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Impact of chitosan coatings on shelf-life, nutrient elements and biochemical qualities of country beans (*Phaseolus lunatus* L.) at postharvest storage

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ABSTRACT

Purpose: In Bangladesh, postharvest damage to various vegetables is common because of a lack of appropriate technologies. Country beans (*Phaseolus lunatus* L.), one of Bangladesh's main winter vegetables, are cultivated throughout the country, which provides numerous health advantages. A research experiment was performed to measure the impact of chitosan covering on weight loss, shelf-life, and some nutritional characteristics of country beans at postharvest storage. **Research Method:** The experiment was set up in a completely randomized design (CRD) with three replications and four treatments at room temperature (≈23-25ºC), and the treatments were: T0 (control), T1 (coating with 0.10% solution), T2 (coating with 0.20% solution) and T3 (coating with 0.30% solution). **Findings:** The use of 0.20% chitosan prevented weight reduction by 1.59% as compared to the control and extended the shelf-life up to 23.3% in country beans at 10 and 12 days after postharvest storage (DAPS), respectively. Chlorophyll-a, chlorophyll-b and total chlorophyll contents varied from 0.75-1.59, 1.36-2.86 and 2.11-4.45 mg g^{-1} tissue at 5 DAPS and 0.61-1.26, 1.10-2.27 and 1.70-3.53 mg g^{-1} tissue at 10 DAPS, respectively. Chitosan treatment T2 significantly enhanced calcium (0.77%) and phosphorus (0.51%) contents in the country beans during postharvest storage at 5 DAPS. Additionally, treatment T3 significantly increased total phenolics (3.06 mg $100g^{-1}$ tissue) in the country beans during postharvest storage at the same DAPS. **Research limitations:** The study could not measure some traits (i.e., anti-radical activity, the activity of antioxidant enzymes, etc.) due to a lack of laboratory facilities. **Originality/Value:** This experiment revealed that country beans covered with 0.20% chitosan solutions could be utilized to enhance several nutritional properties, check weight loss, and prolong the shelf-life.

INTRODUCTION

Among the major winter vegetables of Bangladesh, country beans (*Phaseolus lunatus* L.) are cultivated throughout the country. Dietary intake of beans is associated with numerous health advantages. It is an excellent nitrogen source and several vital micronutrients, including iron, zinc, magnesium, and potassium (Messina, 2014). Campos-Veja et al. (2010) stated that "beans are rich in protein, carbohydrate, dietary fiber, and are a good source of antioxidants, as well as vitamins and minerals." Furthermore, a diet that combines animal proteins with vegetable proteins is important to get the complete pool of amino acids (Melo et al., 2012). The acreage and annual production of beans were 45029 acres and 110116 metric tons in the 2013-14 fiscal year, which were 56941 acres and 170067 metric tons in the 2021-22 fiscal year (BBS, 2021; 2023) indicating an increasing trend for both areas and production of beans in Bangladesh. A similar observation was also reported by Hasan et al. (2014), and they stated that bean production is profitable, and farmers are interested in cultivating more beans in the Mymensingh and Comilla districts of the country.

Vegetables are usually purchased as a fresh agricultural product for human consumption but every year, a large volume of various vegetables decomposed in our country because of a lack of effective postharvest management solutions. Postharvest losses of vegetables include damage due to spillage and degradation during handling, storage, and transportation after harvesting to distribution. Additionally, other factors including damage from insects and mites, diseases brought on by non-infectious pathogens, and pathological rots all contribute to the postharvest loss of vegetables (Katiyar et al., 2015). However, the use of suitable chemicals throughout the pre and postharvest stages may occasionally increase the product's shelf-life and make it available over an extended period of time (Yahaya & Mardiyya, 2019; Duan et al., 2019).

Deacetylated chitosan is partially dissolved in water, which can easily be dissolved in a weak acid solution. Therefore, its application in postharvest storage of fruits and vegetables is harmless, making it a potential preservative for coating various agricultural commodities (Hosseinnejad & Jafari, 2016; Romanazzi et al., 2017; Duan et al., 2019). According to Liang et al. (2017), it is the only alkaline natural polysaccharide with biologically interchangeable and biodegradable properties. Green beans have a high rate of respiration and heat emission, which affect metabolic processes, speed up decomposition, and reduce shelf-life after harvest (ElSayed et al., 2017; Liu et al., 2022). Thus, a partially permeable chitosan layer all over the green bean regulated the interior temperature, lowered transpiration losses, and maintained constant levels of enzyme (peroxidase and polyphenol oxidase) activities, which led to normal free radicals' production, enhanced the biochemical properties and storage of the green beans (ElSayed et al., 2017; Liu et al., 2022).

Furthermore, chitosan has antifungal properties against a variety of fungi. Its application showed a significant increase in different enzymes, viz. β-1,3-glucanase, chitosanase, and chitinase, which play a role in preventing fungal growth (Zhang & Quantick, 1998). Meanwhile, chitosan′s broad-spectrum antimicrobial activities have been extensively documented (Liu et al., 2022; Sun et al., 2018; Hosseinnejad & Jafari, 2016; Alvarez et al., 2013) and *in vivo* investigations have shown that it can check or suspend the postharvest deterioration of different agricultural commodities (Sultana et al., 2019; Zakir et al., 2022). Laboratory trials showed that covering tomato fruits and carrots with chitosan solution (0.2% and 0.3%, respectively) extended their shelf-life in postharvest storage by reducing the decomposition of fruits and weight loss (Sultana et al., 2019; Zakir et al., 2022).

Due to a lack of adequate postharvest technologies, nutritional quality and consumers' acceptability of different vegetables are reduced remarkably in our country. Thus, vegetable

producers/ farmers are deprived both of product loss and financial benefit, which has a lot of harmful effects on the country′s economy (Hossain et al., 2017). It has been well-documented that postharvest chitosan application improves the quality of uncooked green beans (Donsi et al., 2015; Severino et al., 2014). However, to our knowledge, the impact of chitosan treatment on the shelf-life and quality of beans has not been investigated in the context of our country. On the other hand, among the phytochemical substances, chlorophylls, polyphenols, and flavonoids are likely to contribute to health benefits (Grosso et al., 2013; Isabelle et al., 2010). Therefore, the goal of this study was to determine a suitable dose of chitosan for postharvest application in order to extend the shelf-life of country beans, as well as to explore its influence on some nutrients and biochemical features, particularly the contents of chlorophylls and total phenolics.

MATERIALS AND METHODS

Collection and sorting of country beans

Three (3.0) kg of country beans were collected from the grower′s/ producer′s field of the Sadar Upazila of Mymensingh district at full maturity level during January 2020. After that, all bean samples were transported to the Laboratory of Plant Nutrition and Environmental Chemistry, Deptartment of Agricultural Chemistry, BAU, Mymensingh. Then sorting/ screening of country beans was done manually based on their shape, size, and colour. Decomposed and pest-infested beans were discarded at this stage. Finally, almost similar shapes, sizes, and coloured beans were chosen for the laboratory trial.

Treatments and their preparation

Chitosan was obtained from Research-Lab Fine Chem Industries, Maharashtra, India (CAS No. 9012-76-4; Deacetylation >80%) and used in the present study to prepare coating solutions. There were 4 (four) treatments, namely- T0 (no coating solution), T1 (coating with 0.10% solution), T2 (coating with 0.20% solution) and T3 (coating with 0.30% solution). To prepare 0.10, 0.20, and 0.30% treatment solutions, exactly 1.0, 2.0, and 3.0 g chitosan was dissolved in 25 mL of glacial acetic acid (conc.) and then transferred into a 1.0 L volumetric flask containing about 500 mL distilled water. Before making the final volume, the pH of the solution was adjusted to 5.0 with 0.1 M NaOH, following the method adopted by Sultana et al. (2019).

