and biostimulant application significantly impacted the morphological parameters including fresh and dry weight of roots and shoots compared to control plants. Furthermore, the photosynthetic pigments did not differ significantly in two light intensities, but, biostimulant application considerably increased the photosynthetic pigments concentration. The obtained results indicated that the highest value of assimilation rate, transpiration rate and stomatal conductance, and also chlorophyll fluorescence parameters including the highest values of qP, Fv/Fm and (ΦPSII) were connected to plants treated with A.PH + SE biostimulants under full irradiance. **Research limitations:** No limitations were found. **Originality/Value:** Optimizing light condition and combined use of PHs + SE biostimulants due to synergistic effects can improve crop yield and photosynthetic efficiency in violet, when no other sources of fertilizers are available.

INTRODUCTION

Sweet violet (*Viola ignobilis* Rupr.) is a member of the *Violaceae* family is facing extinction because of over-harvesting. *Viola* is the largest genus in this family with approximately 664 species which is used for medicinal and ornamental purposes (Marcussen et al., 2022).

During the last decades, excessive application of chemicals in conventional agriculture has been contaminated environment due to heavy metals, and harmful residues (Alengebawy et al., 2021). Therefore, sustainable agricultural practices such as nature-based solutions can be helpful to enhance production and reduce harmful impacts on the ecosystem (Artmann & Sartison, 2018). It follows that must be attended to the technical aspects of plants growth and development such as temperature, nutrition, humidity, and light condition (Hamidah et al., 2018). In this context, there are novel cultivation methods, that able ameliorate destructive impacts of conventional farming. One of these methods contains the use of naturally derived biostimulants. Du Jardin (2015) describes a plant biostimulant as an organic and inorganic compounds or microorganism that when apply to plants, enhances nutrition efficiency, biotic and abiotic stresses tolerance and crop quality parameters. Biological stimulants reduce the need to use chemical fertilizers by influence on root growth and architecture and consequently increasing the nutrients acquisition (Sun et al., 2024).

Protein hydrolysates (PHs) are natural bio-stimulants consisting of oligopeptides, polypeptides, and free amino acids, which can be produced through chemical and/or enzymatic hydrolysis of obtained organic materials from wastes of plants or animals origin (Carillo et al., 2019). The researchers found that plants easily absorb low molecular size peptides and amino acids, which can significantly influence plant growth and physiology by acting on photosynthesis and mechanisms involved in abiotic stress resistance (Schiavon et al., 2008). These products have ability to improve nutritional uptake, nutrient-use efficiency and boosting yield and quality of treated crops (Polo & Mata, 2018).

Seaweed extracts (SE) and their derivative products are another group of biostimulants. One of the common species is *Ascophyllum nodosum* which is comprised of polysaccharides, primarily alginate, laminaran, polyphenols, betaines, amino acids, and vitamins (Ertani et al., 2018). It has been reported that SE contains essential micro and macro nutrients, phyto-hormones including auxin, ABA and cytokinines and other crucial ingredients, which may have influence on biochemical reaction in plant cells (Baltazar et al., 2021).

Light modulation may also be considered as an effective strategy which different aspects of it consisting quality, intensity and duration strongly influence on plant growth and development (Vitale et al., 2021). Light intensity deeply impact on plant morphogenesis, anatomy, cellular biochemistry, photosynthesis, and secondary metabolite production (Badmus et al., 2022; Tang et al., 2022), thus, optimizing the light condition could be improved yield and physiological responses in medicinal herbs.

Up to now, no research has been published on the subject of the optimal protocol of light requirements of violet (*Viola ignobilis* Rupr.) and also applying the biostimulants on growth and physiology of this important medicinal herb. Moreover, there are a few published studies that describe the interaction effects of light intensity and plant biostimulants. The purpose of the current study was to evaluate the probable effects of the biostimulants application and two light conditions on the growth, photosynthetic pigments, leaf gas exchange, and chlorophyll fluorescence parameters in *Viola ignobilis* Rupr.

MATERIALS AND METHODS

Experimental conditions

The seedlings of *Viola ignobilis* Rupr. were collected at the 4-leaf stage from the valley in Kaleybar County, Eastern Azerbaijan province (38° 51' 59.99" N., 47° 01' 60.00" E., 1144 m a.s.l) and confirmed by the Guilan Agriculture and Natural Resources Research Center. This study was carried out in Roudesar, a city in Guilan province in northern Iran (37° 08' 15.40" N, 50° 17' 16.80" E, 0 m a.s.l) from December 2021 to April 2022.

Treatments and experimental design

The experimental design was a split plot arrangement based on randomized complete blocks with three replicates. The main factor was two light levels included: 100% full sunlight and 50% full sunlight and sub-factors made of three bio-stimulants treatments: animal protein hydolysate (A.PH), vegetal protein hydrolysate (V.PH), seaweed extract (SE) and combination of A.PH + SE and V.PH + SE compared with foliar application of water as controlled treatment. Each treatment consisted of 4 pots with 3 replications. Amounting to a total of 12 experimental unit plots, each plot consisting of 48 plants in each treatment (576 plants in total). The four-leaf stage seedlings were transplanted in December 2021 to 3 L pots (density = 4 plants per pot). The final substrate was prepared from equal proportions of forest soil and leaf mold; pH: 7.35; electrical conductivity: 1.08 $dS \cdot m^{-1}$; organic matter (%): 10; total N (%): 3.1; available P ($mg.kg^{-1}$): 10; exchangeable K ($mg.kg^{-1}$): 145.2.

The protein hydrolysate treatments were started 3 weeks after cultivation (on January 15) and were applied weekly 12 times until the flowering stage on 15 April. The A.PH was obtained from enzymatic hydrolysis of fish under alkaline condition containing 75% free amino-acids. The V.PH was used in this experiment obtained through enzymatic hydrolysis of soybean seeds, containing 48% amino acids and soluble peptides. Foliar spray the above-quoted biostimulants were applied on the leaves of violet at the concentration of 0.2 g L^{-1}, in a solution with distilled water (Cristiano et al., 2018). Moreover, the third biostimulant used in this research was Acadian seaweed (Acadian Plant Health, Canada) extract which is made from the brown seaweed, *Ascophyllum nodosum* is contained amino acid 4.4%, mannitol 4%, alginic acid 10%, and other organic compounds 55%. The elemental composition of Acadian as follows: N 1.5%, K 17%, P 0.2%, sulphur 1%, Mg 0.3%, Ca 0.4%, Fe 150 ppm. The SE was applied directly to the soil (500 mL per pot) every two weeks from 3 weeks after cultivation at the concentration of 2 g L^{-1}. The relative dose of the SE was based on manufacturer recommendations. No fertilizer has been applied and cultivation practices were performed following standard methods.

Shade treatments

Shade treatments were imposed using green shading nets 50% above the wooden frames and fixed at a height of 3 m above the ground to provide a 50% reduction in light. Green agro shade nets with a standard size of 3 m width and 50 m length with 50% shade were used. This was made with high-density polyethylene plastics. Plants were randomly divided into two groups which were subjected to two different light intensities. The mean daily variation in full sunlight from January to April measured by using a HT620 Digital Lux Meter (Habotest, China). To control light condition, light intensity was measured three times a day at 10 am, 12 noon, and 2 pm, and at the end of each month.

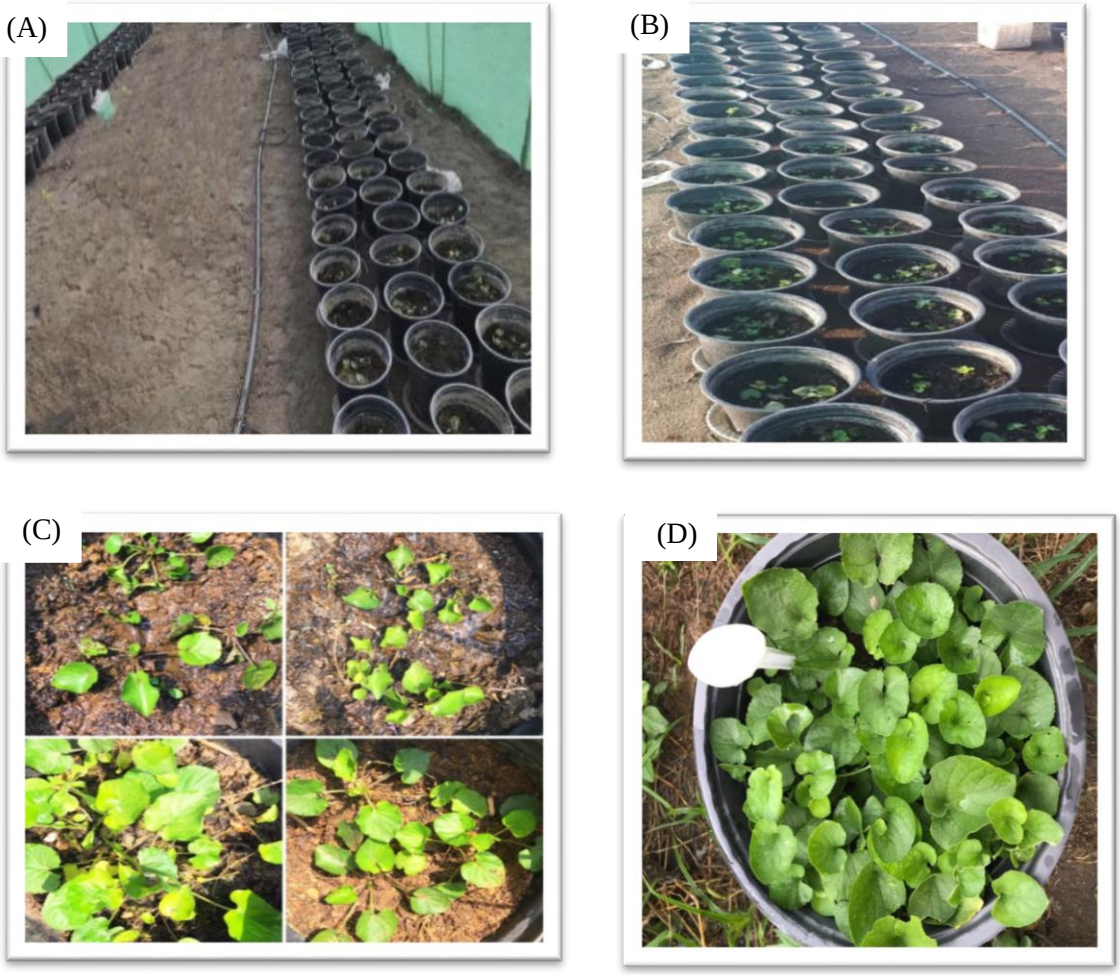

Fig. 1. (A): Shade treatment (50% natural light), (B): 100% light intensity, (C): Different stages of violet growth, (D): Plant growth after 8 weeks.

Morphological parameters

At the end of the flowering stage (121 days after cultivation), six plants were harvested and their morphological parameters were measured. Plants were removed from pots and the growing medium was gently washed from the roots to measure the fresh weight of the aerial part and the root, then, plant samples were dried in an oven at 70°C for 72 h to reach a constant weight for measuring dry weight.

Photosynthetic pigments

The evaluation of total carotenoids and chlorophylls was carried out according to the method reported by Porra (2002). To preparing methanolic extracts, 0.5 g of fresh leaf tissue extracted by grinding leaves in 80% acetone, the samples were kept in the dark at room temperature for 24 hours, and then the absorbance values of the solutions were measured by spectrophotometer at 663.2, 645.4 and 470 nm. The amount of chlorophyll and carotenoid was calculated based on the following formulas (1, 2) and the results were expressed in milligrams/gram FW.

(Chl a) = (12/25 A 663)-(2/79 A645), (Chl b) = (21/21 A645)-(5/1 A663),
(Chl T) = (Chl a) + (Chl b) (1)

Carotenoid = (1000A470)-(1/8 Chl a)-(85/02 Chl b)/198 (2)

Gas Exchange parameters

Photosynthetic parameters were investigated using a portable gas exchange fluorescence system (GFS-3000, Heinz Walz Effeltrich, Germany) to measure gas exchange parameters including assimilation rate (A µmol m^{-2} s^{-1}), transpiration rate (E mmol m^{-2} s^{-1}), and stomatal conductance (G H_2O mmol m^{-2} s^{-1}). The measurements were carried out on a fully expanded leaf from 10:00 am to 2:00 pm (Xu et al., 2020). The cuvette temperature, photosynthetic active radiation and CO_2 concentration were maintained at 28.4° C, 700 µmol m^{-2} s^{-1}, and 578.48 ppm respectively. At each conducted time point, five plants were randomly selected from each replication and analyzed for the mentioned parameters.

Chlorophyll Fluorescence

At the end of flowering stage, measurement of Chlorophyll Fluorescence parameters was carried out on a last fully expanded leaf with a fluorometer GFS-3000 (Heinz Walz GmbH, Effeltrich, Germany). F_o (initial minimal fluorescence) and F_m (maximal fluorescence) were determined after a 30 min dark- adaptation period and the maximum quantum efficiency of PSII was calculated as $F_v/F_m = (F_m - F_o)/F_m$ (Fig. 2A). The plants to be measured were placed in a dark room. Leaves were light-adapted for approximately 20 min before measurements of other parameters were calculated including, qP, F_v/F_m and ΦPSII (Genty et al., 1989). The Chlorophyll fluorescence data presented are means from at least 5 leaves per replication (Fig. 2B).

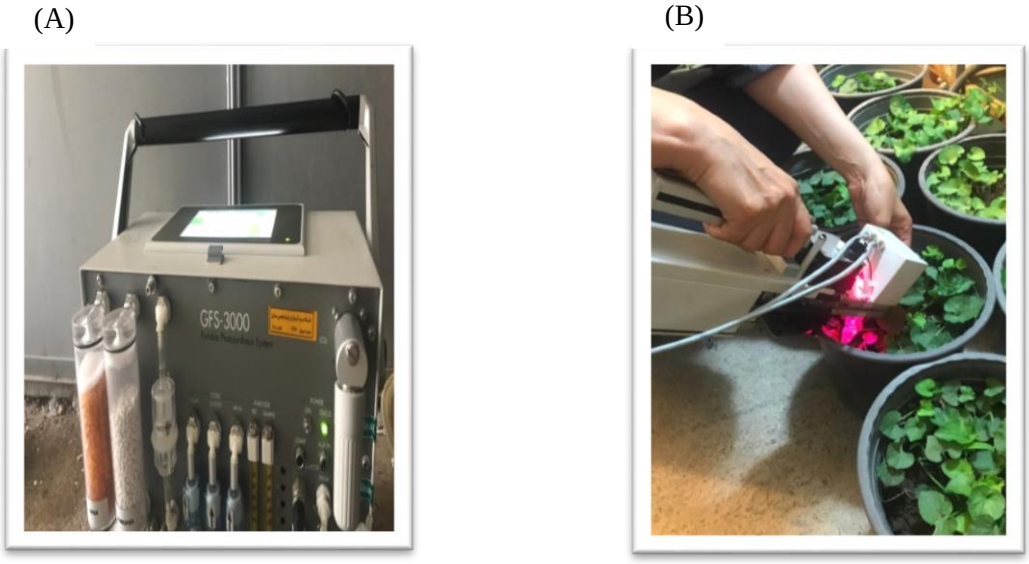

Fig. 2. (A): fluorometer GFS-3000 (Heinz Walz GmbH, Effeltrich, Germany), (B): measurement of Chlorophyll Fluorescence parameters of *Viola ignobilis* Rupr.

Table 1. Chlorophyll fluorescence parameters used in current study.

Parameter	Formula	Description
F_v/F_m	$(F_m - F_0)/F_m$	Maximum quantum yield of PSII photochemistry measured in the dark-adapted state
qP	$(F'_m - F_s)/(F'_m - F'_0)$	Photochemical quenching of PSII
Y(PSII)	$(F'_m - F_s)/F'_m$	Effective quantum yield of photochemical energy conversion in PSII

Ref: Shin et al. (2021).

Statistical analysis

Data Analysis was performed using the ANOVA procedure in SAS version 9.2 (SAS Ins., Cary, NC, USA). Differences between treatment means were separated by the least significant difference (LSD) at the 95% confidence level ($p < 0.05$). All graphs were drawn using Excel software.

RESULTS AND DISCUSSION

Morphological parameters

The experimental results of the collected data revealed that the simple effect of light intensity showed significant influence ($p < 0.01$) on shoot fresh and dry weight, but root biomass appeared to be unaffected by light intensity (Table 2). Also, application of biostimulants improved significantly all studied morphological traits compared to untreated plants in *Viola ignobilis* Rupr ($p < 0.01$). As can be seen from the Table 2, the interaction of light intensity and biostimulants application didn't reveal any significant effect on the morphological parameters.

Table 2. Variance analysis of the effect of light intensity (A), biostimulants (B) and their interaction (A × B) on morphological traits, and photosynthetic pigments of *Viola ignobilis* Rupr.

Mean Squares

S.o.V	df	Shoot FW	Root FW	Shoot DW	Root DW	Chla	Chl b	TChl	Car
R	2	1.6955^{ns}	32.668^{**}	0.9466^{ns}	3.4721^{**}	0.01798^{ns}	0.0018^{ns}	0.0114^{ns}	0.01074^{**}
A	1	37.108^{**}	15.8404^{ns}	0.9801^{**}	2.6028^{ns}	0.07933^{ns}	0.0004^{ns}	0.0756^{ns}	0.01361^{ns}
R × A	2	10.662^{ns}	19.5784^{*}	1.40605^{ns}	1.7230^{ns}	0.00911^{ns}	0.0010^{ns}	0.0870^{ns}	0.00067^{ns}
B	5	338.80^{**}	276.125^{**}	40.8545^{**}	27.990^{**}	0.66426^{**}	0.016^{**}	0.893^{**}	0.11627^{**}
A × B	5	1.5572^{ns}	0.18567^{ns}	0.01027^{ns}	0.0974^{ns}	0.00231^{ns}	0.0002^{ns}	0.0022^{ns}	0.00028^{ns}
Error	20	88.590	95.939333	18.331844	11.39904	1.7225222	0.05034	1.18033	0.035688
Total	35	1852.239	1597.8301	228.34162	164.8307	5.1889638	0.14195	5.93307	0.6549555
CV (%)		7.69	8.21	18.45	10.41	13.14	7.61	8.4	5.87

S.o.V: Source of variation, df: Degree of freedom, CV: Coefficient of variation. Chla: chlorophyll a, Chlb: chlorophyll b, TChl: Total Chlorophyll, Car: Carotenoid. Asterisks (*) represent the level of significance for each factors (A, B) and their interaction (A × B): NS: non-significant; * $p < 0.05$; ** $p < 0.01$.

The fresh and dry weight of violet shoot increased by 6.5% and 7% respectively in 100% light intensity compared with shaded plants. Furthermore, the biostimulant application strongly improved the fresh and dry weight compared to biostimulant-untreated plants. The obtained results exhibited that the highest shoot fresh weight (38.98g), and shoot dry weight (8.52g) were observed in A.PH + SE treatment, without any significant differences with V.PH + SE treatment, also, the untreated plants showed the lowest shoot fresh weight (18 g) and shoot dry weight (2.37g) respectively (Table 3).

As Table 3 shows, the highest root fresh weight (32.57 g) is connected to A.PH + SE, although, had no significant difference with V.PH + SE treatment and the minimum root fresh weight (13.86 g) was belonged to untreated plants. Moreover, the higher root dry weight (8.91 g) was related to A.PH + SE, but no significant differences were found between A.PH +SE and V.PH + SE. The minimum root dry weight (3.21 g) was recorded in untreated plants.

The findings of this experiment indicate that, the fresh and dry weight of shoot and root were increased in full light intensity, although, in terms of root biomass, no statically significant difference was found between two light conditions. In accordance with the present results, previous studies have demonstrated that the plant yield decreased under lower irradiance. Hirano et al. (2019) reported that the total plant mass in *Datura inoxia* and *D. stramonium* decreased under lower light intensity. Szymborska-Sandhu et al. (2020) recorded the highest number of shoots and biomass of *Melittis melissophyllum* L. in full sunlight.

Today, the use of biostimulants in sustainable agriculture is a profitable strategy for improving crop yield and quality (Rouphael & Colla, 2020), hence, many researchers has been focused on effectiveness of new products in order to improving crop production. In this experiment, the use of plant biostimulant enhanced strongly all of evaluated morphological parameters, not only in high light intensity, but also in shade condition compared to untreated plants. Regarding to obtained results, in this work, PHs revealed more strongly effects on morphological parameters of *Viola ignobilis* Rupr. Than SE. Several authors found similar results in agreement with our findings in respect to positive influences of PHs on morphological parameters in various plants. For instance, Carillo et al. (2019) found that the fresh yield of greenhouse spinach was significantly increased in PH-treated plants compared to control. They explained that the great amount of amino acids and small peptides in PH exhibited hormone-like activities on plant which were responsible for increase nutrient acquisition and improve growth. Jolayemi et al. (2023) proved that the protein-based biostimulants increased all agronomic and physiological parameters of sugar beet. In current work, the roots treated with biostimulants considerably improved in comparison with control. Several authors demonstrated that root formation regulated by hormone-like activities of PHs. In a study conducted by Kim et al. (2019) it was shown that PHs extremely increased root growth and development in basil, tomato and chrysanthemum. The current study revealed that the combination of SE and PHs exhibited additive and synergistic effects on enhance growth and physiological traits of *Viola ignobilis* Rupr.