Postharvest application of chitosan

A total of 16 beans were selected for the application of different chitosan treatments. These bean samples were immersed for a half minute in each treatment except T0 (control). In the case of control, beans were likewise immersed in water (distilled) with a pH of 5.0 adjusted with diluted glacial acetic acid or NaOH. Then, all samples were left to air dry beneath a ceiling fan for an hour at a temperature of 25°C. There were three replications and four treatment combinations; thus, a total of 12 (4×3) clusters of beans were handled in this laboratory trial. After applying chitosan solutions, all samples were placed in zip-lock bags following the techniques outlined by Sultana et al. (2019). Finally, all treated samples were stored in a dark place at normal (ambient) temperature (≈23-25°C).

Data recorded at postharvest storage

Data on the shelf-life of country beans were recorded at 8, 10, and 12 days after postharvest storage (DAPS), while the weight loss data of beans were measured at 2, 4, 6, 8, and 10 DAPS. On the other hand, for chemical analyses, four (4) country beans from each replication

were randomly selected at 0 (fresh), 5, and 10 DAPS. However, the shelf-life of country beans was measured by static testing, where bean samples were left on a dark shelf to rot and were then visually counted until 40-50% of a bean was rotten at the respective storage time (days) (Zakir et al., 2022).

$$Shelf-life\ (\%) = \frac{No.of\ beans\ decay\ at\ storage \times 100}{Total\ no.of\ beans\ in\ storage} \qquad (1)$$

Measurement of biochemical quality of country beans

Two (2) country-bean samples from each replication were collected at 0 (fresh), 5, and 10 DAPS to determine total phenol and chlorophyll contents. Total phenol estimation in the country beans was carried out with the Folin-Ciocalteau reagent as outlined by Sadasivam and Manickam (1996). The concentration of phenols in the country beans was calculated against the catechol standard curve and expressed as mg phenols/100 gm material. Chlorophyll is extracted in 80% acetone, and the absorption reading was taken at 663 nm and 645 nm using a spectrophotometer as described by Sadasivam and Manickam (1996). The contents of different types of chlorophylls (per gram tissue) present in the aqueous extract of country beans were measured with the equations mentioned below:

$$mg\ chlorophyll-a\ = 12.7\ (A_{663}) - 2.69\ (A_{645}) \times \frac{V}{1000 \times W} \ldots\ldots\ldots (2)$$

$$mg\ chlorophyll-b\ = 22.9\ (A_{645}) - 4.68\ (A_{663}) \times \frac{V}{1000 \times W} \ldots\ldots\ldots\ldots (3)$$

$$mg\ total\ chlorophyll\ = 20.2\ (A_{645}) + 8.02\ (A_{663}) \times \frac{V}{1000 \times W} \ldots\ldots\ldots\ldots (4)$$

In the above equations, A stands for absorbance at a specific wavelength; v means the final volume of chlorophyll extract in 80% acetone, and w refers to the fresh weight of the sample used to prepare the extract.

Measurement of mineral elements of country beans

To measure the contents of different mineral elements (Ca, Mg, Na, K, P, and S), collected country bean samples were chopped first and then oven-dried at a temperature of approximately 50°C following the techniques as outlined by Singh et al. (1999). The samples were then pulverized in a grinding ball mill and utilized to make an extract applying a combination of HNO_3 and $HClO_4$ at a 2:1 ratio (Singh et al., 1999). Among the mineral elements, calcium and magnesium in the aqueous extract were assessed titrimetrically. On the other hand, phosphorus and sulfur contents in the same extract were measured by spectrophotometry, while the amount of sodium and potassium were estimated by flame photometry (Singh et al., 1999).

Statistical analysis

The data were expressed as the average of three replications and standard deviations using the statistical software Minitab 17 (Minitab Ltd., UK) following analysis of variance (ANOVA) and a general linear model. The least significant difference (LSD) was performed to separate treatment means at a specific time of data collection. Pearson correlation investigation was executed to assess the correlations among the nutritional qualities (total phenol, chlorophyll, and mineral elements) of country beans.

RESULTS AND DISCUSSION

Weight loss of country beans

The impact of different treatments (chitosan coverings) on the reduction of weight loss of country beans at two-days intervals of postharvest storage is shown in Table 1, and in all cases, results were statistically insignificant. Reduction of weight of bean samples ranged from 4.73-5.99, 8.20-9.29, 10.89-12.19, 13.31-14.75, and 16.04-17.63% at 2, 4, 6, 8, and 10 DAPS, respectively. The study results revealed that the degree of weight reduction was greater in the T0 (no coating solution) treatment at all DAPS. While postharvest chitosan coating treatment T2 (0.20%) considerably decreased weight loss in most cases of data recording. However, statistically, there were insignificant variations in weight loss of country beans in between the treatments T1 and T2 during postharvest storage. Compared to the control (T0), treatments T1, T2, and T3 can preserve weight loss by 1.31, 1.59, and 0.43%, respectively, at 10 DAPS.

Regarding obtained data on weight loss of country bean samples, the study found the treatment T2 (i.e., coating with 0.2% chitosan solution) is the best (4.73%, 8.21%, 11.26%, 13.53% and 16.04% at 2, 4, 6, 8 and 10 DAPS, respectively) at normal temperature (≈23-25°C) conditions. Similar observations (0.30% and 0.20% chitosan solution) were also reported by Zakir et al. (2022) and Sultana et al. (2019) in the case of carrot and tomato, respectively stored at room temperature. Furthermore, according to Jianglian and Shaoying (2013), in fresh-cut lotus root, the use of a mixture coating containing chitosan (1%) and phytic acid (1%) reduced weight loss and malondialdehyde content, delayed discolouration (brown), inhibited the functions of enzymes (peroxidase, and polyphenol oxidase), and kept vitamin C contents at a level that was reasonably high. Zhang et al. (2018) stated that the application of gallic acid–chitosan derivatives to preserve cherry tomatoes exhibits great antioxidant ability in scavenging DPPH, hydroxyl, and superoxide anion radicals, which led to longer fruit ripening, decreased weight loss, high hardness, and little change in epidermal colour. Chitosan coatings have the potential to create a partially permeable layer surrounding the green bean that maintains the inner temperature and decreases transpiration losses (ElSayed et al., 2019), resulting in reduced weight loss. Similarly, El-Mogy et al. (2020) reported that fresh fruits and vegetables spend some weight after harvest as a result of respiration and transpiration. Therefore, the country bean coated with chitosan may be able to minimize transpiration and evaporation by producing a tiny, waterproof layer on the outside of the fruits and vegetables (Duan et al., 2019), which might account for the reduced weight loss. In addition, according to Zhang et al. (2011), chitosan treatment reduced the rate of transpiration and preserved water in the vegetables, which resulted in reducing weight loss of green beans. The present study summarized that postharvest application of this polysaccharide (chitosan) covering could be applied to prevent weight loss of country beans.

Table 1. The effect of chitosan coating treatments on weight loss of country beans at different days after postharvest storage (DAPS) at normal temperature (≈23-25ºC).

Treatments	Weight loss (%)					
	Fresh (0 DAPS)	2 DAPS	4 DAPS	6 DAPS	8 DAPS	10 DAPS
T_0	0.0	5.99	9.29	12.19	14.75	17.63
T_1	0.0	5.03	8.20	10.89	13.31	16.32
T_2	0.0	4.73	8.21	11.26	13.53	16.04
T_3	0.0	5.29	8.68	11.40	14.02	17.20
LSD (0.05)	0.0	1.56	1.57	1.59	1.99	1.65
SE (±)	0.0	0.48	0.48	0.49	0.61	0.51
Level of significance	NS	NS	NS	NS	NS	NS
CV%	0.0	15.72	9.71	7.38	7.48	5.21

NS = Non-significant

Storage longevity (shelf-life) of country beans

The pictorial views on the impact of different doses of chitosan coatings on country beans at fresh, 2, and 5 DAPS are shown in Figure 1. However, the storage longevity (shelf-life) of country beans was measured by static testing, where bean samples were left on a dark shelf to decay and were then visually counted until 40-50% of a bean was rotten at the respective storage time (days) and the results were expressed in %. There were significant differences observed in shelf-lives of country beans at 10 ($p<0.01$) and 12 ($p<0.05$) DAPS. The impact of chitosan application on shelf-lives of country beans varied from 93.3-100.0, 50.0-73.3, and 43.3-66.7% at 8, 10, and 12 DAPS, respectively (Table 2). Up to 10 DAPS, the obtained findings demonstrated no significant change in the country beans shelf-life between the treatments T2 and T3. However, treatment T2 had the highest shelf-life at 12 DAPS. When contrasted with the control (T0), the chitosan application at 0.10, 0.20, and 0.30% can extend the shelf-life of beans by up to 3.4, 23.3, and 13.3%, respectively, at 12 DAPS. Sultana et al. (2019) also reported almost similar observations in the case of tomato fruits. According to Liu et al. (2007), gray mould (induced by *Botrytis cinerea*) and blue mould (induced by *Penicillium expansum*) in tomato fruits preserved at room and refrigeration temperature have been greatly reduced by the application of 0.5 and 1% chitosan solution. Furthermore, Romanazzi et al. (2017) stated that both preharvest and postharvest application of chitosan had shown promising effects in disease control. They also stated that chitosan has a dual mechanism of action on pathogens and plants. According to their findings, chitosan inhibits the development of rotten fungi and foodborne microbial growth and persuades resistance reactions in the host plant. ElSayed et al. (2019) stated that low respiration rates during the storage of vegetables and fruits could reduce sugar losses, impacting the shelf-life and storage quality of vegetables. In addition, they also stated that the fresh green bean's active oxygen-scavenging systems were improved by the decrease in enzymes (peroxidase and polyphenol oxidase) activities. Therefore, before storage, green beans treated with chitosan may reduce the production of reactive oxygen species (ROSs) and/or promote the growth of alternative mechanisms for quenching the produced ROSs in the stored beans. Similarly, Hong et al. (2012) supported the idea that applying chitosan coating solution on guava fruits increased antioxidant capability, which is helpful in suspending the ripening process of fruits. Thus, the study results suggested that chitosan covering could be utilized to improve the shelf-life of country beans during postharvest storage, which could be owing to chitosan's ability to suppress postharvest damages of beans due to various pathogens/microbes.

Table 2. The effect of chitosan coating treatments on shelf-life of country beans at different days after postharvest storage (DAPS) at normal temperature (≈23-25ºC). Different letters indicating statistical significance at P-values ≤ 0.01 & ≤ 0.05.

Treatments	Shelf-life (%)			
	Fresh (0 DAPS)	8 DAPS	10 DAPS	12 DAPS
T_0	100.00	96.70	50.00 b	43.30 b
T_1	100.00	93.30	63.30 a	46.70 b
T_2	100.00	96.70	73.30 a	66.70 a
T_3	100.00	100.00	70.00 a	56.70 ab
LSD (0.05)	-	6.28	11.06	15.51
SE (±)	-	1.92	3.39	4.76
Level of significance	-	NS	**	*
CV%	-	3.45	9.16	15.44

** = Significant at 1% level of probability, * = Significant at 5% level of probability, NS = Not significant

Fig. 1. Pictorial views of country bean samples coated with different treatments at 2 and 5 days after post-harvest storage (DAPS) along with fresh samples.

Biochemical properties of country beans at the storage

Total phenolic content

Polyphenols are the most abundant bioactive components in different common bean cultivars. The main phenolic components in common beans are- phenolic acids, flavonoids, and pro-anthocyanidins (Yang et al., 2018). The effect of chitosan coatings on the estimated total phenolic contents in the country beans at different storage times is shown in Table 3. Its content varied from 2.55-3.06 and 3.20-4.18 mg/ 100g sample (fresh wt.) at 5 and 10 DAPS, respectively. The study results revealed that at storage, phenolic contents in the country beans reduced significantly ($p<0.01$), and chitosan coating treatments have no effect on preserving the total phenolic contents, which might be due to the chemical degradation of polyphenol and the production of the phenolic-protein complex (Xu & Chang, 2008). Furthermore, in the beginning, chitosan coating treatments lead to lower exposure to stressful situations; thus, natural defense substances, like polyphenols, were produced lower (Winter & Davis, 2006). The phenolic makeup and antioxidant properties of common bean varieties and their processed products have also been investigated extensively worldwide (Yang et al., 2018; Silva et al., 2018; Mastura et al., 2017; Aquino-Bolanos et al., 2016), and similar results were reported by them. Coelho et al. (2007) reported that long-time storage could change the colour, texture, total phenolic content, and total pro-anthocyanidins contents of beans. Martin-Cabrejas et al. (1997) pointed out that long-term storage could lower total phenolic content while increasing the total pro-anthocyanidins contents of beans. However, interestingly it is evident from Table 3 that the total phenolic content increases at 10 DAPS for all treatments, which might be due to the lower efficacy of chitosan film with higher storage time (Sultana et al., 2019; Zakir et al., 2022). Moreover, due to this reason, increases microbial stressful situations during longer storage time, which accelerates the internal production of natural

defense substances, like polyphenols (Winter & Davis, 2006). However, because of their antioxidant capabilities and capacity to neutralize free radicals, phenolic compounds serve a significant physiological function in boosting plants' stress tolerance and shielding plant tissue from the damaging results of oxidative stress (Sharma et al., 2019). But the behavior of polyphenols to chitosan application depends on the class of compounds investigated as well as the biostimulant's concentration (Sánchez-Hernández et al., 2022). Similarly, Deng et al. (2018) stated that with higher enzyme (polyphenol oxidase) activity oxidation of phenolics may be accelerated. Thus, at 10 DAPS the content of total phenol decreases with increasing doses of chitosan.