Table 3. Means comparison for morphological traits of *Viola ignobilis* Rupr. in response to two light intensities and biostimulants application.

Treatments	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)
Light Intensity				
L1	32.43a	27.30a	5.35a	7.51a
L2	30.45b	25.98a	5.00b	6.97a
Biostimulants				
A.PH	31.90b	28.46b	4.31b	8.14ab
V.PH	31b	27.51bc	4.19b	7.81b
SE	30.32b	25.65c	3.37bc	6.53c
A.PH + SE	38.98a	32.57a	8.52a	8.91a
V.PH + SE	38.15a	31.79a	8.34a	8.71a
H_2O	18c	13.86d	2.37c	3.21d

L1 (100% light intensity), L2 (50% light intensity), A.PH (animal protein hydrolisate), V.PH (vegetal protein hydrolysate), seaweed extract (SE). Plants treated with H_2O served as a control. Different letters within each column indicate significant differences according to the least significant difference (LSD) ($p < 0.05$). Asterisks (*) represent the level of significance for factors (Light, Biostimulant) and their interaction (Light × Biostimulant). NS: non-significant; *: $p < 0.05$; **: $p < 0.01$.

Chlorophyll pigments and carotenoid

As shown in Table 2, the ANOVA analysis demonstrated that the light intensity and the interaction between light intensity and biostimulants had not significant effects on chlorophyll concentration. Furthermore, biostimulant application showed significant impact on all evaluated photosynthetic pigments ($p < 0.01$). As shown in Table 4, the highest concentration of chlorophyll a (2.49 $mg.g^{-1}$ FW) was achieved in A.PH + SE application, although, there was not considerable difference between all biostimulants treatments. Also, the lowest content of chlorophyll a (1.58 $mg.g^{-1}$ FW) was related to control plants. In terms of chlorophyll b, the higher concentration (0.71 $mg.g^{-1}$ FW) was related to treated plants with A.PH + SE, without any significant difference between all biostimulants except for, SE treatment. Moreover, the lowest (0.57 $mg.g^{-1}$ FW) occurred in untreated plants. The maximum amount of total chlorophyll (3.21 $mg.g^{-1}$ FW) was observed in V.PH + SE application, without any significant difference with A.PH + SE. Also, the lowest value (2.15 $mg.g^{-1}$ FW) was belonged to untreated plants (Table 4). Looking at Table 4, it is apparent that the highest carotenoid content (0.81 $mg.g^{-1}$ FW) obtained in plants treated with A.PH + SE treatment, without any significant difference with other treatment except for SE, while, untreated plants showed that, the lowest amount of carotenoid content (0.48 mg g^{-1} FW).

Chlorophyll content is a critical indicator that shows the adaptability of plants to environmental conditions (Liu et al., 2007), indeed plants grown under low light intensity increase pigment density in order to optimize light absorption efficiency (Khoshbakht et al., 2018). In accordance with the present results, previous studies have demonstrated that the highest concentration of chlorophyll a and chlorophyll b were obtained in low light irradiance. For instance, Duan et al. (2018) reported that shading significantly increased the contents of chlorophyll a, chlorophyll b and chlorophyll a+b in *Lespedeza Buergeri* seedlings. Furthermore, He et al. (2019) indicated that the contents of chlorophyll a, chlorophyll b and total chlorophyll of *Castanopsis kawakamii* seedlings were higher in low light intensity in non-gap environment.

In current study application of biostimulants had a positive influence on the chlorophyll contents of *Viola ignobilis* Rupr compared to control plants. However, combination of PHs and SE enhanced chlorophyll content more than when SE and PHs individually were used. These results are in agreement with Caruso et al. (2020) findings which showed both the protein hydrolysates and *Trichoderma* treatments alone or in combination, were led to

increase in chlorophyll content in perennial wall rocket compared to the untreated plants. Munaro and et al. (2024) confirmed that chitosan nanoparticles and microalgae-based protein hydrolysate enhanced chlorophyll and carotenoid in tomato.

In this investigation, the concentration of carotenoid did not differ significantly between two light condition, although, it was higher in full sunlight. In addition to light intensity effects, the carotenoid content can be influenced by biostimulants. Similar to our observation, the positive correlations between carotenoids content and biostimulants application have been reported in several researches. In a research carried out by Rachidi et al. (2020) carotenoid content significantly enhanced in tomato treated by microalgae polysaccharides as a biostimulant. Also Aktsoglou et al. (2021) reported that the PHs is responsible for increasing the content of total carotenoids in spearmint plants.

Gas exchange parameters

Based on the results of analysis of variance (Table 5), the simple effect of light intensity and biostimulant application on the gas exchange parameters was significant ($p < 0.01$), but the interaction of these two factors didn't show any significant influence.

The value of assimilation rate of CO_2 in 100% light conditions was 10.95% higher compared to plants that grew in 50% light intensity (Fig. 3A). The obtained results showed that the highest value of assimilation rate (8.41 µmol m^{-2} s^{-1}) was observed in A.PH + SE treatment and the lowest value (3.36 µmol m^{-2} s^{-1}) related to untreated plant (Fig. 3B).

As it can be seen from Fig. 3C, the transpiration rate of plants under 100% light intensity was 13% higher than plants in 50% light condition. Based on the mean comparison results, the highest transpiration rate (3.75 mmol m^{-2} s^{-1}) was obtained in V.PH + SE treatment, without any significant difference with A.PH + SE application. The lowest rate of transpiration (2.46 mmol m^{-2} s^{-1}) was recorded in control plants (Fig. 3D).

According to the results (Fig. 3E), the stomatal conductance of violet increased by 16.95% at 100% light intensity compared to shade condition. The highest value of stomatal conductance (35.75 mmol m^{-2} s^{-1}) was observed in A.PH + SE treatment without any significant difference with V.PH + SE, furthermore the lowest value (24.82 mmol m^{-2} s^{-1}) was recorded in untreated plants (Fig. 3F).

Table 4. Means comparison for morphological traits of *Viola ignobilis* Rupr. in response to two light intensities and biostimulants application.

Treatments	Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)	Total Chlorophyll (mg g^{-1} FW)	Carotenoids (mg g^{-1} FW)
Light Intensity				
L1	2.27a	0.65a	2.93a	0.73a
L2	2.18a	0.66a	2.84a	0.69a
Biostimulants				
A.PH	2.32a	0.65ab	2.97ab	0.77a
V.PH	2.38a	0.68ab	3a	0.77a
SE	2.18a	0.63b	2.8b	0.73b
A.PH + SE	2.41a	0.70a	3.11a	0.81a
V.PH + SE	2.49a	0.71a	3.21a	0.79a
H_2O	1.58b	0.57c	2.15c	0.48c

L1 (100% light intensity), L2 (50% light intensity), A.PH (animal protein hydrolisate), V.PH (vegetal protein hydrolysate), seaweed extract (SE). Plants treated with H_2O served as a control. Different letters within each column indicate significant differences according to the least significant difference (LSD) ($p < 0.05$). Asterisks (*) represent the level of significance for factors (Light, Biostimulant) and their interaction (Light × Biostimulant). NS: non-significant; *: $p < 0.05$; **: $p < 0.01$.

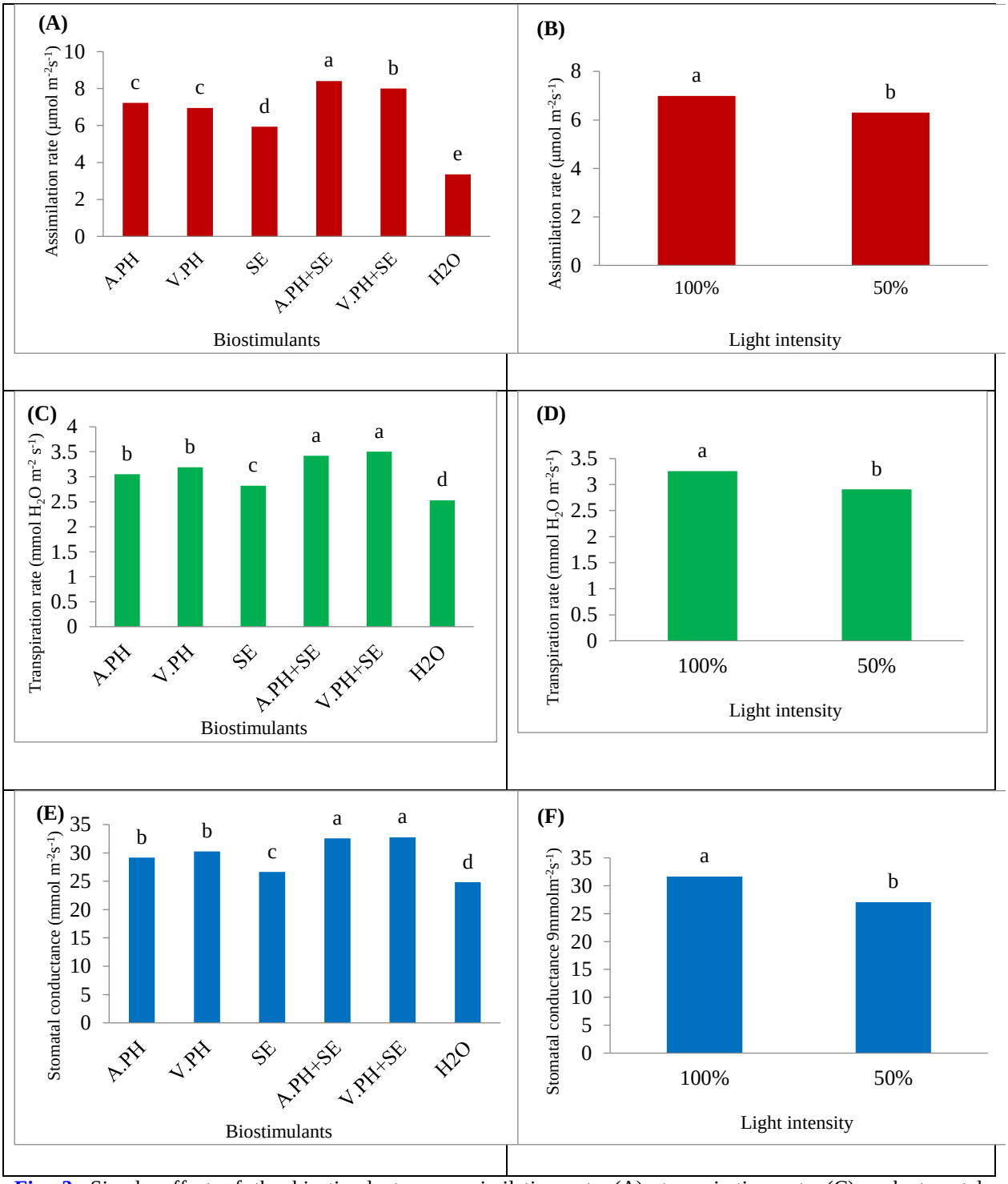

Fig. 3. Simple effect of the biostimulants on assimilation rate (A), transpiration rate (C) and stomatal conductance (E). Simple effect of light intensity on assimilation rate (B), transpiration rate (D), and the stomatal conductance (F). Different letters on bars indicate significant differences at ($p < 0.05$).

Table 5. Variance analysis of the effect of light intensity (A), bio-stimulants (B) and their interaction (A × B) on gas exchange parameters and chlorophyll fluorescence traits of *Viola ignobilis* Rupr.

Mean Squares							
S.o.V	df	Assimilation rate (A)	Transpiration rate (E)	Stomatal conductance (GH_2O)	qP	F_v/F_m	Yield (ΦPSII)
R	2	0.01757^{ns}	0.02235278ns	1.5606750ns	0.00026178ns	0.00059643ns	0.00019242ns
A	1	4.340277^{**}	1.11654444^{**}	189.2458778^{**}	0.16321600^{**}	0.04956560^{**}	0.04096576^{**}
R × A	2	0.010352^{ns}	0.00713611^{ns}	1.1311694^{ns}	0.00164233^{*}	0.00031228^{ns}	0.00089224^{ns}
B	5	20.04544^{**}	0.80371778^{**}	60.6758267^{**}	0.00914978^{**}	0.00589189^{**}	0.00700321^{**}
A × B	5	0.081864^{ns}	0.04514444^{ns}	0.2625711^{ns}	0.00055653^{ns}	0.00006401^{ns}	0.00021226^{ns}
Error	20	0.0601706	0.01039778	1.6061022	0.00046039	0.00068067	0.00085941
Total	35						
CV (%)		3.687751	3.299390	4.315018	2.515109	3.423534	3.953115

S.o.V: Source of variation, df: Degree of freedom, CV: Coefficient of variationAsterisks (*) represent the level of significance for each factors (A, B) and their interaction (A × B): NS: non-significant; * $p < 0.05$; ** $p < 0.01$.

The respiratory behavior of plants is different among species, in this experiment, all gas exchange parameters of *Viola ignobilis* Rupr. were higher in full sunlight. Importance of stomatal conductance correlated to water and CO_2 exchange between leaves and the atmosphere, which caused an increase in photosynthesis. Overall, plants grown under high light intensity are distinguished by the greatest stomatal conductance than plants grown at low light condition (Warren et al., 2007).

According to finding of Idris et al. (2019) the assimilation rate in some species of Malaysian plants in high light intensity was higher than shaded. Moreover, Proietti et al. (2023) demonstrated that an assimilation rate was three times higher in spinach leaves exposed in high light intensity compared to those at low light intensity. Therefore, these findings indicated that the increasing CO_2 gain improved photosynthetic efficiency and growth in plants.

In addition to light as a main factor in photosynthesis process, the findings indicate that there are a positive relationship between biostimulants application and photosynthetic behavior of plants. Colla (2015) declared that PHs promote the photosynthetic rate and energy supply for metabolic process due to the raise of N assimilation and amino acid biosynthesis in plant cells. Cristiano et al. (2018) indicated that the animal PH use in snapdragon had a positive effect on the photosynthetic parameters related to the leaf gas exchange. As a result of this experiment, the leaf net photosynthesis (+52%), transpiration rate (+55%), and stomatal conductance (+0.8%) significantly increased compared to control plants.

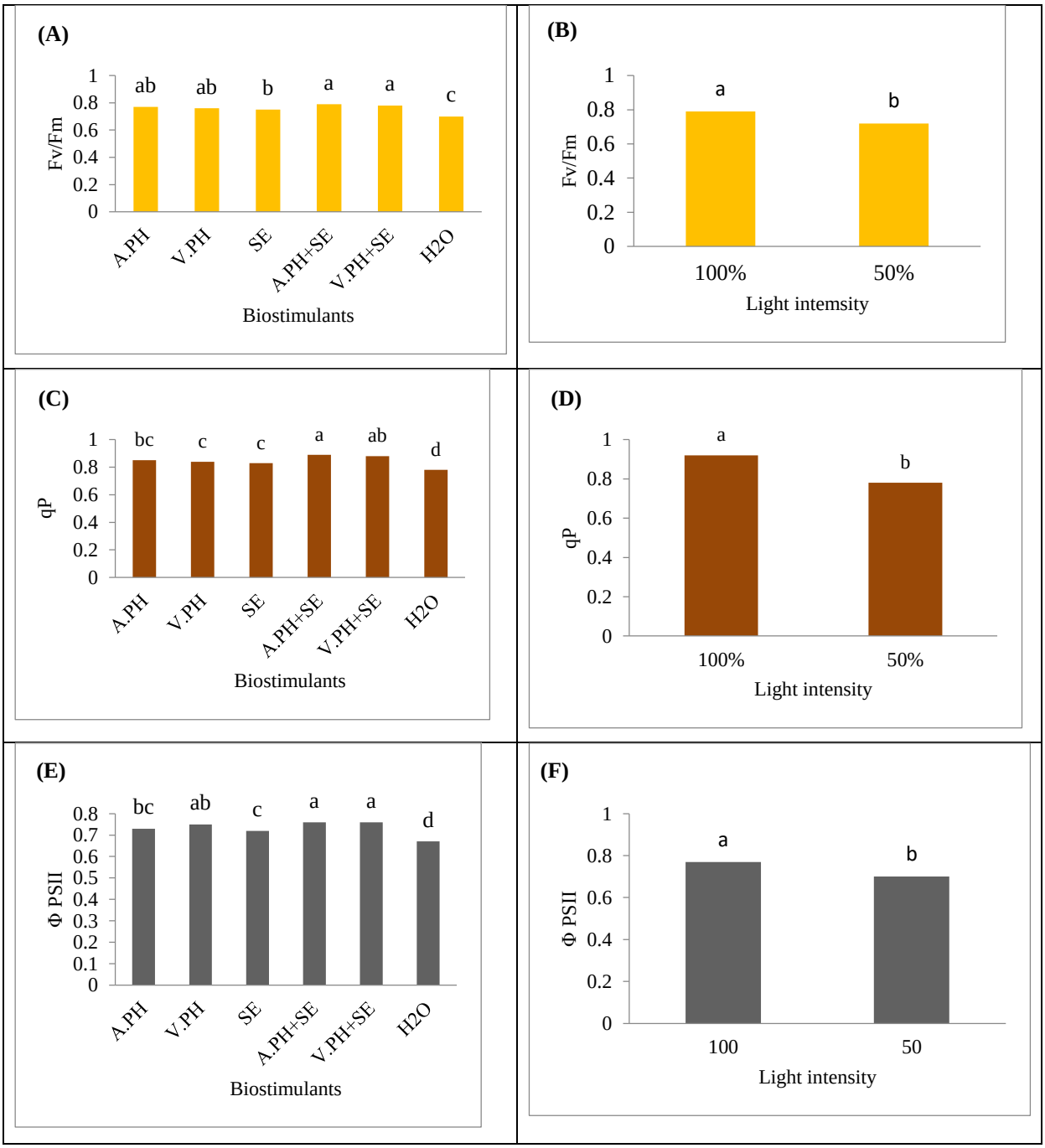

Fig. 4. Simple effect of the biostimulants on the value of F_v/F_m (A), the qP value (C), and the ΦPSII (E). Simple effect of light intensity on the value of F_v/F_m (B), the qP value (D), and the ΦPSII (F). Different letters on bars indicate significant differences at ($p < 0.05$).

Chlorophyll Fluorescence parameters

As shown in Table 5, the simple effect of light intensity and biostimulant application on the Chlorophyll Fluorescence parameters was significant ($p < 0.01$), although did not significantly respond to their interaction.

The value of F_v/F_m increased by 11.26% when the plant was grown in 100% light intensity rather than shaded plants (Fig. 4A). Furthermore, treated plants with biostimulants exhibited significant effect on F_v/F_m value ($p < 0.01$). The highest value of F_v/F_m (0.838) was detected in plants treated with A.PH + SE, on the other hand the lowest value (0.689) was

found in untreated plants. The interaction between light intensity and biostimulant didn't show any significant effect on F_v/F_m value (Fig. 4B).

The qP value increased by 17.94 % at 100% light intensity compared to the plants grown at 50% light intensity (Fig. 4C). The highest qP value (0.89) and lowest (0.78) were recorded respectively in the application of A.PH + SE treatment, and untreated plants (Fig. 4D).

The ΦPSII (quantum yield of photosynthetic electron transport) was 10% greater in plants grown at 100% light intensity (Fig. 4E). The highest value (0.76) of ΦPSII was recorded in plants treated with A.PH+SE and V.PH+SE treatments and minimum value (0.63) was related to control plants (Fig. 4F).

In recent years, chlorophyll fluorescence measurements have been known as useful and non-invasive tools to measures the quantum yield of photosystem II under different light conditions. In plants, PSII is an important component of photosynthesis. Maximum photochemical efficiency of PSII is determined by F_v/F_m ratio. Environmental condition like plant stresses influence on PSII and lead to remarkable decrease in the F_v/F_m ratio (Niari khamsi & Najaphy, 2012). Maximum photochemical efficiency of PSII (F_v / F_m), has been widely used for such researches in various species under different situation (Genty et al., 1989). In this study the higher F_v/F_m ratio was observed for treated *viola ignobilis* Rupr. were grown under full sunlight. All kinds of biostimulants caused to remarkable increase efficiency of photosystem II compared with untreated plant in both evaluated light intensities.

These results indicated that optimum light intensity improves the efficiency of PSII by increasing the energy transport from PSII to PSI. The qP value shows the amount of energy consume by photochemical reactions to the energy absorbed by antenna pigments in PSII and is correlated to CO_2 assimilation. High qP value is advantageous for electron transport and PSII yield (Guo et al., 2006).