Chlorophyll content

The presence of chlorophyll pigments in the chloroplasts of plant tissues gives the green colour. It plays a crucial function in the metabolism of light energy and catalyzes the production of carbohydrates in collaboration with other pigments such as carotenoids. This study measured chlorophyll-a (chl-a), chlorophyll-b (chl-b), and total chlorophyll (total chl) contents in the country beans, and the impact of chitosan coatings on the estimated chlorophyll contents at 0 (fresh), 5 and 10 DAPS is presented in Table 3. Chlorophyll-a, chl-b and total chl contents varied from 0.75-1.59, 1.36-2.86 and 2.11-4.45 mg/g tissue at 5 DAPS and 0.61-1.26, 1.10-2.27 and 1.70-3.53 mg/g tissue at 10 DAPS, respectively. The maximum amounts of all types of chlorophylls were obtained by the application of T3 treatment (coating with 0.3% chitosan solution) at 5 DAPS. It is apparent from Table 3 that all types of chlorophyll contents in the country beans were minimum at control treatment, while increased considerably with the higher application doses of chitosan coatings at both 5 and 10 DAPS. There, the chitosan coating may reduce respiration rates, leading to slower rates of chlorophyll breakdown. This finding is consistent with that of ElSayed et al. (2019), who found that even after 7 days of storage, green beans coated with chitosan (1.5%) had the maximum concentration of total chlorophyll. The application of chitosan nanoparticles along with gibberellic acid was most effective in enhancing leaf area and levels of chlorophylls and carotenoids in beans (Pereira et al., 2017). Chitosan has been shown to boost chlorophyll content in stressful environmental conditions (Katiyar et al., 2015). On the contrary, Lai et al. (2007) stated that the loss of chlorophyll under stress conditions could be due to the oxidation of chloroplast lipids and proteins or the degradation of pigment-protein complexes that defend the photosynthetic machinery in plants. So, it can be summarized that the application of chitosan coatings can be used at postharvest storage to enhance chlorophyll contents in the country beans and the maximum benefit of chlorophylls can be achieved at 5 DAPS.

Table 3. The effect of chitosan coating treatments on biochemical constituents of country beans at different days after post-harvest storage (DAPS) at normal temperature (≈23-25ºC).

Treatment	Moisture (%)		Total phenol (mg 100g^{-1})		Chlorophyll-a (mg g^{-1})		Chlorophyll-b (mg g^{-1})		Total chlorophyll (mg g^{-1})	
	5 DAPS	10 DAPS	5 DAPS	10 DAPS	5 DAPS	10 DAPS	5 DAPS	10 DAPS	5 DAPS	10 DAPS
T0	91.05	88.52	2.575b	4.184a	0.752c	0.607d	1.357c	1.094c	2.109c	1.701d
T1	91.77	89.35	2.548b	3.905a	1.189b	0.765c	2.145b	1.379b	3.334b	2.144c
T2	91.39	89.62	2.748b	4.081a	1.239b	1.259a	2.236b	2.269a	3.475b	3.528a
T3	91.18	89.07	3.059a	3.204b	1.588a	1.208b	2.863a	2.178a	4.450a	3.386b
$LSD_{0.05}$	0.92	0.93	0.27	0.22	0.13	0.04	0.19	0.12	0.31	0.14
SE (±)	0.280	0.290	0.082	0.068	0.040	0.013	0.059	0.036	0.096	0.043
Level of significance	NS	NS	**	**	**	**	**	**	**	**
CV%	0.53	0.05	5.02	3.45	5.76	2.25	4.73	3.63	4.95	2.77
Fresh bean (0 DAPS)	92.85 ± 1.02		6.242 ± 0.324		0.654± 0.021		1.199± 0.036		1.853± 0.063	

Different letters indicating statistical significance at P-value < 0.01. ** indicating at p < 0.01, and NS means non-significant.

Mineral element contents

The common bean is a widely consumed legume, which contains high levels of minerals and protein (Nchimbi-Msolla & Tryphone, 2010). The impact of chitosan application on several mineral elements in the country beans at different days after postharvest storage is shown in Table 4. The concentration of Ca, Mg, Na, K, P and S in the country beans ranged from 0.44-0.77%, 0.37-0.46%, 0.50-0.63%, 2.22-2.62%, 0.32-0.51%, and 0.14-0.17% (dry wt.) at 5 DAPS and 0.48-0.59%, 0.48-0.56%, 0.49-0.59%, 1.90-2.12%, 0.34-0.38%, and 0.12-0.15% (dry wt.) at 10 DAPS, respectively. Alternatively, the mean content of Ca, Mg, Na, K, P and S in fresh country bean samples was 0.372, 0.271, 0.330, 2.136, 0.330, and 0.141%, respectively. It can be seen from Table 4 that the treatment T2 (i.e., coating with 0.2% chitosan solution) produced the highest amounts of different elements (viz. Ca at 5 DAPS, Mg at 10 DAPS, Na at both DAPS, K at 10 DAPS, P at 5 DAPS, and S at both DAPS) in beans. According to Khazaei and Vandenberg (2020), fava beans contain 0.093-0.103% Ca, 0.123-0.148% Mg, 0.002-0.016% Na, 1.084-1.206% K, 0.434-0.606% P and 0.183-0.205% S. However, a few things needed to be more consistent in the mineral element content of the country bean that was used for analysis in the present study, which may be connected to sample size, maturity level, and number of seeds in the bean.

However, there were comparatively higher amounts of minerals except for Na and S in the country bean samples after postharvest storage, although the differences in most cases were insignificant (Table 4). Similar observation was also reported by Sultana et al. (2019) for postharvest storage of tomato. However, they could not provide any information as to why the mineral content of tomato fruits increased during storage. On the other hand, according to Youwei and Yinzhe (2013), the respiration rate, the amount of free radicals, and the level of disease resistance all dropped when the surface of the beans was coated with chitosan. As a result, the majority of nutrients are conserved to the maximum.

Table 4. The impact of chitosan treatments on concentration (± SD) of different mineral elements in the country beans when stored at normal temperature (≈23-25°C).

Treatment	Ca (%)		Mg (%)		Na (%)		K (%)		P (%)		S (%)	
	5 DAPS	10 DAPS	5 DAPS	10 DAPS	5 DAPS	10 DAPS	5 DAPS	10 DAPS	5 DAPS	10 DAPS	5 DAPS	10 DAPS
T0		0.586	0.421	0.478	0.496	0.496	2.215	2.109	0.317b	0.341	0.147	0.141
T1	0.699a	0.476	0.408	0.517	0.605	0.491	2.521	2.124	0.447a	0.339	0.183	0.146
T2	0.773a	0.484	0.377	0.557	0.632	0.592	2.344	2.115	0.509a	0.342	0.163	0.137
T3	0.533b	0.488	0.461	0.421	0.589	0.495	2.262	1.995	0.455a	0.312	0.139	0.112
$LSD_{0.05}$	0.11	0.15	0.07	0.27	0.13	0.11	0.37	0.25	0.11	0.09	0.04	0.04
SE (±)	0.033	0.046	0.022	0.083	0.041	0.032	0.110	0.080	0.034	0.028	0.011	0.012
Level of significance	**	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS
CV%	9.45	15.61	9.30	29.14	12.14	10.82	8.38	6.37	13.57	14.46	12.21	15.31
Fresh bean (0 DAPS)	0.372 ± 0.026		0.271 ± 0.011		0.330 ± 0.058		2.136 ± 0.173		0.330 ± 0.035		0.141 ± 0.017	

Treatment T0= Control (no coating solution); T1= coating with 0.10% solution; T2= coating with 0.20% solution and T3= coating with 0.30% solution. ** indicating significant at $P < 0.01$, * indicating significant at $P < 0.05$, and NS means non-significant.

Correlations among the nutritional qualities of country beans

Pearson correlation coefficients among the nutritional qualities of country beans harvested at 5 and 10 DAPS are summarized in Tables 5 and 6, respectively. Total phenol contents of beans were positively correlated to Mg (r = 0.545) at 5 DAPS, while it showed highly significant positive relationships to Mg, K, and S (r = 0.791, 0.950, and 0.820, respectively) at 10 DAPS. But among the nutrient elements, Ca and P demonstrated highly significant negative relationships (r = -0.936 and -0.963, respectively) to the total phenol contents of beans. On the other hand, total phenol contents of beans were positively correlated to chlorophyll contents at 5 DAPS but negatively correlated to the same at 10 DAPS (Tables 5 and 6). Thus, it can be inferred that total phenol contents in beans negatively affected chlorophyll contents at a longer storage period. This might be due to the increase of total phenolic content at longer storage (Table 3) for all chitosan treatments, and lower efficacy of chitosan film with higher storage time. Thus, increases microbial stressful situations, which accelerates the internal production of natural defense substances, like polyphenols. The study also revealed highly significant positive correlations between each other of different types of chlorophyll contents (chlorophyll-a, chlorophyll-b, and total chlorophyll), which indicated that they were strongly associated with each other, and chitosan coating has the ability to boost chlorophyll content in stressful environmental conditions. Chitosan has also been identified by Katiyar et al. (2015) as a natural bioactive chemical to improve prospective physiological changes in crops. Furthermore, different types of chlorophylls also showed significant positive correlations to Na and P at both DAPS (Tables 5 and 6). Among the mineral elements, Na, K, P, and S were positively correlated with Ca (r = 0.900, 0.634, 0.838, and 0.834, respectively), and K and P showed the same relationships with Na (r = 0.548 and 0.979, respectively) at 5 DAPS (Table 5). Similarly, Mg demonstrated significant positive relationships with Na, K and S (r = 0.589, 0.921 and 0.976, respectively); Ca with P (r = 0.996), and K with S (r = 0.956) at 10 DAPS (Table 6). On the other hand, at 10 DAPS, Mg, K and S were negatively correlated with Ca (r = -0.935, -0.999, and -0.968, respectively), and Mg, K, and S exhibited the same correlations with P (r = -0.906, -0.999 and -0.943). However, such inverse relationships indicate that the content of these mineral elements is moved in the reverse direction, i.e., when the content of any one of these elements is increased, the other is decreased with the same magnitude and vice-versa.

Table 5. Pearson correlation coefficients for nutritional qualities of country beans collected at 5 days after postharvest storage (n=12).

	Total phenol	Chl-a	Chl-b	T-Chl	Ca	Mg	Na	K	P
Chl-a	0.813**								
Chl-b	0.821**	0.998**							
T-Chl	0.818**	0.999**	1.000**						
Ca	-0.128^{ns}	0.417^{ns}	0.377^{ns}	0.392^{ns}					
Mg	0.545*	0.230^{ns}	0.286^{ns}	0.266^{ns}	-0.719**				
Na	0.273^{ns}	0.765**	0.740**	0.749**	0.900**	-0.369^{ns}			
K	-0.463^{ns}	0.097^{ns}	0.103^{ns}	0.101^{ns}	0.634*	-0.280^{ns}	0.548*		
P	0.430^{ns}	0.838**	0.810**	0.821**	0.838**	-0.323^{ns}	0.979**	0.365^{ns}	
S	-0.530^{ns}	-0.113^{ns}	-0.164^{ns}	-0.145^{ns}	0.834**	-0.978**	0.518^{ns}	0.458^{ns}	0.446^{ns}

Notes: Chl = Chlorophyll; T-Chl = Total chlorophyll; ns non-significant; * significant at P < 0.05 and ** significant at P < 0.01

Table 6. Pearson correlation coefficients for nutritional qualities of country beans collected at 10 days after postharvest storage (n=12).

	Total phenol	Chl-a	Chl-b	T-Chl	Ca	Mg	Na	K	P
Chl-a	-0.605*								
Chl-b	-0.701**	0.991**							
T-Chl	-0.703**	0.991**	1.000**						
Ca	-0.936**	0.458 ns	0.573*	0.571*					
Mg	0.791**	-0.116 ns	-0.247 ns	-0.244 ns	-0.935**				
Na	0.228 ns	0.635*	0.534*	0.532*	-0.319 ns	0.589*			
K	0.950**	-0.486 ns	-0.599*	-0.597*	-0.999**	0.921**	0.301 ns		
P	-0.963**	0.512 ns	0.623*	0.621*	0.996**	-0.906**	-0.284 ns	-0.999**	
S	0.820**	-0.286 ns	-0.408 ns	-0.403 ns	-0.968**	0.976**	0.402 ns	0.956**	-0.943**

Notes: Chl = Chlorophyll; T-Chl = Total chlorophyll; ns non-significant; * significant at P < 0.05 and ** significant at P < 0.01

CONCLUSION

The application of chitosan coating at different doses showed a remarkable positive effect to preserve weight loss and increase the shelf-life of country beans. Similarly, chitosan coatings also enhance different types of chlorophyll contents in the country beans during postharvest storage. Furthermore, the study found that chitosan coverings have the ability to enhance some mineral nutritional aspects. However, total phenolic contents in the country beans decreased considerably at storage, and they were not able to be protected by the chitosan application. Finally, this research found that chitosan covered with a 0.20 percent solution is suitable to maintain the nutritional values of country beans while also preventing weight loss and extending shelf-life. The effect of chitosan application on the contents of different mineral elements of fruits and vegetables is limited, and the cause of the observed phenomena is still unclear. Thus, a more thorough and critical quantitative investigation should be conducted, taking into account all factors to determine the actual status of mineral elements during postharvest storage of chitosan-treated fruits and vegetables, which will ultimately contribute to improved farming techniques and nutritional standards.

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Edible coatings maintained postharvest quality and increased shelf life of guava fruits

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ABSTRACT

Purpose: Guava is believed to be the most important commercial fruit crop in Bangladesh. Guava fruit exhibit very short storage life mainly due to high respiration rate, susceptibility to various pathogens and mechanical damages which can rapidly reduce the quality. However, the experiment was conducted to study the edible coatings effects on postharvest quality and shelf life of guava. **Research Method:** Commercially mature guava fruits (Swarupkathi and Thai) were treated with six edible coatings viz., (i) T_1 : Control, (ii) T_2 : Aloe vera gel (25%), (iii) T_3: Carboxy methyl cellulose (CMC) (1%), (iv) T_4: Chitosan(1%), (v) T_5: Aloe vera gel (25%) + Chitosan (1%) and (vi) T_6: Green tea leaf extract. The two-factor experiment was designed with a Completely Randomized Design and three replications. **Findings:** The results showed that, Thai Piara with Chitosan 1% treatment recorded the minimum weight loss (6.28%), the highest vitamin C content (191.44 mg/100gFW), the lowest pH (5.30), the maximum total soluble solids content (6.77 ^{o}Brix) and the highest titratable acidity (2.04%) at 10 days after storage compare to untreated Swarupkathi piara. Thai Piara treated with Aloe vera gel 25 % + Chitosan 1% exhibited the highest shelf life (13.00 days) followed by (12.67) in Chitosan (1%) treatment. **Research Limitations:** The study did not focus on ethylene and respiration rate determination. **Originality/Value:** The study demonstrated that Thai Piara, treated with Chitosan 1% solution showed better performance followed by Aloe vera gel 25% + Chitosan 1% solution for maintaining postharvest quality and shelf life of guava.