Furthermore, the application of biostimulants separately and in combination with together enhanced qP value compared with untreated plants in both light intensities. Di mola et al. (2021) revealed that the application of PH effectively mitigated the impacts of salinity with regard to maintenance of higher F_v/F_m, and ΦPSII at salinity level, as a result of this, improve the photosynthetic productivity. A study set out by Asadi et al. (2022) to assess the effects of *Arbuscular Mycorrhizal* fungus and SE foliar application on growth and physiological traits of *Lactuca sativa* L. in this experiment the combination of AMF and SE enhanced photochemical efficiency of PSII (F_v/F_m). Overall, these results in terms of the maximum values of F_v/F_m, qP and ΦPSII indicated that full sunlight is necessary for normal growth of *Viola ignobilis* Rupr.

CONCLUSION

In current study, a sustainable approach was used in order to sustain medicinal plants productivity without the use of chemical fertilizer. The finding indicated that the plants were grown in full sunlight revealed maximum photosynthetic efficiency and yield. Moreover, the application of all kind of biostimulant, separately and in combination with each other resulted in an improvement in morphological and photosynthetic traits in both of light conditions. Overall, combined PHs and SE provided additive and synergistic effects on growth and development of *Viola ignobilis* Rupr. Future studies on the current topic are therefore recommended on other valuable medicinal plants.

Conflict of interest

The authors declare that there is no conflict of interest.

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Screening of some grape (*Vitis vinifera* L.) genotypes responses to drought stress using physiological and biochemical traits in greenhouse condition

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ABSTRACT

Purpose: Grapes (*Vitis vinifera* L.) are among the most significant agricultural products cultivated in various regions of Iran, boasting high nutritional value. This study focuses on assessing the genetic diversity of grape genotypes from vineyards in the West Azarbaijan province. Drought is an important environmental factor that limits plant growth and production. Given the abundant grape germplasm in Iran, there is potential to select cultivars and high-yielding genotypes possessing valuable genetic traits to use as resilient bases in commercial grape cultivars. **Research Method:** This research involved the evaluation of 16 grape genotypes in a single phase. For this purpose, 16 grape genotypes were grouped and compared in various dry conditions including (PEG0%, PEG2% and PEG4%). **Findings:** The results showed that vegetative traits, relative water content, and membrane stability decreased in all cultivars, but this decline was less pronounced in the «Garashire, Gezel, and Fakhri genotypes». Protein content and the activity of protective enzymes in the roots and leaves increased significantly across all 16 genotypes, with particularly notable levels observed in the «Garashire genotype». Drought stress had a marked effect on the accumulation of malondialdehyde and hydrogen peroxide in the Asgari and Reddish Tabriz genotypes. The levels of these compounds were higher in these genotypes compared to others, indicating increased lipid peroxidation and reduced stability against drought**. Research limitations:** There was no limitation. **Originality/Value:** The adverse effects of drought were more pronounced at the end of the stress period, especially under a high dose of PEG (4%). Overall, the «Garashire genotype» exhibited the highest tolerance, while the Asgari genotype was the most sensitive to drought.

INTRODUCTION

Grapes, scientifically classified as *Vitis vinifera* L., are part of the Vitaceae family, which is alternatively known as the Sarmentaceae or Ampelidaceae family (Keller & Tarara, 2010; Rasouli et al., 2014; Rasouli et al., 2015; Doulati Baneh, 2015; Jahnke et al., 2021; Kupe et al., 2021, Mirfatah et al., 2024a). Experts suggest that grape cultivation has been prevalent in Iran for at least 2000 years before the Common Era (Akram et al., 2021; Doulati Baneh, 2015; Jahnke et al., 2021; Kupe et al., 2021). Grapes are among the most essential fruits consumed by humans since ancient times. Alongside apples, citrus fruits, and bananas, grapes rank among the most crucial horticultural plants cultivated worldwide (Kupe et al., 2021). Climate plays a significant role in shaping grape diversity and production within specific regions. The ability to precisely select among plant varieties is crucial for breeding and developing new strains, a process reliant on recognizing existing varieties and their diversity. Investigating genetic diversity within plant populations and pinpointing suitable traits for the production and introduction of superior genotypes is essential (Mirfatah et al., 2024b, Zahedi et al., 2023). In the realm of screening, numerous studies and experiments have been carried out in Iran and other nations to identify drought-tolerant or resistant genotypes. The quest for resilient cultivars and genotypes against abiotic and biotic stresses stands as a critical strategy for managing such adversities (Razi et al., 2021). By defining appropriate morphological, physiological, and molecular characteristics for screening, it becomes viable to select cultivars and genotypes suited to the climatic conditions of each particular region (Amiri & Eslamian, 2010). In a study conducted by Haddadinejad et al. (2013), a screening process for drought-tolerant genotypes involved 698 genotypes across three stages (Haddadinejad et al., 2013). These studies play a critical role in identifying genotypes with enhanced tolerance, paving the way for their use as the foundation for commercial cultivars to enhance water efficiency in crop production (Zahedi et al., 2023). In a study by Rasouli et al. (2014), the phenotypic diversity of 32 grape varieties and genotypes was examined over three years. The research encompassed morphological and pomological traits, including phenolic content, surface anti-cancer composition of resveratrol, revealing significant diversity among the studied cultivars and genotypes in terms of various measured traits (Rasouli et al., 2014). In another experiment focusing on morphological variation, 36 grape cultivars and genotypes were assessed using the International Grape Descriptor to select superior genotypes based on traits like spike weight, dry spike weight, berry weight, rachis weight, seed weight, and skin color, revealing substantial variation and high diversity coefficients among cultivars and genotypes (Rasouli et al., 2015; Razi et al., 2021). Additionally, Kazemi et al. (2022) evaluated the phenotypic diversity of 60 grape cultivars and genotypes in the tropical and subtropical region of Khuzestan province in Iran. Their findings showcased significant diversity among grape cultivars and genotypes in Khuzestan province, with local cultivars like «Soltani (Sultan), Bangi (Red), and Yershi Dar» displaying superiority in certain traits compared to foreign cultivars (Kazemi et al., 2022). Asadi et al (2018) found that «Chifte» and «Khalili»cultivars along with Askari and «Pearlet» are relatively better in drought stress tolerance and can be cultivated in areas with limited access to water. These cultivars may also serve as a basis for breeding programs aimed at improving drought resistance.

Polyethylene glycol (PEG), mannitol, and sorbitol have been used to stimulate osmotic stress for in vitro selection (Darko et al. 2019), but PEG has been most frequently used to impose water-deficit stress in plants (Siaga et al., 2016). One of the advantages of using PEG is that it does not penetrate the apoplast, causing water withdrawal not only from the cell but also from the cell wall, replicating the effects of water deficit stress (Shirani Bidabadi et al., 2023). Studies have shown that increased drought stress induced by PEG can lead to a

significant reduction in tissue moisture content. Despite its widespread use, there has been a limited focus on investigating the effects of PEG-6000-induced drought stress specifically in viticulture. It is worth noting that PEG-induced osmotic stress can result in a decrease in cell water potential, a key indicator of the stress imposed on plant cells (Elmaghrabi et al., 2017). This property of PEG makes it a valuable tool for researchers and breeders seeking to understand plant responses to drought stress and to select for drought-tolerant plant varieties (Darko et al., 2019).

The current research conducted aimed to explore the morphological diversity among grape cultivars and genotypes sourced from vineyards in West Azarbaijan province, particularly Urmia city in northwest Iran. By delving into the physiological and biochemical responses of these grape cultivars and genotypes to simulated drought stress using PEG 6000, the study aimed to shed light on the mechanisms underlying drought tolerance in these varieties.

Furthermore, the research aimed to highlight and promote genotypes that exhibit promising traits associated with drought resistance, potentially offering valuable insights for grape cultivation in regions prone to water scarcity or drought conditions like Urmia city in Iran.

MATERIALS AND METHODS

Plant materials and growth conditions

Cuttings from 16 grape genotypes «including Lal, Ghezel, Asgari, Grashireh, Siah Sardasht, Tabarzeh Sefid, Tabarzeh Ghermez, Bidaneh Sefid, Bidaneh Ghermez, Fakhri, Hosseini, Rish Baba, Pastili, Hybrid H6, Chugao and Taefi» were initially prepared. After disinfection with 1.5% benomyl, the cuttings were treated with indole butyric acid (IBA) at a concentration of 100 ppm for 5–10 seconds. The treated cuttings were then placed in a perlite medium within a thermal bedding system, maintained at 22–28°C with 80–100% humidity to promote rooting, which typically occurred within 2–4 weeks.

After rooting, two healthy cuttings were selected and transferred to containers filled with perlite and Hoagland solution. The cuttings were maintained under the same conditions for two weeks, until 3 to 4 fully developed leaves had emerged. The modified Hoagland solution for grapes contained the following nutrient concentrations:0.125 mM KNO_3, 0.125 mM $Ca(NO_3)_2$, 0.05 mM $MgSO_4.7H_2O$, 0.0125 mM KH_2PO_4, 5.75 µM H_3BO_3, 1.34mM $MnCl_2.4H_2O$, 0.1 µM $ZnSO_4.7H_2O$, 0.038 µM $CuSO_4.5H_2O$, 0.025 µM $Na_2MoO_4.2H_2O$, and 8.88 µM Fe-EDTA (Abbaspour et al., 2013). After the two-week period and the development of four leaves, drought stress treatment was initiated using polyethylene glycol (PEG 6000) at concentrations of 0%, 2%, and 4%, in combination with the Hoagland nutrient solution. These treatments were applied to the four-leaf plants grown in 2-liter pots filled with perlite for duration of two weeks. To prevent osmotic shock, PEG 6000 was gradually added to the Hoagland nutrient solution until the target concentration was reached (Mohsen et al., 2020). The experiment followed a randomized complete block design with three replications, where each treatment included three replicates, and each replicate contained two plants. The length, wet and dry weight of shoots and roots were determined. Dry and fresh tissues of roots and leaves were kept at -80°C until biochemical assessments.

Leaf relative water content (LRWC)

The LRWC was assessed following the methodology detailed by Shams et al. (2019). Leaf segments, each measuring 10 mm, were initially weighed to establish the fresh mass (FM). These segments were then immersed in distilled water at room temperature and left to float

for a period of 24 hours to determine the turgor mass (TM). Subsequently, the leaf segments were subjected to oven-drying at 70°C for 48 hours and the resulting weight was recorded as the dry mass (DM). The LRWC percentage was calculated using the following equation 1:

$$LRWC(\%) = \frac{FM - DM}{TM - DM} \times 100 \qquad (1)$$

Electrical conductivity (EC)

The quantitative assessment of these indices was carried out using the method outlined by Sairam (1994). In this procedure, 2 grams of leaf tissue were first washed in bi-distilled water to eliminate any electrolytes attached to the surface. The samples were then immersed in a water bath at 40°C for 80 minutes, during which the electrical conductivity (EC) was gauged using an EC meter. Subsequently, the samples were transferred to a water bath at 70°C for 75 minutes, and the electrical conductivity was once again measured. The desired indices were calculated based on the following equation 2:

$$EC(\%) = \frac{EC1}{EC2} \times 100 \qquad (2)$$

Oxidative stress markers (MDA, H_2O_2)

Malondialdehyde (MDA) was determined following the protocol established by Rao and Sresty (2000). Frozen grape leaves (300 mg) were homogenized in 0.1% trichloroacetic acid (TCA) on ice. The homogenized solution was then centrifuged at 4°C for 10 minutes at 10,000g, and the resulting precipitate was extracted twice using the same solvent. Subsequently, 0.5 ml of the supernatant was combined with 1.5 ml of 20% TCA, followed by the addition of 0.5% thiobarbituric acid. The mixture was heated at 95°C for 25 minutes, cooled to room temperature (RT), and then centrifuged at room temperature for 10 minutes. The sample was measured at 532 nm and adjusted for non-specific absorption at 600 nm.

Hydrogen peroxide (H_2O_2) levels were assessed according to the method of Alexieva et al. (2001). A homogenization process involving 300 mg of leaf sample with 3 ml of 0.1% (w/v) TCA was carried out in a cool environment. Following this, centrifugation was performed for 15 minutes at 21,000×g. A mixture of 1 mL of 1 M potassium iodate and 500 µl of 10 mM K_2PO_4 buffer (pH 7) was combined with 500 µL of the supernatant. The absorbance was then measured at 390 nm to determine the concentration of hydrogen peroxide.

Total Protein

The total protein content was determined using the method outlined by Bradford in 1976. To conduct this analysis, 0.25 g of leaf tissue was homogenized with 2.5 mL of 50 mM KH_2PO_4 buffer (pH=7), and the resulting homogenate was centrifuged at 15,000 g for 20 minutes at 4°C. Subsequently, 2.5 mL of Coomassie Brilliant Blue G-250 was added to 20 µL of the supernatant and vortexed. After an incubation period of 10 minutes, the samples were measured at 595 nm. Bovine serum albumin (BSA; Sigma A7906) served as the standard protein for calculating the total protein content in the leaf samples.

Antioxidant enzymatic activities

For the antioxidant enzyme assays, Grapevine leaves (0.2 g each) were ground to a fine powder using liquid nitrogen and then extracted in 3 mL of a buffer comprising 50 mM KH_2PO_4 (pH=7.0), 0.1 mM EDTA, and 1% polyvinylpyrrolidone (PVP) (w/v). The homogenate was subsequently filtered and centrifuged at 4°C for 15 minutes at 15,000×g. The

supernatant was used for Ascorbate peroxidase (APX) and Catalase enzyme activity (CAT) and Guaiacol peroxidase (GPX) activity assay (Ulusu et al., 2017).

Ascorbate peroxidase (ASPX, EC 1.11.1.11)

Analysis of ASPX activity was determined using the method of Karabal in (2003). The reaction was monitored by observing a decrease in absorbance at 290 nm over a 3-minute period. The reaction mixture, with a total volume of 3 mL, comprised 1450 µL of 50 mM phosphate buffer (pH=7), 750 µL of ascorbic acid, 750 µL of 30% H_2O_2, and 50 µL of plant extract. The enzyme activity was computed using the extinction coefficient of ascorbate (2.8 mM^{-1} cm^{-1} at 290 nm).

Catalase (CAT, EC 1.11.1.6)

activity was tested in a reaction mixture (final volume: 3 mL) containing 1450 µl of 50 mM KH_2PO_4 buffer (pH=7), 1500 µl of 30% H_2O_2 and 50 µl of plant extract. The disappearance of H_2O_2 was followed at 240 nm (3 min) (Schimadzu UV-1800, Japan) (H_2O_2: 0.036 $\mu mol^{-1}cm^{-1}$) (Ulusu et al., 2017).

Gayacol peroxidase (GPX, EC 1.11.1.7)

The activity of GPX was measured using Chance and Maehly method (1955). The reaction mixture includes 100 mM potassium phosphate buffer solutions (pH=7), guaiacol 10 mM dissolved in double distilled water, 70 mM hydrogen peroxide dissolved in 100 mM potassium phosphate (pH=7), sterile double distilled water and enzyme extract It was measured for 180 seconds. The mixture without enzyme extract was used as a blank. The specific activity of the peroxidase enzyme was reported as the number of micromoles of hydrogen peroxide decomposed per minute per milligram of protein.

Statistical analysis

Statistical analyses were done using the Statistical Package for Social Sciences (SPSS). The data were subjected to analysis of variance (ANOVA) and the means were compared with Duncan's multiple range test ($P \leq 0.05$). Significant differences between treatment means are indicated by different letters.

RESULTS

Based on the obtained results, characteristics such as fresh weight, dry weight, length, relative water content, ion leakage, malondialdehyde, hydrogen peroxide, protein and antioxidant enzymes in aerial and terrestrial organs showed significant variation among genotypes. Considering the diversity observed in these traits, performing more detailed statistical analyzes can be useful for evaluating traits with significant diversity in different cultivars and genotypes.

Physiological parameters

The impact of drought stress on the growth characteristics of grape genotypes is outlined in Table 1. As the percentage of PEG increased, the fresh weight, dry weight of shoots and roots, and stem height of all 16 grape genotypes decreased. It is noteworthy that in most genotypes, root height notably increased with higher PEG percentages. The Garashireh, Fakhri, and Gezel genotypes exhibited the highest root heights (38.54 cm, 37.63 cm, and 36.53 cm respectively), while the Asgari, Tabarzeh Ghermez, Taefi, and Tabarzeh Sefid genotypes showed lower root heights compared the others (10.32 cm, 16.21 cm, and 16.16 cm

respectively). In the Siah Sardasht genotype, the height decreased while the root height remained unchanged. Overall, the analysis of variance indicated that PEG-induced stress led to a reduction in the fresh weight of both aerial and terrestrial organs. Bidaneh Ghermez and Pastili genotype exhibited the highest percentage of shoot fresh weight reduction (PEG4%: 68%) compared to the control (PEG0%). The Asgari genotype showed the lowest dry weights for both shoot (PEG4%: 0.38g) and root (PEG4%: 0.2g). Conversely, the Garashireh genotype displayed the highest dry weights for shoot (PEG4%: 1.10g) and root (PEG4%: 1.03g) (Table 1).

Table 1. Comparison of the average effects of drought stress induced by PEG6000 on the fresh weight, dry weight, and height of 16 grape genotypes.

Genotype & Dry (PEG)	Root length (cm)	Shoot length (cm)	Root dry weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Shoot fresh weight (g)
Hosseini						
0%	23±2h-m	35±2.6g-k	0.8±0.05efg	1.1±0.09def	7.8±0.3e-i	12.7±0.4def
2%	28±2.6d-i	34±2.9g-l	0.8±0.09e-h	1±0.03f-j	6.9±0.2g-l	7.6±0.9i-m
4%	31±2a-f	34±2h-l	0.4±0.03n-r	0.8±0.07i-m	5.9±0.4j-o	5.6±0.8k-p
Ghezel						
0%	22±2i-n	47±8.1bc	1.1±0.05c	1.6±0.07bc	11.7±1.9b	18.34±0.1b
2%	32±2.7a-f	41±2.6c-g	0.8±0.06e-j	1.1±0.06e-h	10.3±0.6bc	10.3±0.8fg
4%	36±2.1abc	31±3.1e-i	0.4±0.03n-r	0.9±0.08g-k	7.7±0.1e-j	9.3±0.3gh
Tabrzeh Sefid						
0%	29±1.6d-i	20±2.1q-u	0.4±0.01n-s	0.8±0.09i-m	5±0.2l-s	4.6±0.08n- t
2%	20±1.8j-n	17±1.2s-v	0.2±0.01stu	0.8±0.07i-m	4.4±0.5m-t	3.4±0.2p-w
4%	25±1.2f-l	16±1.2tuv	0.2±0.01u	0.6±0.02mno	4.4±0.7n-t	1.7±0.4vwx
Tabarzeh Ghermez						
0%	22±1.4i-n	33±2h-l	0.36±0.04p-t	0.7±0.01lmn	4.9±0.1l-s	2.9±0.3r-x
2%	18±1.1mno	28±0.8k-p	0.33±0.01q-u	0.6±0.03mno	3.6±0.3q-u	1.9±0.1vwx
4%	16±0.8m-p	13±0.8uv	0.2±0.01tu	0.5±0.06opq	3.2±0.1stu	1.7±0.2wx
Bidaneh Sefid						
0%	22±1.2i-n	48±3.1bc	0.9±0.07de	1.3±0.04cd	8.6±0.7c-g	12±0.9de f
2%	32±2.3a-f	42±4.2b-f	0.49±0.02m-p	1.2±0.03de	6.4±0.1i-m	8.3±1g-j
4%	34±3.4a-f	35±2.8g-k	0.41±0.04o-s	0.8±0.08i-m	6.1±0.6i-n	5.2±0.6i-q
Bidaneh Ghermez						
0%	30±1c-h	65±2.8a	0.8±0.03def	1.1±0.08e-h	8.6±0.7c-g	15.7±0.6c
2%	30±0.8b-g	44±4.3b-e	0.6±0.01klm	0.7±0.07lmn	6.4±0.1i-m	7±0.5i-m
4%	31±1.2a-f	28±3.3k-p	0.3±0.03q-u	0.6±0.06mno	6.1±0.6i-n	5±0.3j-s
Fakhri						
0%	31±1.8a-f	65±2.6a	0.9±0.06de	1.4±0.08bc	10±0.7bc	18.3±1.3b
2%	35±0.8a-d	43±1.8b-e	0.7±0.05h-l	1.2±0.005de	9.6±0.7cde	9±0.3ghi
4%	37±1.2ab	27±1.8k-p	0.5±0.02lmn	0.9±0.08f-j	9.5±0.7cde	6.6±0.5j-n
Siah Sardasht						
0%	31±2.9a-f	42±2.3b-f	0.9±0.05def	1.3±0.06de	8.3±0.5d-h	8.9±0.6ghi
2%	30±2.6b-g	35±2.3b-f	0.5±0.04mno	0.9±0.06h-l	6.1±0.8i-n	7.3±0.6h-l
4%	31±2.6b-f	33±2.3h-l	0.4±0.02m-p	0.6±0.04mno	4.9±0.5l-s	4.8±0.9m-s
Pastili						
0%	20±1.8j-n	27±1.4l-q	0.8±0.06f-j	1±0.08h-l	6.6±0.6k-l	11.9±0.6ef
2%	27±2.3f-j	24±0.8m-s	0.7±0.04i-l	0.8±0.07mno	5.6±0.2k-p	4.1±0.6o-v
4%	26±1.6f-j	22±2.6o-t	0.4±0.03o-s	0.7±0.04mno	4.3±0.7n-t	3.7±0.7o-w
Lal						
0%	15±0.8nop	31±0.8i-m	0.7±0.02f-j	0.9±0.03 h-l	5.9±0.5i-o	6.6±0.4j-n
2%	19±3.4lmn	24±2.6m-r	0.5±0.05mno	0.6±0.05mno	4.2±0.5n-t	3.1±0.6q-x
4%	23±5.4j-m	19±0.8r-v	0.4±0.01n-q	0.6±0.05mno	4.1±0.3o-t	2.6±0.2s-x
Chugao						
0%	20±2j-n	36±2f-j	0.8±0.1e-j	1±0.06f-i	7.5±0.2f-k	5.9±0.9k-o
2%	26±2.4f-k	31±2.3i-m	0.6±0.02jkl	0.8±0.03j-m	5.9±0.7i-o	4.9±0.3i-s