INTRODUCTION

Guava (*Psidium guajava* L.) belongs to the family Myrtaceae, is believed to be the most important commercial fruit crop in Bangladesh. It is one of the most popular fruit in Bangladesh due to its comparative low price, high nutrient value, good taste and high health benefit than some other fruits (Bose, Ahmed, Howlader, & Ali, 2019). Guava is the major source of vitamin C and Pectin. Guava contains moisture 80-83%, acid 2.45%, reducing sugar 3.5-4.45%, non-reducing sugar 3.97-5.23%, TSS 9.73-14.23, Potassium 0.48%, vitamin C 260mg per 100 g of edible' potion (F. Islam, Islam, Al Munsur, & Rahim, 2008). It is an excellent source of dietary fibers and minerals such as potassium, manganese, magnesium and phosphorus (Soares, Pereira, Marques, & Monteiro, 2007). Guava is consumed along with its seeds which are rich in omega fatty acids and fiber (Meena et al., 2021).

The per capita availability of fruits is reduced due to high level of postharvest losses. Approximately 40% fruit goes waste during postharvest handling, storage and ripening (FAO, 2018). At harvesting stage high respiration and quick ripening of the guava fruits leads to perishable during storage interval. To supply fresh and quality fruits to the consumers during the entire year, it is important to develop postharvest technologies related to quality maintenance and shelf life extension of guava varieties (Chien, Sheu, & Yang, 2007; Qiuping, Wenshui, & Jiang, 2006). To control postharvest decay and increase shelf life of fruits, different synthetic chemicals are used but, consumers prefer more natural, eco-friendly minimally processed products without considerable changes in their fresh characteristics with high nutritional quality and longer shelf life (Bose, Howlader, Jia, Wang, & Yin, 2019). Guava is climacteric fruit higher rate of respiration and ethylene production, very susceptible to mechanical injury that limits its postharvest shelf-life at room temperature (Azam et al. 2020). Various postharvest treatments were used to enhance the storage life and quality of guava such as fruits treated with edible coatings (Silva et al., 2018), gamma-irradiation and calcium chloride (Javed, Randhawa, Butt, & Nawaz, 2016; Pandey, Joshua, Bisen, & Abhay, 2010), ascorbic acid (Azam et al., 2020), 1-MCP (Phebe & Ong, 2010), control atmosphere storage (Teixeira, Júnior, Ferraudo, & Durigan, 2016), low temperature storage (Mahajan, Gill, & Dhaliwal, 2017) and packaging types (Rana & Siddiqui, 2018).

Recently edible coatings are used as novel food preserving compounds which help to maintain food quality (Ergun & Satici, 2012). These compounds do not have side effects and due to presence of antimicrobial compounds, increases the food quality and storage period (Ashwini & Desai, 2018). A number of edible coatings have been used and discussed by the scientists and efforts are still going on to find the best one (Abbasi, Iqbal, Maqbool, & Hafiz, 2009; Zhu, Wang, Cao, & Jiang, 2008). Application of edible coating is one of the low cost and proven technologies which have attained wide popularity among the researchers. They prevent the entry and exit of moisture and gases, controls the growth of microorganisms, retain the original color of the fruits, and effectively extend the shelf-life of the product (Vania, 2011). So, edible coating might be the alternative of chemical preservative and one of the best solutions for preserving guava fruit quality and shelf life.

However, in Bangladesh, there is limited information and experience to use edible coatings as postharvest treatment to extend the shelf life of guavas. Therefore, the present experiment was undertaken to study the effect of edible coating in maintaining the postharvest quality of guava.

MATERIALS AND METHODS

Experimental chemicals and materials

Two varieties of guava, namely Swarupkathi and Thai piara was used as experimental materials for the experiment. Swarupkathi guava is oval to round shape, upper surface rough, green and yellowish green in mature and ripe stage respectively. Flesh is whitish, very sweet, juicy and pleasant aroma. Fruits were harvested at turning stage and immediately after harvest fruits were transferred in the laboratory. Thai piara is ovate shape; flesh is white, yellowish green in mature and ripe stage. The guavas were collected from farmer's field at Swarupkathi in Pirojpur district, Bangladesh. Commercially mature fruits of guava with uniform size, shape and maturity were harvested and used for the experiment. The fruits were cured just after harvesting to make sure the temperature of the fruits was stable. Then the skin of the fruits was cleaned with soft cloth and water. The different treatments were selected on the basis of previous studies. The experiments consist of six treatments viz., T_1 = control; T_2 = aloe vera gel (25%); T_3 = carboxy methyl cellulose (CMC) (1%); T_4 = chitosan (1%); T_5 = aloe vera gel (25%) + chitosan (1%); T_6 = tea leaf extract (green tea leaf extract).

Coating application

The 25% aloe vera gel was prepared from collected fresh aloe vera leaves. The colorless hydro parenchyma was mixing with distilled water with a ratio of (1:3), then homogenized in a blender machine. The gel was then filtered by sieve to remove all unwanted lump and to get 25 percent fresh aloe gel. CMC (1%, w/v) was prepared by solubilizing 1 g of CMC powder in 100 mL of water–ethyl alcohol mixture (3:1 L/L) at 75 $^{\circ}$C under magnetic stirring for 15 min. For preparation of 1% chitosan solution, 10g of chitosan was taken and slowly added to the beaker with 50 ml glacial acetic acid and 1L water placed on magnetic stirrer which was already stirring and gradually heating up. After adding full amount of chitosan powder to the beaker, 1L chitosan solution was prepared. Green tea leaves are heated with distilled water at 90 $^{\circ}$C for 10 minutes at a ratio of 1gm to 5ml and filtered using Whitman No.1 filter paper. glycerol (10%) was used as additive.

A total of 360 fresh guava fruits of two varieties were used for this experiment. Ten fruits from each variety were selected for individual treatment. Then the selected fruits were individually dipped into each solution for 2 minutes and allowed to air dry for a period of 10 min and then kept on brown paper for observation at 22±2 $^{\circ}$C and 70-85% relative humidity. During the entire storage period, the fruits used for experiment will be keenly observed everyday but data will be recorded on physico-chemical changes during 2, 4, 6, 8 and 10 DAS influenced by different edible coatings.

Color, firmness and weight loss analyses

Changes in skin color were recorded during storage by matching the pericarp colors with a standard color chart. Digital penetrometer along with a measuring probe (5 mm diameter stainless steel) was used for firmness determination. Fruit firmness was measured from two opposite sides of each fruit by penetrating the probe at a distance of 5 mm into the fruit with pre- and post-test speed 1mms^{-1}. The firmness was calculated as maximum penetration force reached during tissue breakage and expressed as Newton (N).

The percent weight loss was calculated by the following formula (1) by Ranganna (1979):

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

[Here, IW= Initial/Fresh weight (g), FW= Final weight (g)]

Titratable acidity (TA) and pH determination

Titratable acidity was determined according to the method by Ranganna (1977) with minor modification. Ten grams of guava pulp tissues were homogenized with 40 ml of distilled water by using a Kitchen blender for two minutes and filtered through a Whatman filter paper No.2. Five milliliters of the guava juice extract solution were taken in a 100ml conical flask. Two to three drops of phenolphthalein indicator solution were added and then the conical flask was shaken vigorously. The sample was titrated with 0.1 M sodium hydroxide (NaOH) solution until the color changed to pink and persistent for at least 15 seconds. The titer volume was recorded and the result was expressed as percentage citric acid, which was calculated using the following formula (2):

$$\text{Citric acid (\%)} = \frac{\text{Titre (mL)} \times \text{NaOH normality (0.1N)} \times \text{vol.made up (50 mL)} \times \text{citric acid eq.weight (64 g)} \times 100}{\text{Vol.of sample for titrate (5 mL)} \times \text{wt.of sample taken (10 g)} \times 1000} \quad (2)$$

The remaining juice extract from TA measurement was used to measure the pH of the fruit pulp. The pH was determined by using a glass electrode pH meter.