4%	30±1.1c-h	29±2.3j-n	0.3±0.02o-t	0.9±0.04k-n	5.5±0.4l-q	4.3±0.2n-u
Rish Baba						
0%	18±2.3lmn	31±2i-m	0.7±0.01h-k	0.9±0.05h-l	5.3±0.5l-r	5±0.7i-r
2%	26±2.4f-k	21±1.7p-t	0.7±0.04h-k	0.9±0.07h-l	4.5±0.1m-t	2.7±0.2s-x
4%	23±2h-m	16±0.8tuv	0.4±0.02n-r	0.7±0.08lmn	4.4±0.5n-t	2.2±0.1u-x
Hybrid H6						
0%	20±1/5j-n	49±2.8b	0.8±0.03e-j	1±0.07f-j	6.9±0.7g-l	7.6±0.8h-k
2%	22±2.4i-n	31±1.3i-m	0.7±0.02g-k	0.9±0.06f-j	5±0.6l-s	5.1±0.8i-r
4%	27±2.4e-i	24±2.6m-r	0.6±0.05klm	0.9±0.05h-l	4.1±0.3o-t	3.9±0.5o-w
Taefi						
0%	22±1.2i-n	28±1j-o	0.3±0.03o-t	0.8±0.05i-m	5.3±0.9l-r	5.3±0.7i-q
2%	19±0.8k-n	22±1.4n-t	0.29±0.01stu	0.7±0.02lmn	4.3±0.6n-t	2.4±0.5t-x
4%	20±1.4j-n	15±1tuv	0.20±0.02u	0.5±0.03nop	3.7±0.5p-u	1.7±0.3vwx
Asgari						
0%	18±1.5mno	20±1q-v	0.3±0.01r-u	0.5±0.05nop	3.4±0.3r-u	2±0.2u-x
2%	12±1.5op	16±0.9tuv	0.25±0.01tu	0.4±0.03pq	2.8±0.5tu	1.5±0.1w-x
4%	10±1.2p	13±1v	0.2±0.01u	0.3±0.02q	1.9±0.2u	1±0.1x
Garashireh						
0%	32±1.4a-f	63±2.9a	2.5±0.04a	1.9±0.06a	18.9±0.5a	23.5±1.5a
2%	35±1.7a-d	46±2.1bcd	1.6±0.06b	1.6±0.04bc	10±0.9bcd	17.7±1.2b
4%	38±2a	39±1.7d-h	1±0.03cd	1.1±0.05e-h	9±0.4c-f	14.2±0.7cd

Means followed by different letters in each column indicate significant difference at $p \leq 0.05$ (Dunkan test).

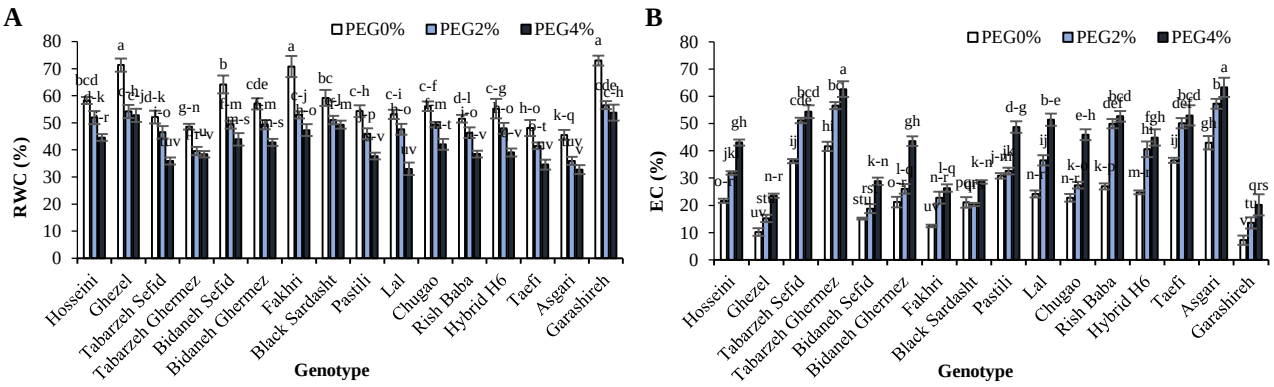

Fig. 1. LRWC (A) and EC (B) in leaves of 16 grape genotypes under different drought treatments (PEG 0%, 2% and 4%). Bars are mean ± standard error.

Drought stress significantly reduces the LRWC and EC in the leaves of all genotypes (Fig. 1). Under PEG4% stress, the Garashireh genotype exhibited the highest RWC percentage at 53.76%, while the Asgari genotype had the lowest LRWC percentage at 32.77%. There was a statistically significant difference in EC percentage among genotypes and treatments ($P \leq 0.05$). The lowest EC percentage under PEG4% stress was observed in the Garashireh (20.20%), Fakhri (26.32%), and Ghezel (23.54%) genotypes, while the highest percentage was recorded for the Asgari (63.29%) and Tabarzeh Ghermez (62.64%) genotypes.

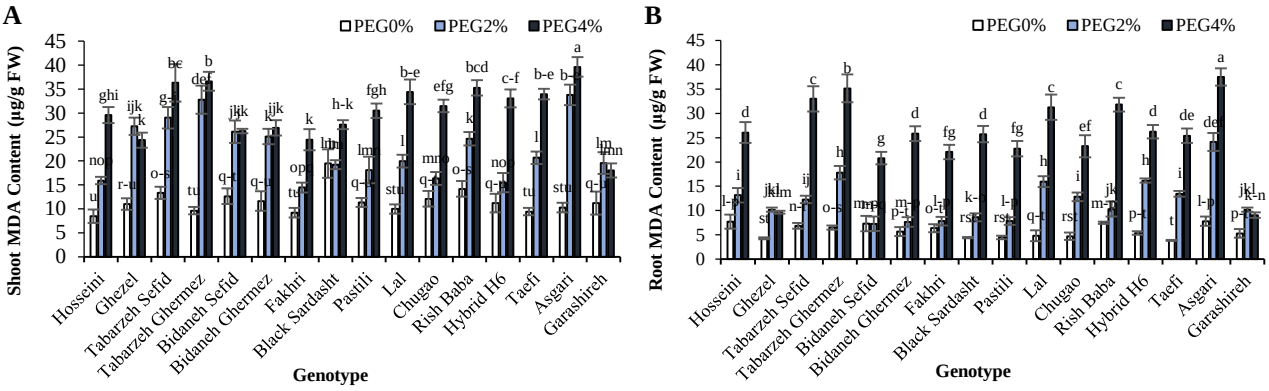

Fig. 2. MDA content (µg.g^{-1} FW) in leaves (A) and roots (B) of 16 grape genotypes under different drought treatments (PEG 0%, 2% and 4%). Bars are mean ± standard error.

MDA, H_2O_2 and protein contents

The effect of drought stress on malondialdehyde (MDA) content of grape genotypes is shown in Figure 2. MDA content increased significantly (P≤0.05) in the roots and leaves of all genotypes with increasing drought stress. However, this increase in the leaves of Asgari (37.49µg.g^{-1} FW), Tabarze Ghermez (35.16µg.g^{-1} FW), Tabarze Sefid (33µg.g^{-1} FW), and Lal (31.25µg.g^{-1} FW) was relatively more than other genotypes. The roots and leaves of Grashireh, Ghezel, Fakhri and Pastili showed lower MDA content compared to other genotypes (Figure 2). Variance analysis showed that the difference in MDA content in root and leaf between genotypes, treatments and genotype × treatment interaction was statistically significant (P≤0.05).

The contents of H_2O_2 were investigated in 16 grape genotypes (Fig. 3). Under well-watered conditions, there were no significant changes in the contents of H_2O_2 in the leaves and root. However, as the drought stress prolonged, the contents of H_2O_2 gradually increased in the grapevine leaves. A notable observation in the Asgari genotype is the substantial increase in H_2O_2 levels in the roots (5.08µg.g^{-1} FW) under PEG4% stress conditions.

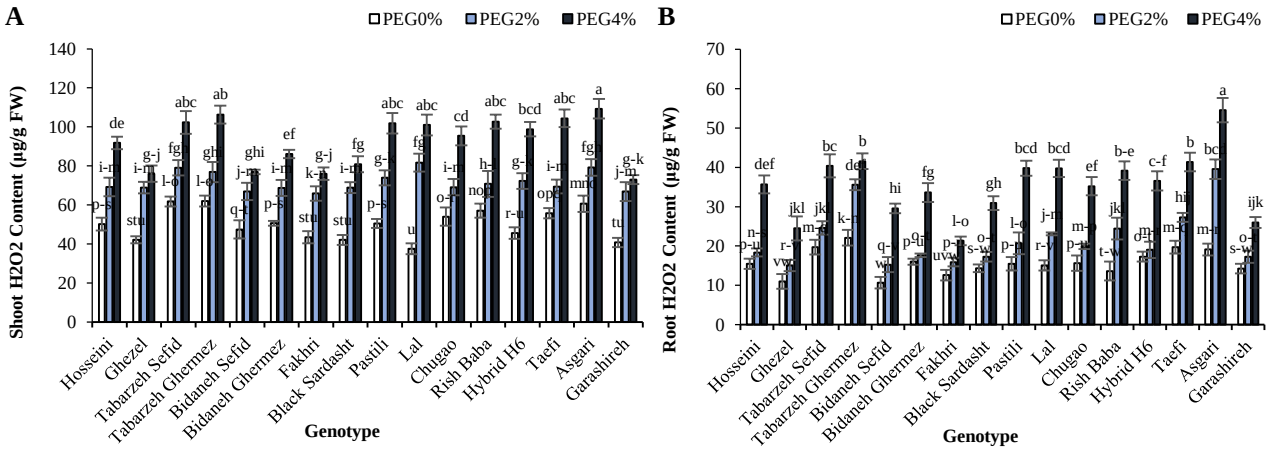

Fig. 3. H_2O_2 content (µg.g^{-1} FW) in leaves (A) and roots (B) of 16 grape genotypes under different drought treatments (PEG 0%, 2% and 4%). Bars are mean ± standard error.

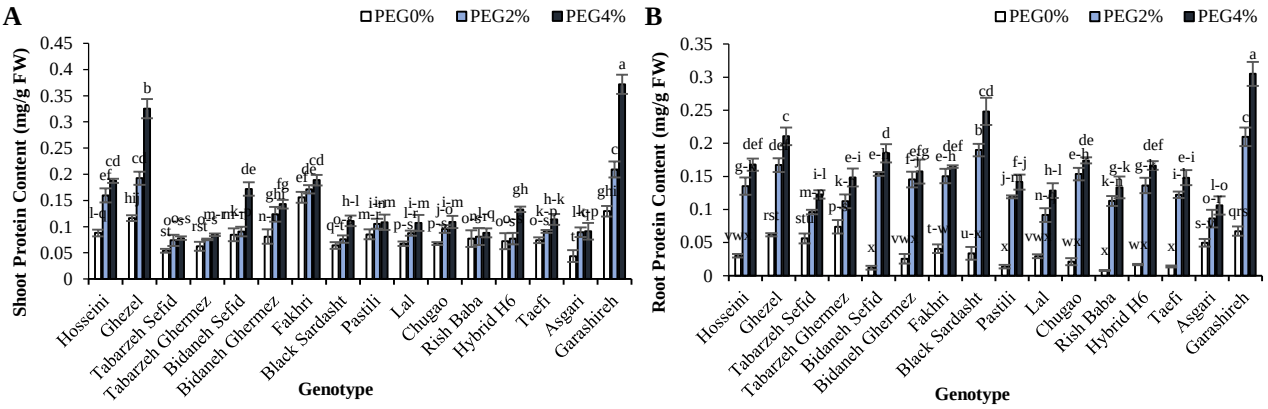

Fig. 4. Protein content (µg.g^{-1} FW) in leaves (A) and roots (B) of 16 grape genotypes under different drought treatments (PEG 0%, 2% and 4%). Bars are mean ± standard error.

Specifically, the amount of H_2O_2 surged by 186% when compared to the control group subjected to PEG (0%) stress. This significant rise in hydrogen peroxide content indicates an elevated level of oxidative stress in the roots of the Asgari genotype under severe osmotic stress induced by PEG (4%) (Fig. 3).

Based on the comparison of average data, a significant difference was found between the 16 genotypes in terms of total protein levels in both the shoot and roots. Additionally, it was observed that as the percentage of PEG increased, the total protein content also increased. The highest amount of protein was observed in the aerial parts of the Garashireh (0.37µg.g^{-1} FW) and Ghezel (0.32µg.g^{-1} FW) genotypes, as well as in the root parts of the Garashireh (0.30µg.g^{-1} FW), SiahSardasht(0.24µg.g^{-1} FW), Ghezel (0.21µg.g^{-1} FW), and Bidaneh Sefid (0.18µg.g^{-1} FW) genotypes under PEG4% stress conditions. Conversely, there was no significant difference in the protein content among the remaining genotypes under the same stress conditions (Fig. 4).

Antioxidant enzyme activities

The experiment results indicate that drought stress leads to an elevation in antioxidant enzyme levels, particularly noticeable at higher PEG concentrations. Across all genotypes, the activity of the APX enzyme was generally higher in the shoots compared to the roots. While PEG2% stress did not show significant differences, a pronounced disparity was observed at PEG4% stress in the leaves of genotypes such as Garashireh (1.93 Unit.mg protein $^{-1}$), Fakhri (0.82 Unit.mg protein $^{-1}$), Bidaneh Sefid (0.54 Unit.mg protein $^{-1}$), Ghezel (1.26 Unit.mg protein $^{-1}$), Hosseini (0.51 Unit.mg protein $^{-1}$), and Bidaneh Ghermez (0.42 Unit.mg protein $^{-1}$), compared to the PEG-free control plants. Notably, Asgari (0.19 Unit.mg protein $^{-1}$), Tabreze Ghermez (0.22 Unit.mg protein $^{-1}$), Rish Baba (0.36 Unit.mg protein $^{-1}$), and Lal (0.23 Unit.mg protein $^{-1}$) genotypes exhibited the lowest APX enzyme activity levels (Fig. 5A and B). A significant divergence in CAT enzyme level increments was noted between the shoots and roots of the genotypes, with the roots of Garashireh (9.19 Unit.mg protein $^{-1}$), Fakhri (10.19 Unit.mg protein $^{-1}$), SiahSardasht (3.44 Unit.mg protein $^{-1}$), Bidaneh Sefid (4.49 Unit.mg protein $^{-1}$), Bidaneh Ghermez (3.57 Unit.mg protein $^{-1}$), Ghezel (5 Unit.mg protein $^{-1}$), and Hosseini (2.40 Unit.mg protein $^{-1}$) genotypes displaying higher CAT activity levels. CAT enzyme levels notably increased with escalating drought stress in the leaves of Garashireh (7.35 Unit.mg protein $^{-1}$), Hosseini (4.38 Unit.mg protein $^{-1}$), Ghezel (5.02 Unit.mg protein $^{-1}$), Fakhri (7.32 Unit.mg protein $^{-1}$), Bidaneh Sefid (7.08), Bidaneh Ghermez (6.28 Unit.mg protein $^{-1}$), Siah Sardasht (4.17 Unit.mg protein $^{-1}$), and Hybrid (H6) (3.34 Unit.mg protein $^{-1}$) raisin plants. However, no significant differences were observed in other genotypes as drought stress levels

increased (Fig. 5 C and D). Furthermore, the GPX enzyme activity showed a significantly higher level in shoots compared to roots. The most substantial increase under PEG4% stress was observed in the leaves of the Garashireh genotype (1.06 Unit.mg protein $^{-1}$) and in the roots of the Fakhri (0.43 Unit.mg protein $^{-1}$) genotype (Fig. 5 E and F).

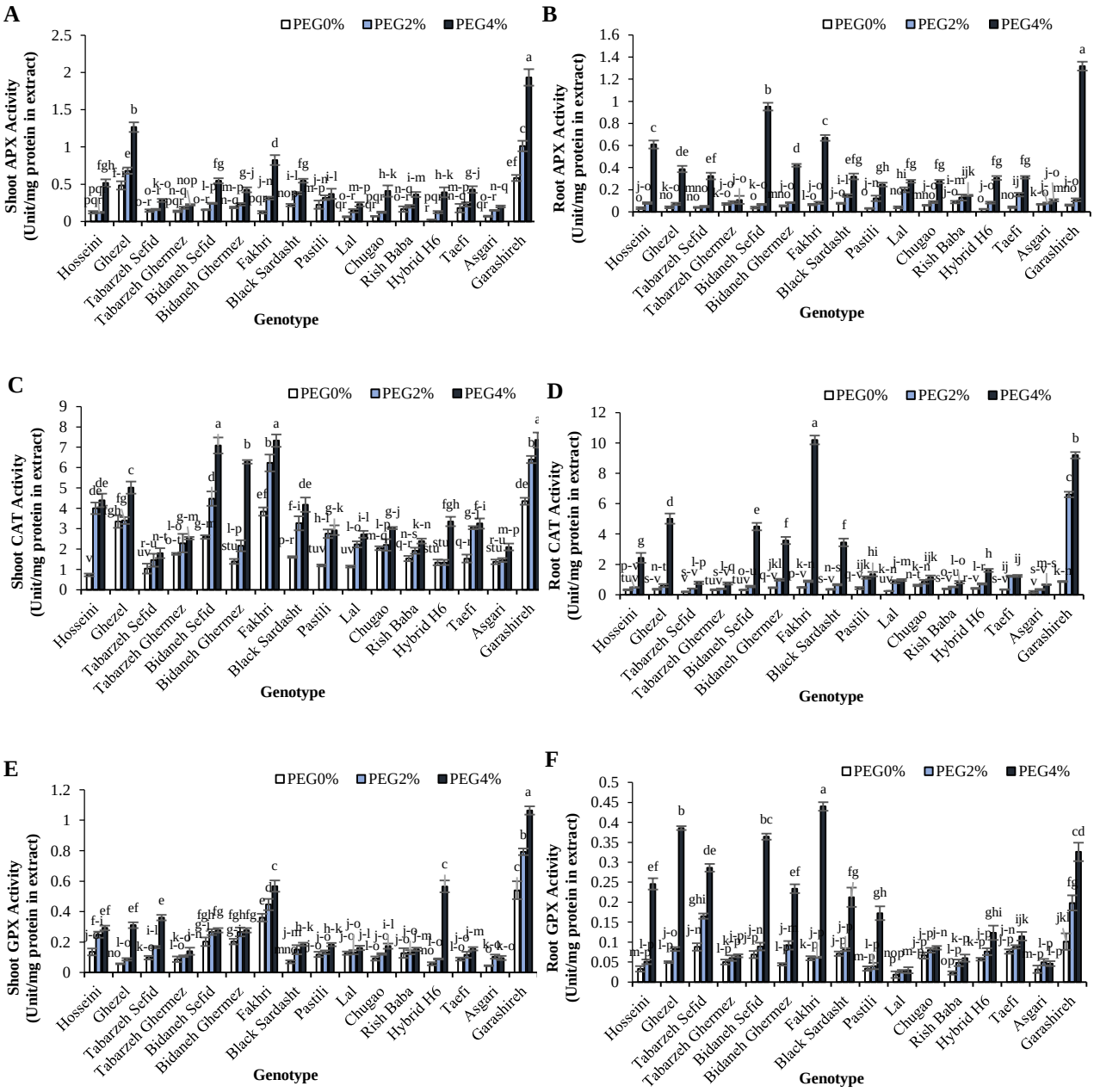

Fig. 5. Ascorbate peroxidase (APX) activity in leaf (A) and root (B), Catalase (CAT) activity in leaf (C) and root (D) and Guaiacol peroxidase (GPX) activity in leaf (E) and root (F) of 16 grape genotypes under different drought treatments (PEG 0%, 2% and 4%). Bars are mean ± standard error.

DISCUSSION

Drought stands out as the predominant abiotic stress factor that significantly constrains the growth and productivity of crop plants. Consequently, focusing on breeding programs aimed at developing genotypes that exhibit tolerance to drought stress plays a crucial role in mitigating the substantial yield losses associated with this stressor (Levitt, 1980; Karimi et al., 2012). Through targeted breeding efforts, the cultivation of drought-tolerant crop varieties can help safeguard agricultural productivity and food security in the face of drought-induced challenges. The study examined 16 grape cultivars under in vitro conditions subjected to PEG-induced drought stress. Efforts have been directed towards identifying stress-tolerant plants under in vitro conditions across a diverse array of plant species, encompassing cereals, vegetables, fruits, and other economically significant plant varieties (Rai et al., 2011; Bigdeloo et al., 2018). By evaluating grape cultivars in this controlled environment, the research aims to elucidate stress responses and potentially identify drought-tolerant genotypes that could be valuable for future cultivation practices. The study observed significant reductions in fresh and dry weight stem and root length as the PEG concentration in the culture medium increased. Oukabli et al. (2008) also noted restricted growth under drought stress conditions. PEG serves to lower water potential, mimicking drought stress without inducing toxic effects or plant uptake (Rumbaugh & Johnson, 1981; Kent & Lauchli, 1985; Bigdeloo et al., 2018). LRWC is regarded as a crucial indicator for evaluating plant tissue water status (Kramer, 1983). Numerous studies have demonstrated a decline in LRWC in response to drought stress (Augé et al., 2003; Liu et al., 2008; Sarvari et al., 2017). In the current investigation, LRWC decreased with escalating PEG concentrations in the medium. Notably, the Garashireh genotype exhibited a smaller decline in LRWC with increasing stress levels compared to other genotypes, with a decrease ranging from 26% to 20% in contrast to the control group. Conversely, the Siah Sardasht variety displayed lower LRWC levels than Garashireh under normal conditions, yet its LRWC decreased by 16% under PEG-induced 4% stress. This suggests that the Siah Sardasht genotype coped better with PEG stress in terms of LRWC compared to the Garashireh genotype. According to the research by Asadi et al. (2019), the Galati and Melai cultivars demonstrate better drought tolerance compared to the Fakhri variety. These cultivars can be cultivated in areas with limited water availability.

It has been observed that water stress reduces the relative water content in grapes. These findings are consistent with the results reported by other researchers (Khalil & Badr Eldin, 2021; Fahim et al., 2022; Zeng et al., 2022; Fayek et al., 2022; Bidabadi et al., 2023). Among others. These results indicate that reduced water availability can create challenges for plant roots in terms of water and soil uptake, forcing plants to regulate water loss through transpiration. This adjustment may lead to a decrease in LRWC in grape leaves, which may not necessarily indicate optimal conditions for grape plants (Al-Tabbal et al., 2020).

Leaf RWC serves as a crucial indicator of plant water status, closely tied to cell volume and reflecting the balance between water supply to leaves and their transpiration rate (Deka et al., 2018). It is considered a valuable metric for assessing the level of water stress experienced by leaves. RWC is an indicator of the metabolic activity within plant tissues (Yan et al., 2016) and typically exhibits a significant decrease under water stress conditions. When the soil moisture levels are inadequate and roots face water scarcity, plants may struggle to compensate for water loss through transpiration. Consequently, the RWC of leaves tends to decrease as a result of this water stress (Al-Tabbal et al., 2020). Monitoring RWC can provide insights into the water status and physiological condition of plants, especially under challenging environmental conditions like water scarcity.

Drought stress can lead to an increase in leaf electrolyte leakage, which is often indicator of cellular injury. Under drought conditions, reactive oxygen species (ROS) accumulate due to the stress on cell membranes, ultimately causing damage. This stress-induced ROS production can result in membrane lipid peroxidation, leading to increased membrane permeability and ion leakage, disrupting membrane structure and function (Hnilickova et al., 2018). The extent of this damage can be assessed by measuring electrical conductivity resulting from ion leakage. Research of Min et al. (2019) indicated that polyethylene glycol (PEG) can elevate electrolyte leakage in leaves and young grapevine seedlings. Studies of Zeng et al. (2022) demonstrated that drought stress in grape leaves increases the production of hydrogen peroxide (H_2O_2) and free radicals, leading to higher levels of malondialdehyde (a byproduct of membrane lipid peroxidation) and increased ion leakage, aligning with similar findings in other studies. Furthermore, Fahim et al. (2022) observed a significant increase in electrolyte leakage in 14 commercial grape varieties under heightened drought stress, echoing the results of previous studies. Altinci and Cangi (2019) research on six commercial wine grape varieties in vitro revealed membrane destruction, cellular damage, and inactivation of ion pumps on the cell membrane due to drought stress, resulting in elevated electrolyte leakage. These findings collectively underscore the impact of drought stress on membrane integrity and ion balance in grapevines.

In the current study, it was observed that increasing the concentration of PEG induced drought stress in grapevines led to an increase in the activity of antioxidant enzymes such as ascorbate peroxidase (APX), catalase (CAT), and guaiacol peroxidase (GPX). These findings align with previous reports on grapes (Pontin et al., 2021; Fahim et al., 2022; Shirani Bidabadi, 2023). Fahim et al. (2022) noted that under decreasing water levels, antioxidant enzymes like superoxide dismutase (SOD) and CAT, as well as enzymes from the glutathione-ascorbate cycle such as APX and POD, increase to counteract the effects of reactive oxygen species (ROS).The activation of enzymatic defense mechanisms, including the increase in antioxidant enzyme activity, in response to drought stress is crucial for detoxifying ROS and combating the rise in free radicals within plant cells under stressful conditions, often linked to H_2O_2 production (Fahim et al., 2022). Inducing enzyme activity in antioxidants represents a general adaptation strategy employed by plants to mitigate oxidative stress-induced damage (Noctor & Foyer, 2016). Plant cells are equipped with a free radical scavenging system comprising both antioxidant enzymes and non-enzymatic antioxidants for protection against oxidative damage (Keivanfar et al., 2019). In research of Zeng et al. (2022), it was found that drought stress significantly increased SOD activity while decreasing CAT activity compared to the control group of the examined grapes. Another study reported by Shirani Bidabadi et al. (2023) indicated a decrease in CAT enzyme activity in grape leaves under drought stress, which was attributed to a reduction in iron concentration, a cofactor for the CAT enzyme. Conversely, an increase in CAT activity was observed in Cabernet Sauvignon grapes following severe drought stress for 16 days, as reported by Shirani Bidabadi (2023).

CONCLUSION

The main goal of measuring the traits was to evaluate diversity and identify superior grape cultivars for breeding programs. Limited water access compromises plants' water absorption, leading to decreased cell water potential, which can produce damaging free radicals. These radicals can harm cell membranes, degrade photosynthetic pigments, and reduce photosynthesis efficiency, ultimately stunting growth and productivity. Thus, adequate water availability is critical for healthy plant growth. The «Garashireh genotype» has been

identified as drought-tolerant among tested grapevines. Its notable drought tolerance mechanisms include:

- Reduced Malondialdehyde (MDA) Content: Lower MDA indicates less oxidative stress and better cellular integrity.
- Enhanced Antioxidant Enzymes: Increased activity of these enzymes helps scavenge reactive oxygen species (ROS), protecting cells from damage.
- Lower Ion Leakage: This suggests better membrane stability and reduced cell damage under drought stress.
- Decreased Hydrogen Peroxide Levels: This reduction indicates effective ROS scavenging, mitigating oxidative stress.
- Tolerance to PEG Stress: The genotype can withstand various PEG doses, showing adaptability to osmotic stress.
- Maintenance of Vegetative Apparatus: The «Garashireh genotype» sustains growth despite drought, demonstrating resilience.

These mechanisms underscore the «Garashireh genotype» adaptability to drought, making it a strong candidate for regions facing water scarcity.

Conflict of interest

The authors have no conflict of interest to report.

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Avoiding endocarp lesion and abscission of pistachio nut using lecithin-enriched calcium nitrate

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ABSTRACT

Purpose: The pistachio nut is an important product primarily traded as dry nut in-shell kernels. Any damage to the shell during growth and development can cause shell staining and kernel decay in pistachios, rendering them unsuitable for sale. This study aimed to mitigate these issues by evaluating the effects of various calcium nitrate solutions. **Research method:** Fruit samples from two pistachio cultivars, 'Akbari' and 'Kaleh-Ghoochi,' were collected from 18-year-old trees with moderate tree vigor in a commercial orchard. The calcium nitrate solutions included a control sample (distilled water), 0.4% calcium nitrate, and 0.2% calcium nitrate enriched with lecithin, foliar applied two weeks after full bloom. **Findings:** Calcium nitrate treatments, both alone and enriched with lecithin, significantly reduced physiological disorders such as endocarp lesions and fruit abscission. In 'Akbari,' calcium nitrate treatments reduced issues such as nut ounce, shell staining, deformed nuts, blank nuts, endocarp lesions, early-split nuts, hull decay, and hull cracking. In contrast, Kaleh-Ghoochi also benefited from reduced occurrences of deformed nuts, early-split nuts, hull decay, and hull cracking. However, when treated with calcium nitrate enriched with lecithin, there was a notable reduction in blank nuts in Kaleh-Ghoochi, whereas calcium nitrate alone led to an increase in blank nuts. These calcium nitrate treatments resulted in positive outcomes and reduced fruit defects, thereby enhancing the overall quality and marketability of pistachios. Notably, the combination of calcium nitrate and lecithin had a more pronounced impact on Akbari, improving the pistachio nut's hull appearance, firmness, and reducing water activity. **Research limitations:** There were no limitations. **Originality/Value:** These findings suggest that calcium plays a significant role in enhancing the yield, quality, and marketability of pistachio fruit, providing practical insights for farmers aiming to improve their pistachio production practices.

INTRODUCTION

The pistachio nut is a valuable produce primarily traded as dry nut in-shell kernels (Sheikhi et al., 2019). Therefore, enhancing quality can create better consumption and marketing opportunities (Singh et al., 2022). The pistachio kernel is edible and sits in the center, encased by a hard, lignified shell and a green hull (Toghiani et al., 2023). Any damage to hull and shell during growth and development can cause shell staining and kernel decay in pistachio, rendering them unsuitable for sale (Hasanshahi et al., 2023).

Pistachio can be affected by various physiological disorders, including inflorescence bud and fruit abscission, blank nuts, non-split nuts, endocarp lesion, deformed nuts, early-split nuts, alternate bearing, and low productivity (Khezri et al., 2010; Pourahmadi et al., 2019). Some of these physiological disorders affecting pistachios occur within their skins. Therefore, maintaining the quality of the pistachio hull and shell can help address many physiological disorders. Nutrient deficiency is associated with increased physiological disorders in pistachio trees (Tadayon & Hosseini, 2022). Calcium is generally recognized as safe (GRAS) and is used to reduce physiological disorders in fruits (Mirshekari & Madani, 2022). Products with low calcium content are more susceptible to physiological disorders (Khademi & Khoveyteri-Zadeh, 2022). It plays a crucial role in various aspects of plant growth, including cell division, cell elongation, and fruit development (Sadr et al., 2019). Therefore, foliar application should be considered during the period of peak nutrient demand. This is based on the premise that soil supply and root uptake may be inadequate to meet demands, even with adequate soil-applied fertilizer (Tadayon & Hosseini, 2022). Moreover, the presence of essential nutrients, particularly calcium, plays a crucial role in fruit development during the initial growth stages (Sen et al., 2010; Zeraatgar et al., 2019). Remarkably, the thickening process of the fruit surface can impact nutrient absorption when employing foliar application (Madani et al., 2014). Calcium is transported through the xylem via the transpiration stream (Sajadian & Hokmabadi, 2011). In fact, the competition between leaves and fruit for calcium significantly affects the amount of calcium acquired by the fruit (Sen et al., 2010). Interestingly, despite leaves not exhibiting symptoms of calcium deficiency, the fruit (particularly the endocarp) can still suffer from severe calcium deficiency and lead to endocarp lesion in pistachio nuts. Furthermore, an imbalance in the calcium-to-magnesium ratio results in inadequate calcium absorption by the plant (Sajadian & Hokmabadi, 2011). Calcium nitrate has been used in various studies as a source of calcium (Khademi & Khoveyteri-Zadeh, 2022).

Fruit abscission in pistachio trees is an important problem that leads to significant economic losses in pistachio orchards. It occurs from the millet stage until the hardening of the endocarp. Fruit abscission in pistachio trees starts with the browning and blackening of the skin of the tip of the fruit. This complication is known as endocarp lesion (Sajadian & Hokmabadi, 2011). The inner endocarp surface becomes white, while the border between healthy and infected regions turns brown. Infected pistachios often shrink and drop due to endocarp damage, with fruit abscission occasionally exceeding 80% of the total. As the endocarp hardens and cotyledons begin to develop, the infected endocarp area becomes soft and flexible, potentially leading to deformation during harvesting. A characteristic symptoms of pistachio endocarp lesion is the blackening of the endocarp from the top to the bottom, with black spots appearing on the green pericarp (Adibfar et al., 2012; Sadr et al., 2019) (Fig. 1). Normally, pistachio trees produce abundant flowers and pollen grains. However, during early fruit development, numerous flowers and fruits fall and only a few remain on pistachio clusters. The physiological states and nutrient availability from the previous year have a significant effect on fruit preservation. Previous research has shown that calcium deficiency

can lead to endocarp lesion and fruit abscission in pistachio nuts (Sajadian & Hokmabadi, 2011).

The formation and strength of the pistachio endocarp depend significantly on the availability of calcium ions (Adibfar et al., 2012; Sadr et al., 2019; Sadr et al., 2020). Calcium plays a crucial role in maintaining the texture and firmness of fruits by forming calcium pectate, which stabilizes the cell walls (Hossain et al., 2021). When administering calcium nitrate and calcium chloride sprays to pistachio fruit, they effectively reduce the incidence of endocarp lesion disorder. Calcium treatments help fortifies the structural integrity of the endocarp (Pourahmadi et al., 2019). Similarly, the study by Sajadian and Hokmabadi (2011) and Sajadian (2016) demonstrated that the incidence of endocarp lesion decreases with the application of calcium treatments, including calcium sulfate, calcium nitrate (soil application), and calcium chelate foliar spray, during the rapid growth of the endocarp. Adibfar et al. (2012) reported that spraying a calcium chloride solution in pistachio orchards reduces endocarp lesion.

Recent studies by Pourahmadi et al. (2019) have shed light on the role of calcium in the development of deformed nuts. Microscopic observations indicate that pistachio deformation occurs approximately two weeks after full bloom, coinciding with the destruction of parenchymal cells in the endocarp (Pourahmadi et al., 2019). Calcium plays a crucial role in cell division and elongation, and it is essential for maintaining the integrity of the plant cell wall (Tapia-Rodriguez et al., 2021). Additionally, calcium plays a crucial role in influencing the activity of auxin, a plant hormone. Auxin, in turn, plays a significant role in shaping the fruit.

It has been shown that the primary cause of blank pistachio nut is associated with inadequate differentiation and damage to various components of the embryo sac, leading to embryo miscarriage during the growing season (Mohammadi et al., 2017; Pourahmadi et al., 2019). Interestingly, the application of calcium appears to mitigate blank nut formation by safeguarding the embryo sac (Desouky et al., 2009). The occurrence of non-split nuts and early-split nuts is also associated with optimal nutrition and kernel development in pistachio (Khezri et al., 2010). Early-split nuts refer to pistachio nuts in which the hulls and shells have split prematurely (Pourahmadi et al., 2019). This rupture of the hull often results in dark staining of the shell (Sedaghati & Alipour, 2005). External calcium application plays a crucial role in mitigating fruit cracking (Mohammadi et al., 2017). Approximately 60% of calcium is present in the cell wall. Calcium acts as an intermolecular connector, stabilizing the pectin complex in the middle lamella of plant cells. By inhibiting ethylene production, calcium prevents the activation of cell wall hydrolysis enzymes. Consequently, it contributes to maintaining cell wall integrity and firmness in fruits (Sajadian & Hokmabadi, 2011).

According to Adibfar et al. (2012), the hardiness of pistachio endocarp is influenced by the availability of calcium in fruit. One effective method for enhancing the calcium status of fruit involves using substances that facilitate calcium absorption. Lecithin, acting as a natural emulsifier, assists calcium in better penetrating plant cells. This strategy can be particularly beneficial for fruit like pistachio, which heavily depend on calcium for their growth and development (Pirozzi et al., 2020). Lecithin role is to limit spray drifting, reduce surface tension, and prevent droplet evaporation by encapsulating the active ingredient within each droplet (liposomes), thereby enhancing assimilation (Vannini et al., 2021). It is a highly polar compound with a lipophilic fatty acid backbone and a hydrophilic choline head could potentially facilitate the movement of calcium through the waxy cuticle of the apple. However, lecithin formulations dry into a thin, even film after application, and it is possible that the close contact between the calcium and the fruit's surface aids in calcium movement (Reid & Padfield, 1975). Pistachio producers worldwide encounter various physiological

disorders that impact performance and quality. However, there is limited evidence regarding the pistachio tree's response to calcium nitrate in controlling these disorders. The lack of crucial information represents the main limiting factor in this context. The objective of this study is to assess the effectiveness of foliar calcium nitrate and lecithin application as a pre-harvest treatment for controlling pistachio physiological disorders during the initial growth stages.

Fig. 1. Symptoms of endocarp lesion on a pistachio cluster (Iran-Rafsanjan, June).

MATERIALS AND METHODS

Plant material and experiments

Fruit samples from two pistachio cultivars (Akbari and Kaleh-Ghoochi) were collected from 18-year-old trees with moderate tree vigor in a commercial orchard at Rafsanjan, Iran (30.41334539°N, 55.98884582°E). These trees are grafted onto the Badamizarand rootstock and are planted at an optimal distance of 1.6 meters from each other. The trees receive irrigation once every 45 days and are trained in an open center formation. The samples underwent the following treatments: control samples (distilled water), calcium nitrate (0.4%), and calcium nitrate (0.4%) with lecithin (0.2%) two weeks after full bloom, with three trees per treatment. Spraying was done in the early morning using a backpack sprayer. Calcium nitrate was dissolved in a 0.5% solution of Tween 20 (v/v) and used immediately. Approximately 1.5 liters of solution was used per tree.

Assessment of nut disorder status and nut characteristics

Six relatively homogeneous shoots on each tree are selected and labeled to monitor the fruit abscission, hull decay, hull cracking, early-split disorder, and endocarp lesion. Each selected shoot contains one cluster. Two weeks after full bloom, the number of fruit in each cluster is counted. To evaluate the effect of treatments on endocarp lesions, six pistachio clusters were sampled from each randomly selected tree within each treatment group. Both non-contaminated and contaminated nuts were counted in mid-June with three replications (Sajadian & Hokmabadi, 2011). The percentage of early split nut disorders, hull cracking, and hull decay in each cluster was calculated four weeks before harvest, by quantifying the proportion of fruit with abnormalities relative to the total number of fruit sets (Pourahmadi et al., 2019). Additionally, the fruit abscission percentage was determined by dividing the number of fallen fruit in each shoot by the initial number of fruits set in that shoot (Tadayon & Hosseini, 2022). Other pistachio disorders were evaluated at the time of harvest for each cluster. Pistachio nuts were harvested when the nut-shells were easily detached from the endocarp (Ferguson & Haviland, 2016). After harvest, all fruit were removed from the

clusters and manually sorted into categories: blank, deformed, and those with internal shell staining. The internal shell staining refers to the endocarp with white and brown halos within the shell. The disorders are quantified as the percentage of fruits with abnormalities out of the total number of fruit sets (Tadayon & Hosseini, 2022). To calculate the pistachio nut ounce for each replicate, the number of fruits per 28.3 grams of dry fruit is determined, following the method described by Tadayon and Hosseini (2022).

At harvest, external shell staining was assessed by examining 20 fruits per replicate. Superficial brown spots on the outer skin were identified as indicators of browning. The severity of symptoms was visually assessed based on a six-stage scale: 0 (no browning), 1 (browning covering ≥20% of the fruit surface), 2 (browning covering ≥20% but <40% of the fruit surface), 3 (browning covering ≥40% but <60% of the fruit surface), 4 (browning covering ≥60% but <80% of the fruit surface), and 5 (browning covering ≥80% of the fruit surface). The browning index (BI) was calculated as follows (1) (Wang et al., 2006):

$$BI = [(BI\ level) \times (number\ of\ fruit\ at\ the\ BI\ level)] / (6 \times total\ number\ of\ fruit\ in\ the\ treatment) \quad (1)$$

Statistical Evaluation

The experiment is designed as a factorial, considering two variables: cultivar and treatments. It follows a randomized complete block design with three repetitions. Data analysis is performed using SAS software, employing analysis of variance (ANOVA). To compare the means, Duncan's multiple range tests at the 5% probability level was used. Graphs are created using Excel software.