Total soluble solids (TSS) (˚Brix) analysis

The total soluble solids of the thoroughly mixed guava fruit pulp was directly recorded by using hand refractometer (Model BS Eclipse 3-45) at room temperature (Nanda, Sarkar, Sharma, & Bawa, 2003). Fruits were homogenized in a kitchen blender for two minutes and filtrated through four layers of muslin cloth. A drop of fruit extract was placed on the prism of refractometer and reading was observed. The results were expressed as percent soluble solids (°Brix).

Determination of total sugar content of guava pulp

Sugar content was estimated by determining the volume of unknown sugar solution of guava pulp required for complete reduction of standard Fehling's solution. Fifty gram of fruits was used to calculate percent reducing, non-reducing and total sugar content using the following formulae (3, 4):

$$\text{\% Reducing sugar} = \frac{I \times D \times 100}{T \times D \times 1000} \quad (3)$$

(Where, I = mg of invert sugar required to reduce to known volume of Fehling's solution, D = Dilution, T = Titre and W = wt. of the sample)

$$\text{\% Non-reducing sugar} = (\text{\% Total invert sugars} - \text{\% reducing sugars originally present}) \times 0.95 \text{ (conversion factor)} \quad (4)$$

3. % Total sugars = % reducing sugar + % non-reducing sugar

Estimation of vitamin C content

Ascorbic acid content of guava was estimated by titration method using 2, 6-dichlorophenol indophenol dye solution described by Ranganna (1986). The method of estimation involves the reduction of 2, 6-dichlorophenol indophenol dye to a colorless form by ascorbic acid in an alkaline solution. The reaction is quantitative and particularly specific for ascorbic acid in solution in the pH range of 1-3.5. Then the ascorbic acid content of the sample calculated by the following formula (5):

$$\text{Vitamin C (mg/100g fruit)} = \frac{T \times D \times V1}{V2 \times W} \times 100 \quad (5)$$

(Here, T= Titre, D= Dye factor, V_2= Volume made up, V_1= Volume taken for titration, W= Weight of the sample taken for estimation)

Shelf life

Shelf life of guava fruits as influenced by different storage treatments and variety was calculated by counting the days required to ripe fully as to retaining optimum marketing and eating qualities**.**

Statistical analysis

The experiment was carried out in a Completely Randomized Design (CRD) with three replications. The collected data were significantly analyzed by Analysis of variance method using SPSS software. The significance of difference between pair of means will be tested by the Least Significant Difference (LSD) test at 5% and 1% levels of probability (Gomez & Gomez, 1984).

RESULTS AND DISCUSSION

Color

Guava is a green fruit that does not change color much during storage. Instead, it loses water and turns slightly brown, followed by skin softening. This is common under control conditions.

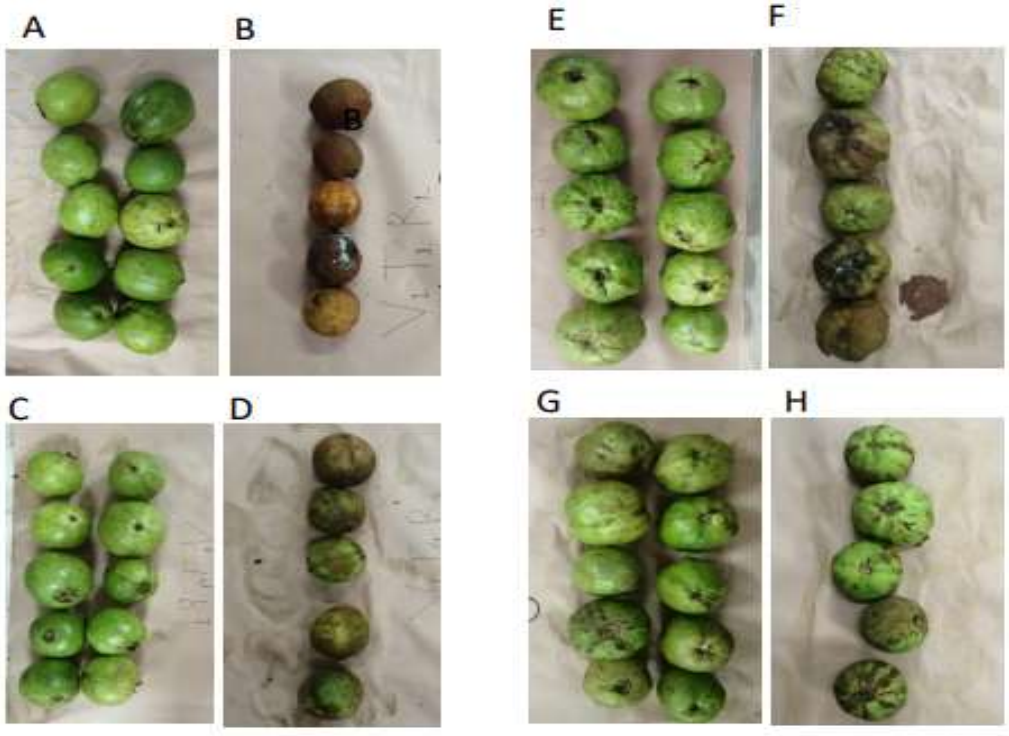

Fig. 1. Color changes of guava fruits during storage. Here, (A & B): Uncoated Swarupkathi piara after 2 and 10 days of storage, (C & D): Swarupkathi piara coated with chitosan (1%) after 2 and 10 days of storage, (E & F): Uncoated Thai piara after 2 and 10 days of storage, (G & H): Thai piara coated with chitosan (1%) after 2 and 10 days of storage.

However, when different postharvest treatments were used, a noticeable difference was observed in each case. In terms of color change, when two varieties of guava were combined with edible coatings, all of the treatments showed better results than control. The best result was found when Thai piara treated with chitosan (1%) and the lowest was noted from Swarupkathi piara without edible coating (Fig. 1). The color changed into brown in Swarupkathi piara after 6 days of storage when fruits were uncoated whereas Thai piara treated with chitosan (1%) able to retain its color up to 12 days followed by aloe vera gel (25%) + chitosan (1%) (11 days).

Firmness and weight loss

In respect of firmness, there was a significant variation was observed between two varieties. However, decreasing trend in firmness was found during storage. At 2 DAS, the highest firmness was 4.30 N which decreased to 3.60 N at 10 DAS in Swarupkathi while in Thai piara, at 2 DAS and 10 DAS, the firmness was 4.40 and 3.76 N, respectively (Fig. 2a). The results on firmness showed that there was a significant variation among the postharvest treatments of guava in relation to storage duration. Higher rate of decreasing trend in firmness was recorded only on control treatment while slow decreased rate on firmness was recorded for other treatments especially in case of carboxy methyl cellulose (1%) (Fig. 2b). The combined effect of varieties and treatments on firmness was non-significant at 2, 4 and 6 DAS but it was significant in 8 and 10 DAS. Decreasing trend of firmness was recorded for increasing of storage duration for all the treatment combinations. At 2 DAS, the highest firmness (4.68 N) was noted from Thai piara coated with carboxy methyl cellulose (1%) whereas the lowest firmness (3.81 N) was recorded from uncoated Swarupkathi piara which was significantly different from other treatment combinations (Table 1). Similar results were observed and they demonstrated that treatment with 2.0 percent chitosan greatly slowed weight loss and firmness loss over the course of a 12-day storage period. (Hong, Xie, Zhang, Sun, & Gong, 2012).