RESULTS

Endocarp lesion

As shown in Table 1, endocarp lesion was influenced by the simple effects of cultivar and treatment. All applied calcium nitrate treatments, when as compared to control samples, reduce the occurrence of endocarp lesion. The lowest level is observed in lecithin-enriched with calcium nitrate, which does not exhibit a statistically significant difference with calcium nitrate (Fig. 2a). Additionally, Akbari shows a higher amount of endocarp lesion as compared to Kaleh-Ghoochi (Fig. 2b).

Table 1. Variance analysis of physiological disorders in pistachio cultivars under different calcium nitrate treatments.

		Mean squares									
Sources of variation	df	Endocarp lesion	Blank nut	Deformed nut	Early-split nut	Internal shell staining	External shell staining	Hull cracking	Hull decay	Fruit abscission	Pistachio nut ounce
block	2	3.61	11.93	0.02	0.13	0.15	0.0002	8.94	0.62	1.45	7.76
C	1	56.60*	34.72*	0.08^{ns}	25.52**	0.34*	0.0008^{ns}	43.58^{ns}	11.10**	67.74**	66.90*
T	2	499.80**	117.48**	3.38**	21.45**	0.73**	0.0020*	522.59**	188.19**	531.60**	52.70*
C×T	2	5.28^{ns}	95.57**	0.60**	20.17**	0.34*	0.0018*	85.41**	12.53**	2.54^{ns}	58.70*
Error	10	7.61	6.29	0.02	0.06	0.06	0.0003	9.89	0.51	6.38	10.18
Coefficient of variation		18.60	29.45	27.21	20.44	28.24	11.63	23.75	17.88	16.85	9.07

* and ** show significance at the 5% levels, and ns means no significant difference.

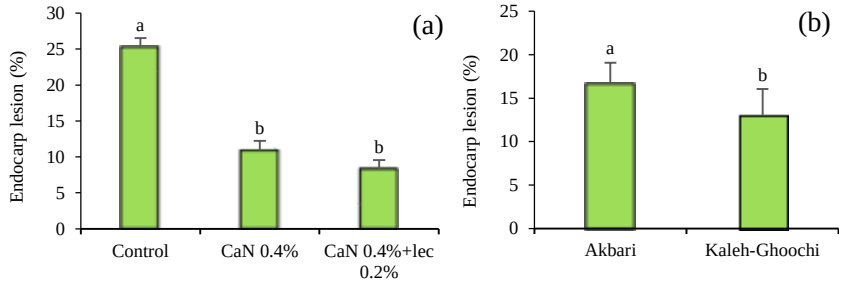

Fig. 2. Endocarp lesion of pistachio fruit treated with CaN and CaN + lec (a), and in different cultivars (b). The same letters indicate no significant difference in the 5% probability level for Duncan's multiple range tests. Vertical bars represent the SE.

Blank nut

As shown in Table 1, blank nut was influenced by the simple effects of cultivar, treatment, and their interaction. In Akbari, calcium nitrate and calcium nitrate enriched with lecithin reduced the occurrence of blank nut as compared to control samples. However, no significant difference was observed within the calcium nitrate treatments. In contrast, in Kaleh-Ghoochi, only calcium nitrate enriched with lecithin effectively reduced the occurrence of blank nuts. Interestingly, calcium nitrate increases the occurrence of blank nuts as compared to control samples (Fig. 3a).

Deformed nut

Table 1 indicated that the simple effects of treatment and their interaction significantly influenced deformed nut (P≤0.01). In Fig. 3b, it is clear that applying calcium nitrate and calcium nitrate enriched with lecithin effectively reduces the occurrence of deformed nut as compared to the control in Akbari cultivar. Notably, a similar effect was observed in Kaleh-Ghoochi, that calcium nitrate treatments also reduced deformed nut. Interestingly, calcium nitrate enriched with lecithin exhibited the lowest incidence of deformed nuts.

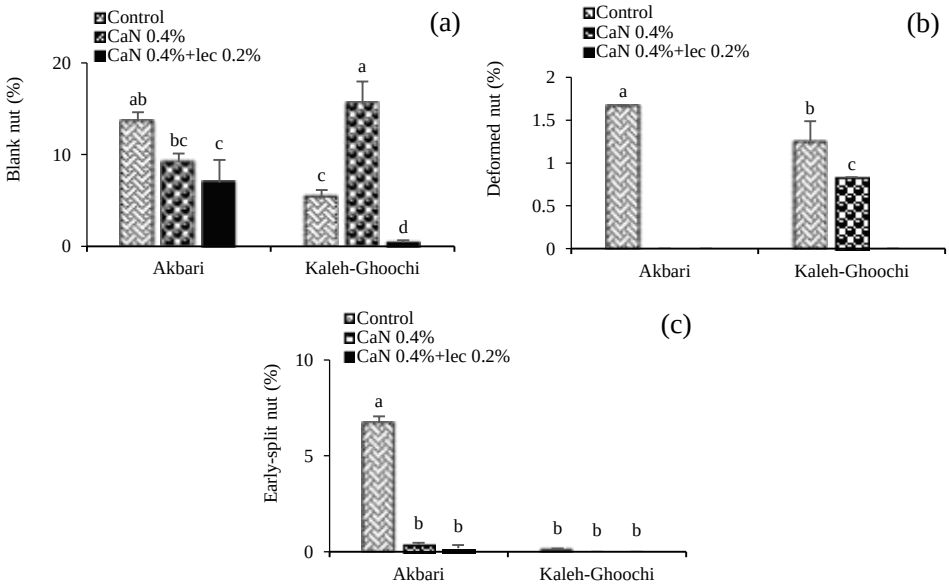

Fig. 3. Blank nut (a), deformed nut (b), and early-split nut (c) of pistachio fruit treated with CaN and CaN + lec. The same letters indicate no significant difference in the 5% probability level for Duncan's multiple range test. Vertical bars represent the SE.

Early-split nut

As shown in Table 3, early-split nut was affected by the simple effects of cultivar, treatment, and their interaction (P≤0.01). The application of calcium nitrate treatments, both alone and with lecithin, significantly reduced the percentage of early-split nut in Akbari pistachios as compared to control samples. Although there was no statistically significant difference between the calcium nitrate treatments, the mean of these treatments was lower than the control samples. In contrast, there was no significant difference between the calcium nitrate treatments and control samples regarding the occurrence of early-split nut in Kaleh-Ghoochi pistachios. However, early-split nut is generally very low in Kaleh-Ghoochi, indicating that calcium nitrate treatments are particularly effective for improving the quality of Akbari pistachios (Fig. 3c).

Internal shell staining

According to the results in Table 1, internal shell staining in dried fruits was affected by the simple effect of treatment and the interaction effects of cultivar and treatment. The occurrence of internal shell staining was decreased with the application of calcium nitrate treatments in Akbari as compared to control samples. The highest rate of internal shell staining was observed in control samples, whereas the lowest rate occurred in calcium nitrate enriched with lecithin. Notably, in Kaleh-Ghoochi, calcium nitrate enriched with lecithin, calcium nitrate, and control samples exhibit similar effects on the rate of internal shell staining. However, the mean for calcium nitrate enriched with lecithin is lower than that of the calcium nitrate and control samples (Fig. 4a).

External shell staining

As shown in Table 1, external shell staining was influenced by the simple effects of treatment and the interaction effects of cultivar and treatment (P ≤ 0.05). The application of calcium nitrate treatments, especially calcium nitrate, significantly reduces external shell staining in Akbari as compared to control samples. Notably, in Kaleh-Ghoochi, calcium nitrate enriched with lecithin, calcium nitrate, and control samples exhibit similar effects on external shell staining. However, the mean of the calcium nitrate treatments is slightly lower than the control samples (Fig. 4).

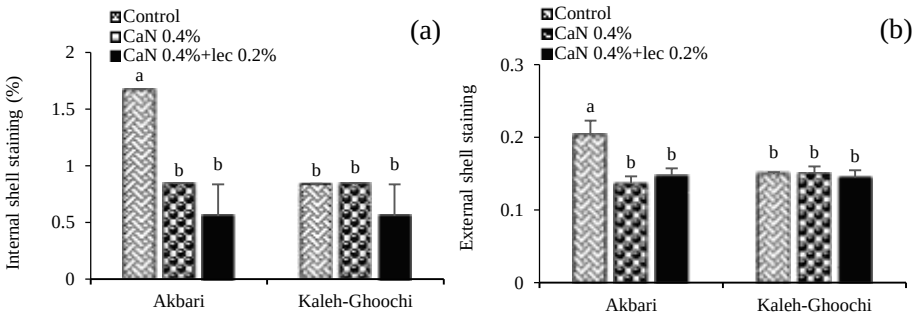

Fig. 4. Internal shell staining (a) and external shell staining (b) of pistachio fruit treated with CaN and CaN + lec. The same letters indicate no significant difference in the 5% probability level for Duncan's multiple range tests. Vertical bars represent the SE.

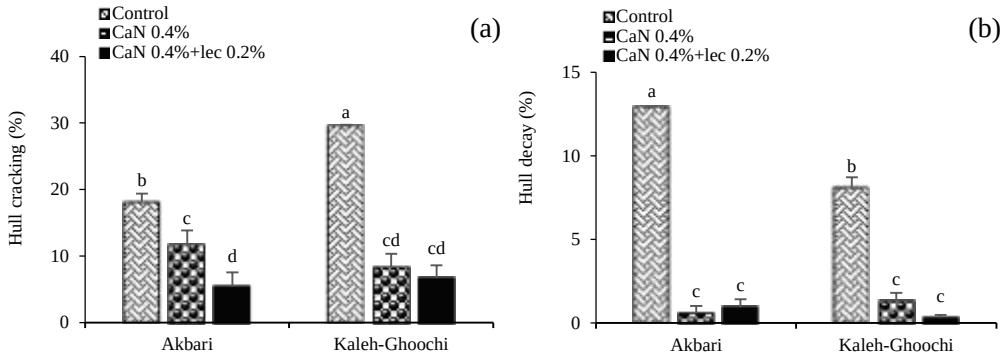

Fig. 5. Hull cracking (a) and hull decay (b) of pistachio fruit treated with CaN and CaN + lec. The same letters indicate no significant difference in the 5% probability level for Duncan's multiple range test. Vertical bars represent the SE.

Hull cracking

The results of the variance analysis table showed that hull cracking was influenced by the effect of treatment and the interaction effect of cultivar and treatment at a significance level of 1% (Table 1). In Figure 5a, it is evident that calcium nitrate and calcium nitrate enriched with lecithin effectively reduced hull cracking in as comparison to the control samples in Akbari. It is noteworthy that a similar effect was observed in Kaleh-Ghoochi, where calcium nitrate treatments reduced hull cracking. In both cultivars, it is evident that calcium nitrate enriched with lecithin had a more favorable effect than calcium nitrate, although there was no statistically significant difference between the two treatments.

Hull decay

As shown in Table 1, the simple effects of cultivar, treatment, and their interaction significantly influenced hull decay in pistachios at a significance level of 1%. The application of calcium nitrate treatments significantly reduces hull decay in Akbari as compared to the control samples. Specifically, calcium nitrate exhibits a more pronounced positive effect. Notably, a similar effect is observed in Kaleh-Ghoochi, with the distinction that calcium nitrate enriched with lecithin has a greater impact than calcium nitrate in reducing hull decay (Fig. 5b).

Fruit abscission

As Table 1 indicated, the simple effects of cultivar and treatment affected fruit abscission at a significance level of 1%. Calcium nitrate enriched with lecithin demonstrated a lower percentage of fruit abscission than control samples, although it did not exhibit a significant difference with calcium nitrate (Fig. 6a). Additionally, Kaleh-Ghoochi demonstrates a higher percentage of fruit abscission as compared to Akbari (Fig. 6b).

Pistachio nut ounce

Based on the analysis of variance table, the effects of cultivar, treatment, and their interaction on pistachio nut ounce were statistically significant ($P \leq 0.05$) (Table 1). The results suggest that in Akbari, calcium nitrate treatments, especially calcium nitrate enriched with lecithin, result in a decrease in pistachio nut ounce as compared to the control samples. Additionally, in Kaleh-Ghoochi, no significant difference was observed between calcium nitrate enriched with lecithin, calcium nitrate and control samples (Fig. 6c).

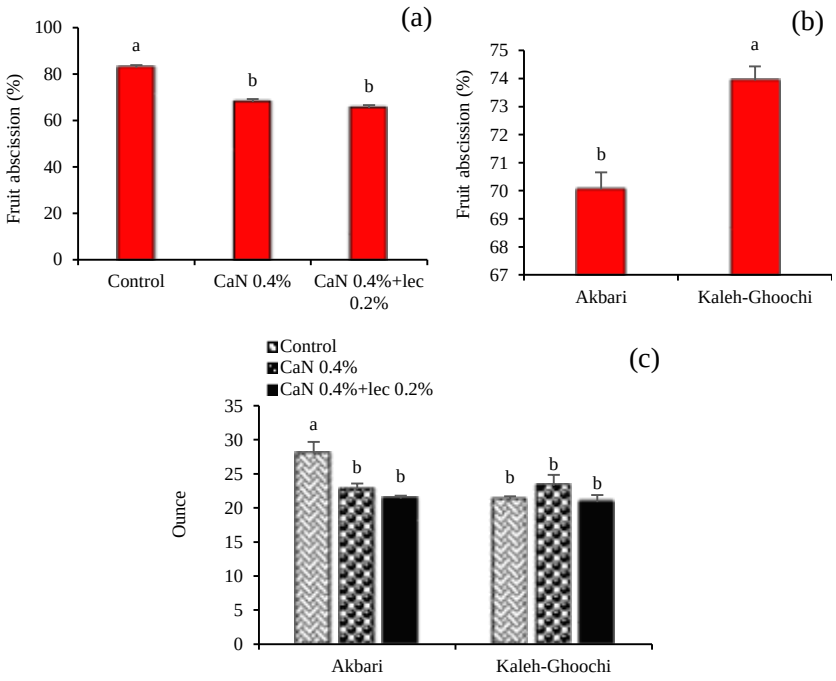

Fig. 6. Fruit abscission (a, b) and pistachio nut ounce (c) of pistachio fruit treated with CaN and CaN + lec. The same letters indicate no significant difference in the 5% probability level for Duncan's multiple range test. Vertical bars represent the SE.

DISCUSSION

Calcium deficiency in fruit can lead to various physiological disorders (Sen et al., 2010). It is employed to avert disorders both pre- and post-harvest (Dunnd & Able, 2006). The formation and strength of the pistachio endocarp significantly depend on the availability of calcium ions (Sadr et al., 2020). Research conducted by Sadr et al. (2019, 2020) demonstrates that applying calcium spray to pistachio fruit effectively enhances the structural integrity of the endocarp. Consequently, improvement in endocarp integrity leads to a reduction in endocarp lesion disorder. In this study, the incidence of endocarp lesion disorder decreases with the implementation of calcium treatments. Similarly, research by Sajadian (2016) demonstrates that applying calcium treatments during the rapid growth of the endocarp decreases the occurrence of endocarp lesions. Additionally, the findings of Pourahmadi et al. (2019) and Sajadian and Hokmabadi (2011) indicate that calcium treatments enhance calcium uptake in pistachio fruits and reduce the incidence of endocarp lesion disorder, which is in line with the results of our study.

Calcium nitrate and calcium nitrate enriched with lecithin in Akbari, have resulted in a reduction in the occurrence of blank nuts. However, in Kaleh-Ghoochi, only calcium nitrate enriched with lecithin demonstrates a reduction in blank nuts. The findings align with the research by Pourahmadi et al. (2019). According to Ferguson and Haviland (2016), blanking occurs during the fruit set stage and persists until the kernel is fully formed. The primary cause of blank is associated with inadequate differentiation and damage to various components of the embryo sac, leading to embryo miscarriage during the growing season (Mohammadi et al., 2017; Pourahmadi et al., 2019). Calcium, as a key regulator, influences various physiological processes in plants (Hashimoto et al., 2012). Furthermore, the

application of calcium mitigates the occurrence of blank nuts by safeguarding the embryo sac (Desouky et al., 2009).

Microscopic observations indicate that pistachio deformities arise due to the destruction of parenchymal cells in the endocarp (Pourahmadi et al., 2019). Calcium, as a vital nutrient, plays a crucial role in cellular functions and membrane stabilization (Sinha et al., 2019). Notably, Fageria (2016) emphasizes that calcium is essential for cell division, elongation, and the structural integrity of plant cell walls and other organelles. Furthermore, calcium significantly influences auxin activity, which contributes to improving fruit shape (Tiwari et al., 2012). It appears that calcium contributes to tissue reconstruction, resulting in a decrease in the percentage of deformed nuts in pistachio fruit (Mohammadi et al., 2017).

In this study, it is evident that calcium nitrate treatments effectively mitigate early-split nuts. The exogenous application of calcium may reduce pistachio fruit cracking by increasing the calcium content in the skin of the fruit and maintaining the structural integrity of plant cell walls (Mohammadi et al., 2017). The increase in early-split nuts could be attributed to irrigation and nutritional stresses within the orchard (Khezri et al., 2010). If the occurrence of early-split nuts is indeed associated with nutritional stresses, calcium nitrate may potentially alleviate the occurrence of early-split nuts (Pourahmadi et al., 2019). On the other hand, ROS can cause damage to membranes, lipids, proteins, and cellular structures (Karray-Bouraoui et al., 2011). Considering that early-split nuts affect the skin of the fruit, it appears that ROS and ethylene contribute to the occurrence of early-split nuts in pistachio trees. The application of calcium nitrate as an anti-ethylene agent reduces the incidence of early-split nuts by increasing calcium levels (Pourahmadi et al., 2019). Similarly, the application of calcium compounds has been shown to decrease early-split nuts and enhance overall yield in pistachios (Mohammadi et al., 2017).

The appearance of fruit serves as a fundamental indicator of post-harvest quality, directly influencing the marketability of the produce. From a horticultural perspective, the external application of essential elements, such as calcium, contributes to improving the quality of fruit (Madani et al., 2014). Calcium plays a crucial role in reinforcing the cell wall structure of fruit and enhancing the integrity of the plasma membrane. By maintaining the structure and functionality of the cell wall, calcium stabilizes the membrane and facilitates signal transmission. The application of calcium leads to improved quality attributes and an increase in bioactive compounds within the fruit. Specifically, calcium ions form cross-links with pectin in the cell wall, thereby protecting cell wall from degradation by polygalacturonate enzymes (Sinha et al., 2019; Fattah et al., 2023). In our research, calcium nitrate improved the visual quality of nuts and reduced shell staining in Akbari. In line with these research findings, the application of calcium chelates via foliar spray, one week before full bloom and again four weeks after full bloom, reduces browning and enhances aril quality (Tadayon, 2021). Applying calcium as a pre-harvest treatment can either reduce internal browning or completely prevent internal browning in pineapples (Herath et al., 2003).

The importance of calcium in influencing fruit growth and maturation, including cell wall characteristics, is widely acknowledged (Wu et al., 2022). It is evident that applying calcium nitrate and calcium nitrate enriched with lecithin effectively reduces hull cracking and blackening compared to the control fruits in both cultivars. Calcium interacts with proteins, influencing their structure and functions within plant cells (Fattah et al., 2023). Pre-harvest calcium treatment enhances fruit quality by strengthening cell walls (Sinha et al., 2019; Fattah et al., 2023). Calcium is employed both pre- and post-harvest to slow down ripening and improve the quality of various fruits (Dunnd & Able, 2006). Applying calcium nitrate to pomegranate trees decreases the incidence of fruit cracking and sunburn, while enhancing fruit firmness (Al-Saif et al., 2022).

In this study, the percentage of fruit abscission decreased significantly with the application of various calcium nitrate treatments. This finding aligns with previous research, which has demonstrated that an enhanced nitrogen supply helps prevent fruit abscission and increases fruit yield in trees (Arias et al., 2005). Under environmental stress conditions, plants respond by generating ROS and ethylene. These substances serve as intracellular signals, activating the plant's defense mechanisms against stress (Xia et al., 2015). Interestingly, an increase in fruit abscission is positively correlated with ethylene levels, and the presence of ethylene reduces the overall percentage of fruit set. However, calcium acts as an anti-ethylene agent during the abscission process, effectively mitigating stress-induced fruit drop (Pourahmadi et al., 2019).

In the context of pistachio trees, numerous valuable studies conducted over the past decade have consistently highlighted nutritional deficiencies, particularly in potassium, zinc, and possibly calcium (Malakouti, 2005). Pourahmadi et al. (2019) reported that using calcium nitrate as a treatment resulted in reduced fruit drop. This reduction in fruit drop can potentially lead to smaller fruit size. When fruit drop decreases, resources distributed among a larger number of fruits, resulting in smaller individual fruit sizes but an increased pistachio nut ounce. Calcium nitrate treatments led to both increased fruit set and a higher pistachio nut ounce compared to control samples in Akbari (Pourahmadi et al., 2019). In pistachio trees, calcium deficiency can lead to endocarp lesion, which in turn causes fruit abscission (Sajadian & Hokmabadi, 2011). Researchers have found that applying calcium treatments during the rapid growth phase of the endocarp can reduce the occurrence of endocarp lesion disorder. Therefore, addressing calcium deficiency can help mitigate fruit abscission (Pourahmadi et al., 2019; Sadr et al., 2020).

CONCLUSION

Calcium deficiency in pistachio fruit leads to various physiological disorders. Managing disorders is crucial for quality fruit production. Applying calcium during the initial growth stage ensures efficient calcium absorption. Notably, findings suggest that calcium nitrate treatments, especially when enriched with lecithin, effectively mitigate physiological disorders by reducing stress conditions. In this study, the application of calcium nitrate and calcium nitrate enriched with lecithin resulted in reduced fruit abscission and endocarp lesions. These treatments have reduced defects (external shell staining, deformed nut, blank nut, internal shell staining, early-split nut, hull decay, and hull cracking) in Akbari, that result in improvement of the appearance of the nuts. In Kaleh-Ghoochi, similar positive effects are observed, although some differences exist. Kaleh-Ghoochi exhibits a lower fruit abscission percentage and fewer defects compared to Akbari. These findings suggest that calcium plays a significant role in enhancing the yield, quality, and marketability of pistachio fruits. However, it is also possible that calcium nitrate treatments have negative effects. For instance, a decrease in the percentage of fruit abscission results in an increase in the percentage of fruit formation but a reduction in the size of each fruit. In conclusion, based on the findings of this study, the use of these treatments has the potential for addressing physiological disorders in pistachios, benefiting economic and marketing aspects.

Conflict of interest

The authors have no conflict of interest to report.

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Effects of pre-storage pectin, cellulose acetate, and sodium alginate coatings on the preservation of papaya (*Carica papaya* L.)

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ABSTRACT

Purpose: The current study examined the impacts of postharvest treatments with different coating solutions to enhance the shelf life of papaya at the least nutrient loss. **Research method:** The study was carried out with mature and fresh shahi papayas (BARI Papaya-1) using Complete Randomized Design. The experiment comprised four treatments namely control (T_1), coating with 2% pectin solution (T_2), 2% cellulose acetate solution (T_3), and 2% sodium alginate solution (T_4). **Findings:** Significant variations among the treatments regarding physicochemical characteristics like color, weight loss (%), moisture content (%), pH, titratable acidity, total soluble solids (°Brix), vitamin C content, and biological parameters like total viable count (TVC), and shelf life were observed for the 12 day storage periods. It was observed that vitamin C content, moisture content, and titratable acidity gave higher values in the treated samples (T_2, T_3, T_4) with the lowest color score, weight loss, total soluble solids, and pH. Among the samples, the papaya treated with 2% sodium alginate solution obtained the longest shelf life with the lowest TVC value. Conversely, the control papaya had the highest microbial load with the shortest shelf life. **Research limitations:** There was no limitation. **Originality/Value:** Among the treatments, 2% sodium alginate solution increased the shelf life of papaya by 16% and decreased post-harvest loss. Therefore, 2% sodium alginate solution treatment seems to be a good substitute for preservation and an effective way to retain the quality of papaya.

INTRODUCTION

The most significant species of Caricaceae is the papaya (*Carica papaya* L.), which is widely produced for use as fresh fruit, as well as for beverages, jams, and candies, and the leaves and flowers may also be used as cooked vegetables (Tamma et al., 2018). It is known as the common man's fruit due to its reasonable price (Dev et al., 2019). It is also a good source of calcium, phosphorus, iron, fair quantities of vitamin C, riboflavin, and niacin (Chukwuka et al., 2013). Papaya is rich in papain and chymopapain, which are often used to soften meat, clarify beer, and treat dyspepsia (Brishti et al., 2013; Chukwuka et al., 2013). Papaya pastes can also be applied topically to heal burns and skin wounds.

Approximately 5.83% of the papaya grown in Bangladesh spoils because of the country's prevailing high temperature and humidity (Bhuyan & Raju, 2018). Once it is fully ripe, the edible and marketing quality deteriorates rapidly. Papaya fruits are prone to numerous fungal infections, which significantly reduces the quality of fruit (Kahawattage et al., 2023). Post-harvest losses can be reduced by increasing the shelf life of papaya. It may be done with packaging material, physical and chemical measures by using modified atmosphere packaging, ethylene scavenging compounds, etc (Josalia et al., 2013).

The use of edible coatings is an emerging technique for extending the shelf life of fruits and is increasingly gaining popularity. These coatings act as barriers to microbial contamination, thereby mitigating detrimental effects on minimally processed fruits (Soliva-Fortuny & Martiin-Belloso, 2003; Correa-Betanzo et al., 2011; Moreira et al., 2011). Thin layers of biopolymers are applied swiftly on the surface of fruits to reduce water loss and the rate of respiration (Hamzah et al., 2013). Several biopolymers have been used as edible coatings, such as alginate, starch, methylcellulose, carboxymethyl cellulose, soy protein, pectin gelatin, and chitosan (Rojas-Grau et al., 2009; Moradinezhad & Firdous, 2025). Since pectin is hydrophilic and forms strong gels when it reacts with multivalent metal cations like calcium, it is a popular ingredient for edible coatings. However, it has a low water vapor barrier and good oxygen and carbon dioxide barrier qualities (Ferrari & Sarantoulos, 2013). Acevedo et al. (2012) stated that due of sodium alginate's special colloidal properties—which include thickening, suspension forming, gel-forming, and emulsion—it is a hydrophilic biopolymer with a coating function. In addition to being cheap, non-toxic, and biodegradable, cellulose acetate can also be used to stop microbial growth and physiological weight loss in agricultural produce after harvesting. Therefore, this study was designed to i) treat papaya fruits with 2% pectin, 2% cellulose acetate, and 2% sodium alginate solution, ii) compare the effect of these coating formulations in the physicochemical parameters of papaya, and iii) enhance the shelf life of papaya.

MATERIALS AND METHODS

The experiment was conducted in the laboratory of the Department of Food Technology and Rural Industries, Bangladesh Agricultural University, Mymensingh-2202. The pectin, cellulose acetate, sodium alginate, and other chemicals and solvents used in the study were of analytical grade and supplied by the Department of Food Technology and Rural Industries. The water used in sampling was collected from the Central Laboratory (BAU, Mymensingh).

Preparation of plant samples

Papaya fruits (BARI Papaya-1) were purchased from Boiragram, located in the Mymensingh division of Bangladesh, approximately at the geographic coordinates of 24.7470° N latitude and 90.4120° E longitude having a suitable climate for harvesting papaya. In this

investigation, papaya fruits were harvested between late spring to early summer at maturity stage 1, defined as a yellow stripe near fruit apex (70%–80% of yellow surface), pulp almost completely orange except near peduncle and still hard (Albertini et al., 2016). The number of papaya fruits was fifty. All fruits were similar in size and without any apparent physical damage.

Experimental design

The trial was set up in a Complete Randomized Design (CRD) with a combination of two factors and three replications. The two factors were 0, 3, 6, 9, and 12 days of storage periods and coating treatments (T_1: Control; T_2: 2% pectin coating; T_3: 2% cellulose acetate coating; T_4: 2% sodium alginate coating).

Coating treatments of Papaya

The experiment was done using the concentration of 2% pectin, 2% cellulose acetate, and 2% sodium alginate solution in three replications. Coating solutions were prepared by dissolving 40g powder separately in 2 liters of hot water and then heating up to 70º C for 2 hours and cooling to 25ºC (Poverenov et al., 2014). Then the Selected papayas were immersed in pectin and cellulose acetate solutions for 2 minutes separately. For 2% sodium alginate coating, papayas were submerged in the alginate solution for two minutes and then submerged in 5% $CaCl_2$ solution to form the gel of alginate molecules (Sigma-Aldrich Co., Steinhein, Germany) and induce cross-linking reaction. The samples were then allowed to air dry for half an hour at room temperature (Valentina & Giovanna, 2016). For control fruits, the samples were washed in water followed by drying for 30 minutes at room temperature.

Storage Conditions

After coating treatments, fruits were dried in the air and further stored at ambient temperature on trays placed on the lab floor. Using a digital monitor, the storage room's temperature and relative humidity were monitored every day of the study period. The minimum and maximum temperatures and relative humidity of the storage room during the study were 26.8 °C, 31.6 °C, 58%, and 86%, respectively.

Physicochemical Quality

Color**,** weight loss, moisture content, dry matter content, pH, titratable acidity, total soluble solids, vitamin C, total viable count, and shelf life of control and treated papaya were analyzed and observed.

Color

Color of the fresh and treated papayas during the study was determined objectively using a numerical rating scale of 1-7, where 1 = green, 2 = mild green, 3 = one-quarter-yellow (< 25%), 4 = two-quarter fruit skin yellow (<50%), 5 = three-quarter yellow (<75%), 6 = fully yellow (75-100%), and 7 = blackened/rotten (fully yellow & black) (Hassan & Gilani, 2006).

Weight loss (%)

Weight loss was determined according to Sharmin et al. (2016). The papayas were weighed using an electric balance, and the data was recorded at every 3-day intervals. The Weight loss of papaya was estimated using the formula (1):

$$\% \text{ Weight loss (WL)} = \frac{IW - FW}{IW} \times 100 \qquad (1)$$

Where, WL = Weight loss (%), IW = Initial weight of papaya (g), FW = Final weight of papaya (g)

Moisture content

AOAC (2009) technique was utilized to determine the moisture content. At first, the weight of 3 empty dry crucibles was taken and 5g of each papaya sample was taken in each dried crucible. The crucibles with the samples were dried in an air oven at 105°C for 24 hrs or more to get constant weight. The crucibles were cooled in desiccators and weighed soon after reaching room temperature. The losses in weight were taken as the moisture loss of the samples and the percent of moisture in the samples was calculated (2) as:

$$\%\text{Moisture} = \frac{\text{Loss in weight}}{\text{Weight of samples}} \times 100 \qquad (2)$$

pH, titratable acidity, and TSS

pH was determined by a PerkinElmer Merion-V pH meter where pure juice was extracted from papaya then put the electrode in juice and the values were recorded in triplicate (Sultana et al., 2020). TSS of papaya was measured according to AOAC (2005) by dropping juice into the prism of a hand refractometer (MASTER-M, Model No 2313and finally, the reading was recorded as ˚Brix. Titratable acidity was also determined using the AOAC (2005) method. 5 g of papaya and distilled water were blended and homogenized. The volume was adjusted to 100 ml and the solution was filtered. Phenolphthalein was used as an indicator and 10 ml of aliquot was titrated against 0.1 N of NaOH. The titratable acidity was calculated from the following formula (3):

$$\%\ \text{Titratable acidity} = \frac{T \times V1 \times N \times E}{V2 \times W \times 1000} \times 10 \qquad (3)$$

Where, T = Titre value; N = Normality of NaOH; V_1 =Volume of the sample; E = Equivalent weight of acid; V_2 = Volume of the sample; W = Weight of sample.

Vitamin C content

Vitamin C content was measured by titration using a 2,6-dichlorophenol indophenol indicator, as described by Ranganna (2004). 5 g of papaya was blended and homogenized with distilled water, and then the volume was increased to 100 ml and filtered. A 10 ml aliquot was pipetted into the beaker and 50 ml of oxalic acid was added. The solution was then titrated with 2,6-dichlorophenol indophenol until the equivalence point. The ascorbic acid content was estimated by the following formula (4) and expressed as mg/100 g fresh weight.

$$\text{Ascorbic acid content (mg/100 ml)} = \frac{\text{Titre value} \times \text{Dye factor} \times \text{volume made up}}{\text{Aliquit taken} \times \text{Weight of sample}} \times 100 \qquad (4)$$

Total viable count (TVC)

The total viable count of papaya was assessed using the method described by Waghmare and Annapurna (2013). For the determination of TVC, 10 µl of each tenfold dilution was transferred and spread on the plate count agar media. The plates were incubated at 35°C for 12 hr. Following the incubation, plates exhibiting 30-300 colonies were counted. The TVC value was calculated as follows (5) and expressed as CFU/ml of the sample (ISO, 1995):

$$TVC = \frac{\text{Number of colonies x Dilution factor}}{\text{Volume of culture plate}} \quad (5)$$

Shelf life

The shelf life was estimated by counting the days required to be fully ripe and acceptable for the consumers. The papaya was considered the final stage of ripening when it became soft and wrinkles developed on the surface. Papaya with flaws or mechanical damage was believed to deteriorate during storage.

Statistical analysis

The collected data on various parameters were statistically subjected to Two-way analysis of variance (ANOVA) using Microsoft Excel 10 and univariate analysis of variance (General Linear Model) following Tukey Paired Comparison Test using IBM SPSS statistics 2022 software with homogeneous subsets of Post Hoc Tests.

RESULTS AND DISCUSSION

Color

A crucial sensory characteristic for customer appeal is color. Fig. 1 depicts the gradual transition from a mostly green to a yellow color. The color of the control and coated samples changed significantly ($P \leq 0.05$) during storage which indicated ripening. Over 12 days, the papaya coated with 2% sodium alginate showed the least color change (1.68), next to the 2% cellulose acetate solution (2.0), 2% pectin solution (2.58), and the control sample (4.14). In the case of treated papaya, no significant ($P \leq 0.05$) differences were found until the 6^{th} day of the storage period. Probably due to reduced respiration, which caused delayed ripening, alginate coating had a positive impact on preserving the papaya's original color, as evidenced by the less dramatic increase in color values near 2% alginate (Narsaiah et al., 2014). As chlorophyll degraded over time, the color altered primarily from green to yellow (Hamzah et al., 2013). By analyzing the color of papaya, it was determined that coating application caused papaya to ripen more slowly, maturing for eating after 12 days as opposed to 3 days for control fruits.

Weight Loss (%)

A vital indicator for evaluating the fruit's shelf life is weight reduction. When fresh plant tissues are kept at a constant temperature with dry air, they often lose moisture and, therefore, gain less weight (Liplap et al., 2013). Fig. 2 shows a difference in the total weight loss among treatments and storage intervals during the observation periods.

The coatings used in this research exhibited less pronounced effects on papaya's total weight loss throughout the storage periods. Fig. 2 shows that as storage time rose, the percentage of weight loss increased significantly in all treatments. Throughout the experimental periods, the maximum weight loss was found in the control sample, which was 17.56%. Among the treatments, the minimum weight loss was found in the 2% sodium alginate treated sample, which was 0.56%, followed by 2% pectin solution and 2% cellulose acetate solution, which were 0.94% and 1.88%, respectively. Slightly ripe fruit lost more weight compared with less ripe fruit handled likewise. Due to respiration, a carbon atom is lost from the fruits in each cycle in the form of CO_2 and the weight loss happens (Parven et al., 2020). The different water vapor permeability of the polysaccharides is the reason for variations in their ability to inhibit weight loss (Chien et al., 2007). On the contrary, the treated papaya showed the lowest weight loss compared to the control on the 12^{th} day because coatings act as a barrier that reduces the moisture loss from the pulp (Alharaty &

Ramaswamy, 2020). A similar outcome in total weight loss was reported by Parven et al. (2020) for aloe vera gel coating on papaya. In this research, the 2% sodium alginate treatment offered a better water barrier property than the other treatments because it acts as a semipermeable barrier against oxygen, carbon dioxide, and moisture. This reduced respiration, water loss, and oxidation reactions, resulting in decreased weight loss for coated papayas during storage.

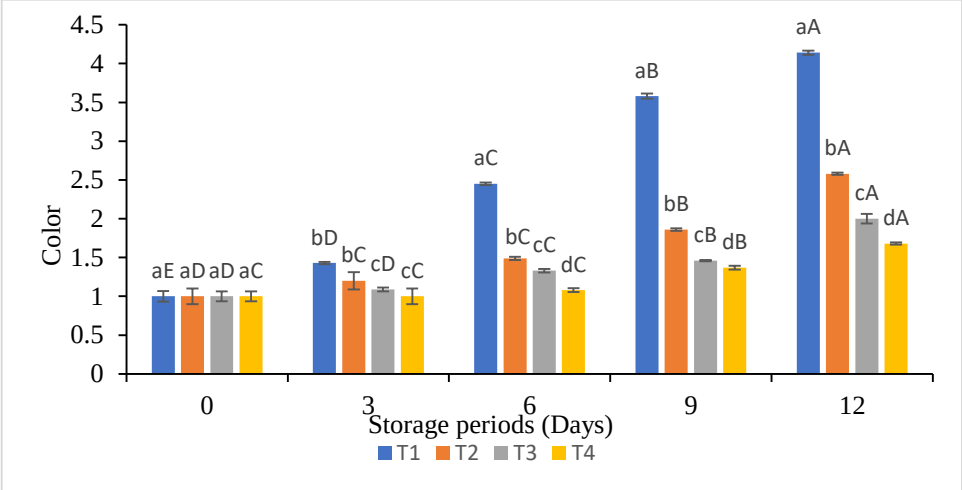

Fig. 1. Effect of coating treatments on the color of papaya during storage. Values followed by different small letters (a-d) in particular storage period indicate differences among the treatments and different capital letters (A-E) in particular treatments indicate differences among the storage intervals (P≤0.05). T_1: Control; T_2: 2% pectin coating; T_3: 2% cellulose acetate coating; T_4: 2% sodium alginate coating.

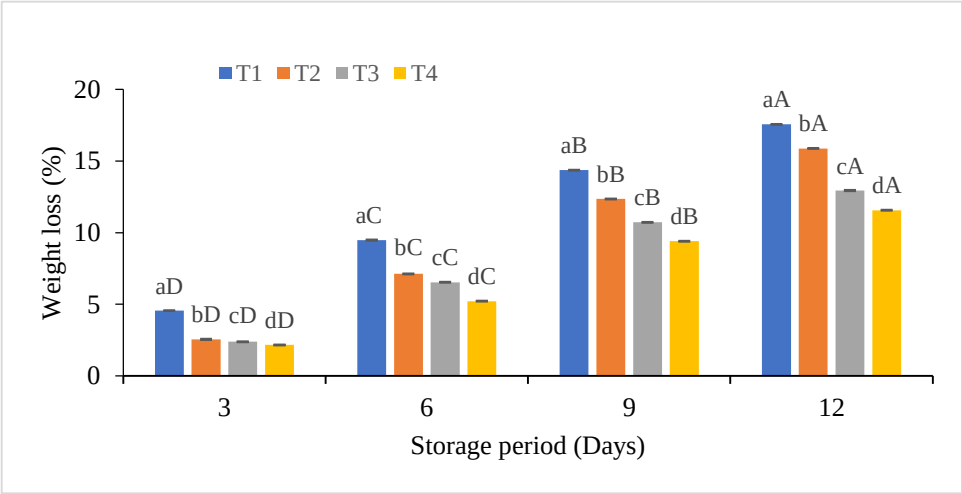

Fig. 2. Effect of postharvest treatments on the weight loss (%) of papaya during storage. Values followed by different small letters (a-d) in particular storage period indicate differences among the treatments and different capital letters (A-E) in particular treatments indicate differences among the storage intervals (P≤0.05). T_1: Control; T_2: 2% pectin coating; T_3: 2% cellulose acetate coating; T_4: 2% sodium alginate coating.

Moisture content (%)

Moisture content is an important parameter of postharvest-treated fruits and vegetables that indicates the shelf life of the product. Figure 3 depicts a significant (P≤0.05) decrease in moisture content throughout the treatment periods. No significant (P≤0.05) difference was found between the 9th and 12th days of treatment periods except for the control and 2% sodium alginate-treated papaya. Among the treatments, the highest moisture content was found in 2% sodium alginate-treated papaya, which was 89.77%, followed by 2% cellulose acetate and 2% pectin solution-treated papaya, which were 87.98% and 86.78%, respectively, and the lowest moisture content was found in control papaya, which was 85.63% over the treatment periods. Throughout the storage periods, moisture content decreased by 7.95% for the control papaya and 3.73% for the 2% sodium alginate-treated papaya, which was about two times lower than the control papaya. The same result was found by Sharmin et al. (2016) for aloe vera-treated papaya. Pathmanaban et al. (1995) also reported on the decrease in moisture content during storage. The decrease in moisture content was most likely brought on by starch hydrolysis as well as transpiration and evaporation loss. These edible coatings serve as primary packaging which is directly in contact with the fruit surface, wrapping it to form a gas and moisture barrier to hold moisture content. Compared to other treatments, sodium alginate film acts as a water-permeable membrane due to the addition of calcium chloride salts and less water evaporates from the surface ultimately leading to held moisture content (Senturk Parreidt et al., 2018).

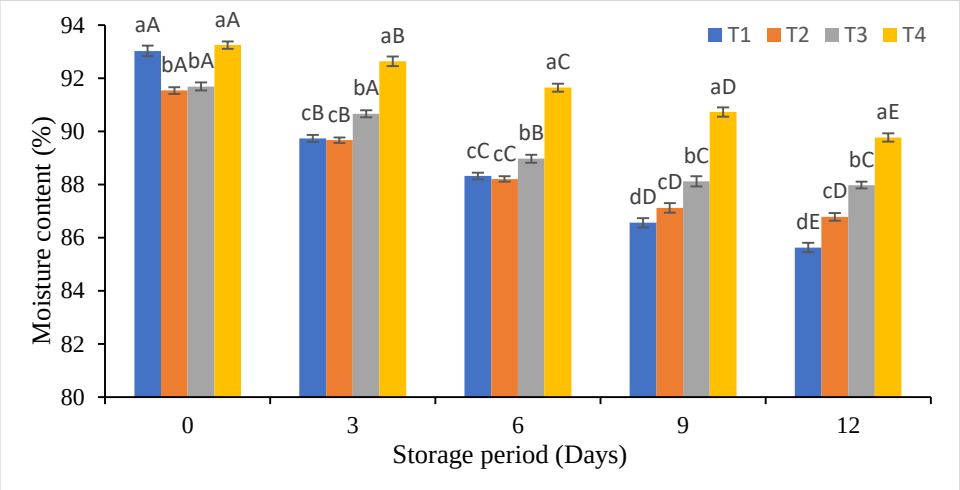

Fig. 3. Effect of postharvest treatments on the moisture content (%) of papaya during storage. Values followed by different small letters (a-d) in particular storage period indicate differences among the treatments and different capital letters (A-E) in particular treatments indicate differences among the storage intervals (P≤0.05). T_1: Control; T_2: 2% pectin coating; T_3: 2% cellulose acetate coating; T_4: 2% sodium alginate coating.

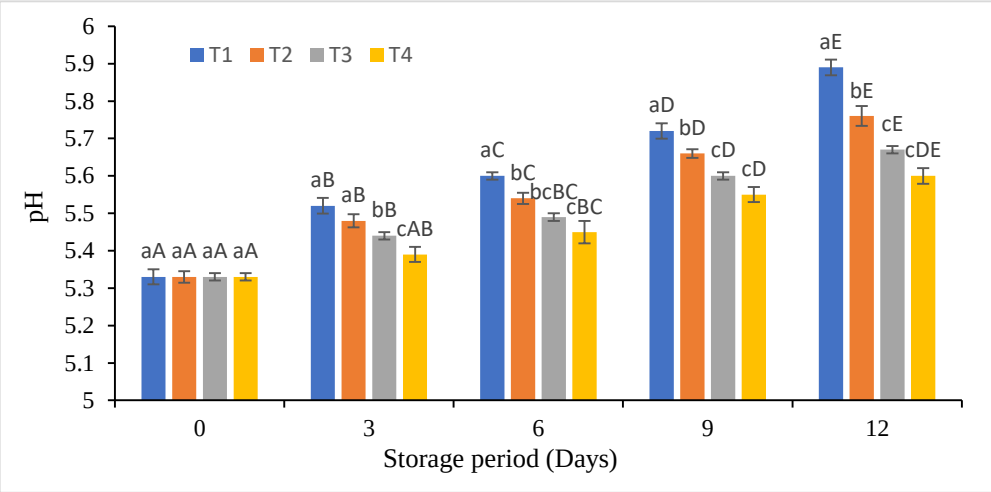

Fig. 4. Effect of postharvest treatments on the pH of papaya during storage. Values followed by different small letters (a-d) in particular storage period indicate differences among the treatments and different capital letters (A-E) in particular treatments indicate differences among the storage intervals (P≤0.05). T_1: Control; T_2: 2% pectin coating; T_3: 2% cellulose acetate coating; T_4: 2% sodium alginate coating.

pH

pH is an indication of the maturity of fruits and vegetables. Figure 4 depicts a significant (P≤0.05) increasing trend in the pH of papayas during the experiment. During the storage periods, the control papaya showed a higher pH (5.89) than the treated samples. Among the treatments, 2% sodium alginate-treated papaya showed a lower pH (5.6) followed by 2% cellulose acetate (5.67) and 2% pectin treated (5.76) papaya. Throughout the storage periods, the control papaya was increased by 10.51% and the 2% sodium alginate-treated papaya was increased by 5.07% which was half of the control papaya. Vieira et al. (2016) observed a comparable response when they coated blueberries with aloe vera gel to maintain their pH as opposed to non-coated ones. According to Ahmed et al. (2013), strawberries treated with alginate maintained their pH during storage. The fruits that were not treated, on the other hand, saw a higher pH shift.

Titratable acidity

An important indicator of the ripening stage is a change in the acidity level. Figure 5 shows a decline in acidity with storage periods. Titratable acidity started to decrease from 3rd day of storage. On the 12th day, the control papaya showed significantly (P≤0.05) lower acidity (0.21%) than the treated papaya. Among the treatments, the 2% sodium alginate-treated papaya showed the highest acidity (0.32%), followed by 2% cellulose acetate (0.3%) and 2% pectin solution-treated papayas (0.27%). Fruit loses acid during ripening and senescence because acids are crucial substrates for respiratory mechanisms (Narsaiah et al., 2015). Valero et al. (2013) reported that the decrease in fruit acidity would increase with increased metabolic respiration and vice versa. According to Alharaty and Namaswamy (2020), the changes in CO_2 and O_2 levels indicate a decrease in the rate of respiration. The alginate coating reduced the acidity by delaying the respiration rate. Acidity decreases at the late stages of fruit ripening due to the use of organic acids during respiration. On the contrary, edible coating reduced the loss of organic acids by reducing oxygen diffusion and respiration rates thus enabled to have higher acid levels than the control fruits at the end of the storage period. Alginate coating delays the utilization of organic acids (Yaman et al., 2002). Similar

trends in the total acid level were also noted by Olivas et al. (2007) in alginate-coated gala apples.

Total soluble solids (TSS)

The solubilization of more complex carbohydrates into simpler ones causes fruit to mature, which is reflected by an increase in total soluble solids concentration (Waghmare & Annapurna, 2013). Figure 6 revealed that TSS increased significantly (P≤0.05) with the increase of storage periods. At the end of the storage periods, the control papaya showed a higher TSS than the treatments. Among the treatments, 2% sodium alginate treated papaya showed a lower value (9.2 °Brix) followed by 2% cellulose acetate (10.4 °Brix) and 2% pectin solution (11.3 °Brix) treated papaya.

The increase in TSS was 28.26% higher in the control papaya as compared to the 2% sodium alginate-treated papaya. According to Narsaiah et al. (2014), this was caused by enhanced ripening, which is the side effect of greater respiration rate, in control papaya when they are sorted. This result was similar to that of the papaya treated with bacteriocin and alginate which was reported by Narsaiah et al. (2014). Kittur et al. (2001) showed for bananas and mangoes treated with polysaccharide-based coatings, the amount of reducing sugar in the samples showed that the treated papayas produced reducing sugars more slowly than the control and other treatments. According to Jiang (2013) and Guillén et al. (2013), the findings of additional studies on alginate-coated mushrooms and aloe vera-coated peaches were consistent with the trends of change in TSS.

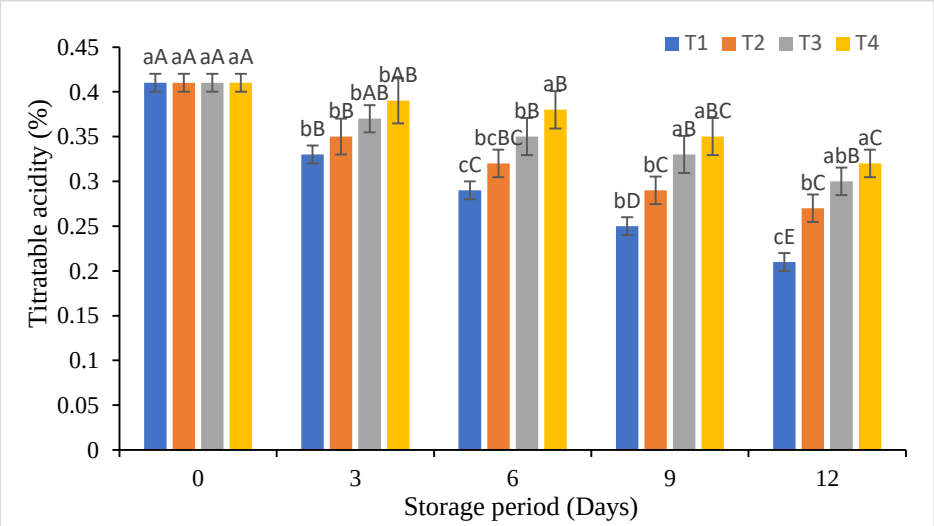

Fig. 5. Effect of postharvest treatments on the titratable acidity (%) of papaya during storage. Values followed by different small letters (a-d) in particular storage period indicate differences among the treatments and different capital letters (A-E) in particular treatments indicate differences among the storage intervals (P≤0.05). T_1: Control; T_2: 2% pectin coating; T_3: 2% cellulose acetate coating; T_4: 2% sodium alginate coating.

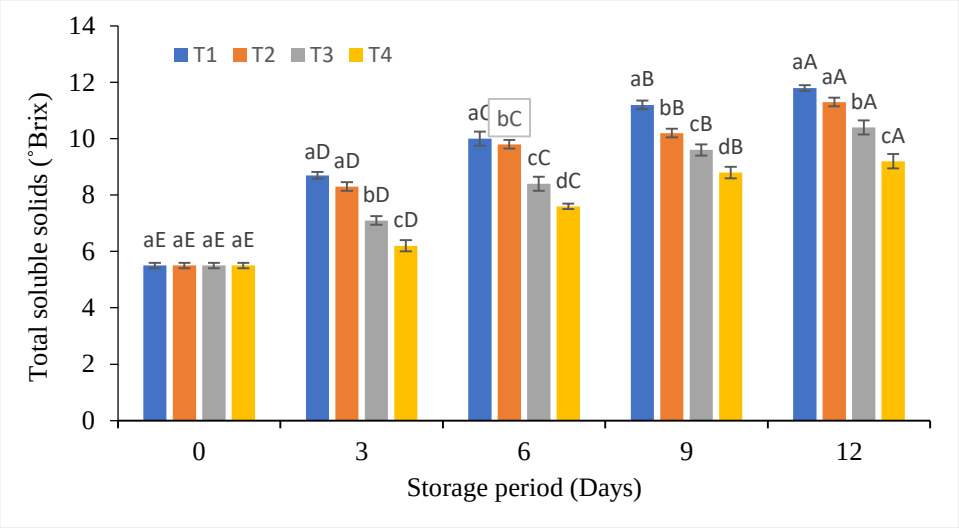

Fig. 6. Effect of postharvest treatments on the total soluble solids (°Brix) of papaya during storage. Values followed by different small letters (a-d) in particular storage period indicate differences among the treatments and different capital letters (A-E) in particular treatments indicate differences among the storage intervals ($P \leq 0.05$). T_1: Control; T_2: 2% pectin coating; T_3: 2% cellulose acetate coating; T_4: 2% sodium alginate coating.

Vitamin C content

Vitamin C content is the distinguishing element in the maturity stages of papayas that are linked to oxidative degradation (Siriamornpun et al., 2017). Figure 7 reveals the retention of vitamin C content throughout the storage periods. Vitamin C started to decrease from the 3rd day of storage. Vitamin C retention was higher in the treatments than in the control sample. The vitamin C contents on the 12th day of storage periods for all samples were statistically ($P \leq 0.05$) similar to those on the 9th day except control. Over the storage periods, Vitamin C content was lowest in control papaya which was 29.5 mg/100g, and the highest was found in 2% sodium alginate treated papaya which was 38.56 mg/100g followed by 2% cellulose acetate (37.38 mg/100g) and 2% pectin solution (35.67 mg/100g) treated papaya. The vitamin C content in 2% sodium alginate treated papaya was 30.71% higher as compared to control papaya. In the presence of oxygen, ascorbic acid auto-oxidizes spontaneously and that's why vitamin C content degrades (Owusu-Yaw et al., 1988). Qamar et al. (2018) also reported a reduced rate of decrease in ascorbic acid content in the case of sodium alginate-based strawberry coating. However, comparatively, vitamin C content decreased quickly in the control papaya.

Total viable count (TVC)

Microbial quality is the most significant component of food because it directly affects the health of the consumer. Figure 8 shows the total viable count of treated and control papaya fruits. The highest TVC was found in the control papaya (7 log CFU/ml). Here control papaya fruits were significantly ($P \leq 0.05$) different from the treatment papaya. Among the treatments, 2% sodium alginate treated papaya showed the least total viable count (6 log CFU/ml) followed by 2% cellulose acetate (6.5 log CFU/ml), and 2% pectin solution (6.32 log CFU/ml) treated papayas. This decrease in the total viable count was 14.29% lower in 2% sodium alginate-treated papaya as compared to control papaya. This is explained by improved release behavior and higher trapping efficiency (Narsaiah et al., 2015). A similar result was obtained in the chitosan and sodium alginate-based edible coating of fresh-cut

nectarines against yeast and molds (Valentina & Giovanna, 2016), and melon incorporated with antimicrobial components (Raybaudi-Massilia et al., 2008).

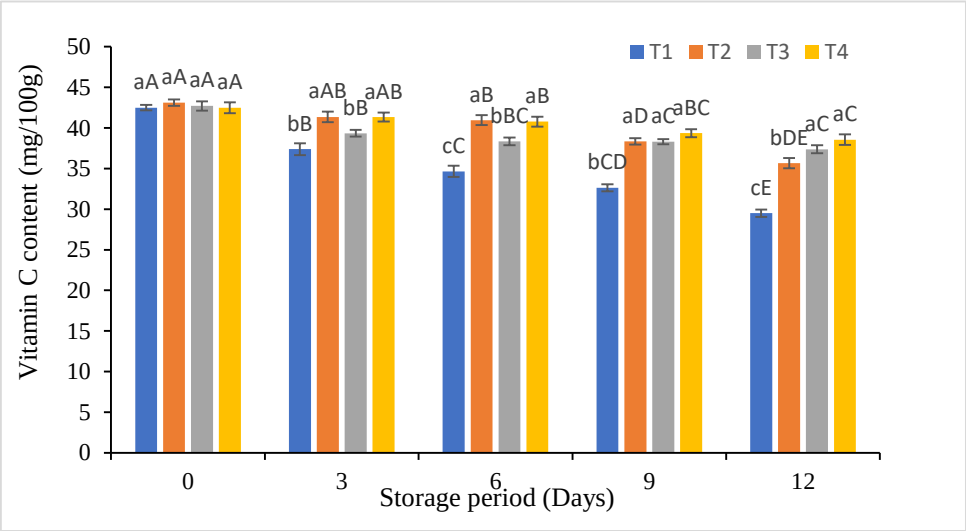

Fig. 7. Effect of postharvest treatments on vitamin C content of papaya during storage. Values followed by different small letters (a-d) in particular storage period indicate differences among the treatments and different capital letters (A-E) in particular treatments indicate differences among the storage intervals (P≤0.05). T_1: Control; T_2: 2% pectin coating; T_3: 2% cellulose acetate coating; T_4: 2% sodium alginate coating.

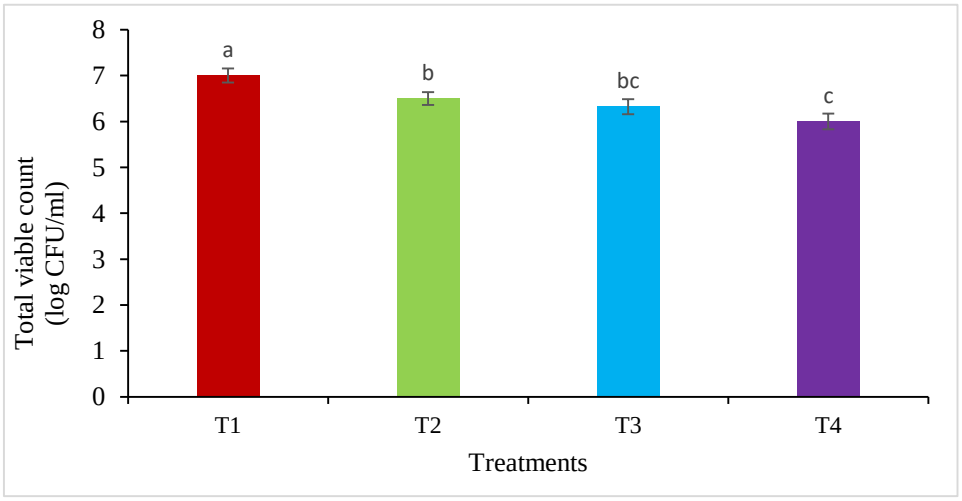

Fig. 8. Effect of postharvest treatments on the total viable count (TVC) of papaya. Values followed by different small letters (a-c) indicate differences among the treatments (P≤0.05). T_1: Control; T_2: 2% pectin coating; T_3: 2% cellulose acetate coating; T_4: 2% sodium alginate coating.

Shelf life

The most crucial factor in the biochemical reaction that causes fruit to rot is its shelf life, which is defined as the time interval from harvesting to the beginning of fruit rotting. Fig. 9 shows the effect of coating on extending the shelf life of papaya and it was statistically significant ($P \leq 0.05$). The maximum shelf life was found in 2% sodium alginate-treated papaya (16^{th} day) and significantly ($P \leq 0.05$) different from the other treatments, whereas the minimum shelf life was found in control papaya (6^{th} day). The addition of calcium chloride salt in sodium alginate coating helps in reducing bacterial growth and physiological disorders. Tabassum and Khan (2020) also reported the extension of shelf life to 12 days in the case of alginate-based edible coating. Delayed fruit ripening, whose changes in weight loss, firmness, total carotenoid, lycopene, and vitamin C were significantly slower than fruit treated with sodium alginate-based coating.

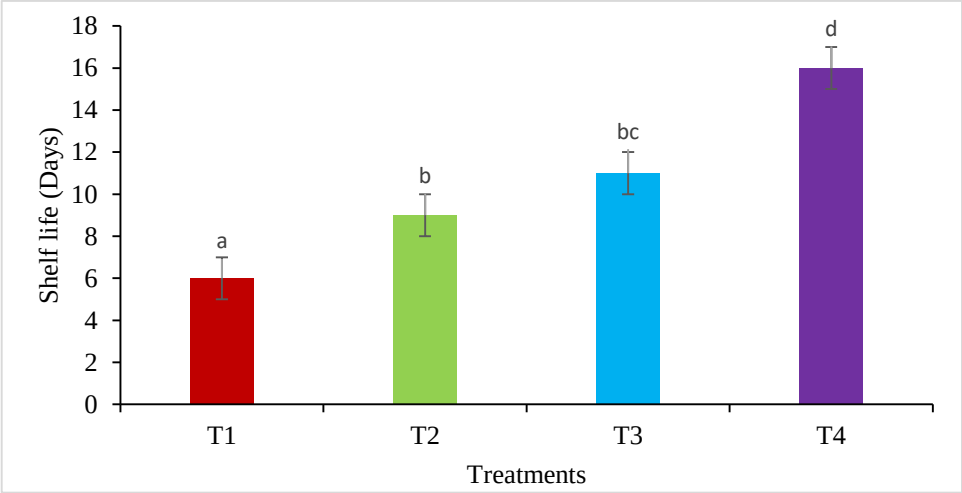

Fig. 9. Effect of postharvest treatments on the shelf life of papaya. Values followed by different small letters (a-c) indicate differences among the treatments ($P \leq 0.05$). T_1: Control; T_2: 2% pectin coating; T_3: 2% cellulose acetate coating; T_4: 2% sodium alginate coating.

CONCLUSION

The current study reported the efficacy of edible polysaccharide coatings such as pectin, cellulose acetate, and sodium alginate solution on papaya fruits to enhance the shelf life of papaya at the least nutrient loss. All the treated papayas showed good results, but 2% sodium alginate solution-coated papaya showed the lowest weight loss, color change, pH, total soluble solids content, and total viable count with the highest moisture content, titratable acidity, vitamin C retention, and shelf life throughout the storage periods followed by 2% cellulose acetate and 2% pectin solution coated papaya. So, 2% sodium alginate Solution coating seems to be a good substitute for preservation and a practical way to increase the quality and shelf life of papaya under commercial circumstances. To determine which edible coatings are optimal for commercial use, more research may be conducted to examine how these coatings affect the texture and sensory qualities of papaya while it is being stored.

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Conflict of interest

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

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