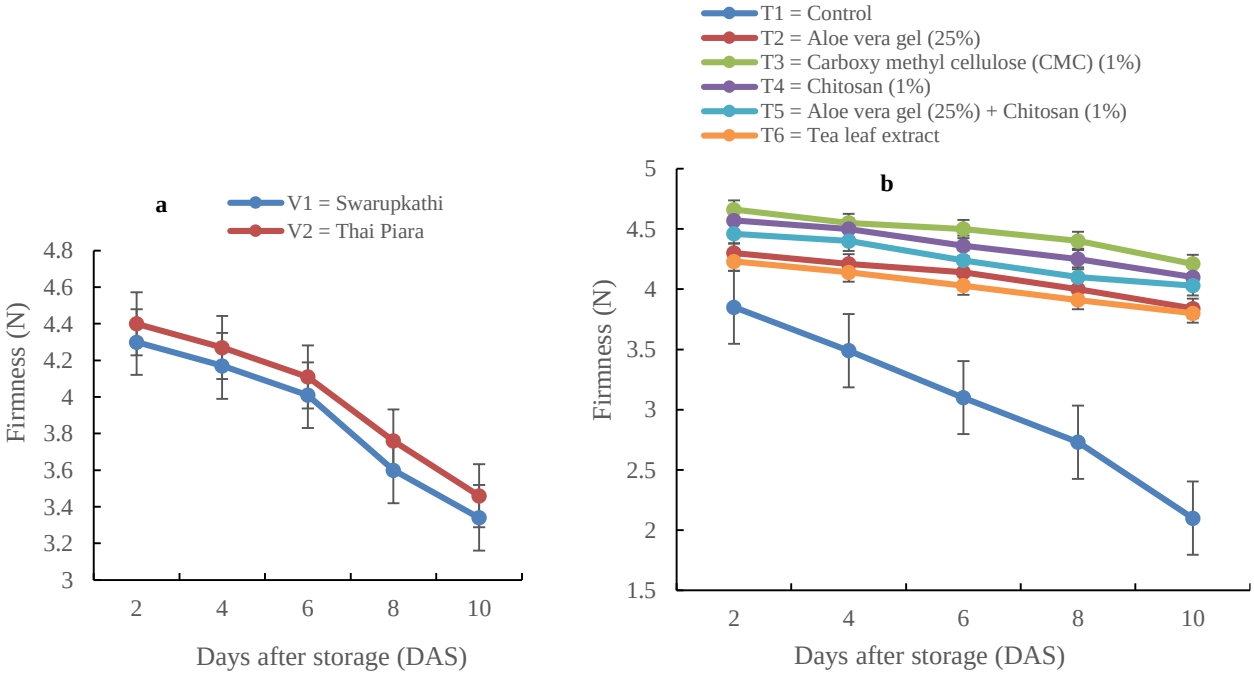

Fig. 2. Effect of variety (a) and treatments (b) on firmness of guava at different days after storage. Vertical bars represent standard error.

During ripening of fruit, softening appears mainly due to middle lamella and cell wall degradation, that mostly occurs in the last stages of the ripening process (He et al., 2019). At the time of ripening of fruit, depolymerization of pectin substances occurs and at the same time increase activities of softening related enzymes such as pectin-esterase and polygalacturonase (Desai & Park, 2006).

Edible coatings restrict the loss of moisture from the fruit to the external environment and to lessen the absorption of the oxygen by the fruit. Its preserve the texture of the fruit by reducing the respiration rate and providing physical protection to the food product. Less availability of oxygen to the coated fruit may be responsible for reduction in the activities of these enzymes and hence retention of the firmness of fruits during storage (Salunkhe, Bolin, & Reddy, 1991).

There was a significant difference between the two varieties in terms of total weight loss. However, it was shown that in both varieties, the rate of weight loss increased as the storage duration lengthened. The weight loss was greater in Swarupkathi (8.12%) compared to Thai Piara (7.63%) at 10th days after storage (Fig. 3a). The current study demonstrated that postharvest treatments greatly showed significant effects in respect of weight loss. The total weight loss was found to be the highest (6.99, 7.69, 8.17, 9.07 and 9.89%) in case of uncoated fruits where the fruit treated with chitosan 1% represented the lowest weight loss (4.32, 4.75, 5.41, 5.98 and 6.32%) at 2, 4, 6, 8 and 10 days after storage, respectively (Fig. 3b). Combined effect of treatments and fruit varieties showed non-significant variation in 2 and 4 DAS. But in case of 6, 8 and 10 DAS, significant variation was observed on overall weight loss.

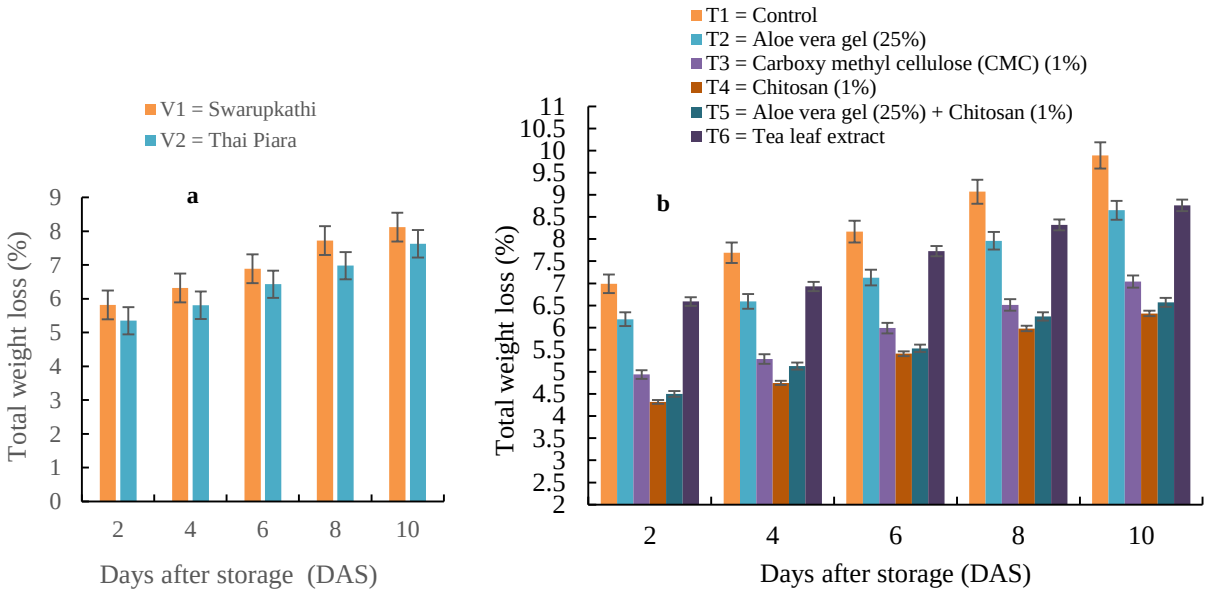

Fig. 3. Effect of variety (a) and treatments (b) on total weight loss of guava at different days after storage. Vertical bars represent standard error**.**

The variety, Swarupkathi under control treatment gave the highest weight loss (6.79, 7.24, 7.80, 8.03 and 8.32% at 2, 4, 6, 8 and 10 DAS, respectively) whereas the lowest weight loss (4.21. 4.63, 5.47, 5.93 and 6.28 % at 2, 4, 6, 8 and 10 DAS, respectively) was found in Thai Piara treated with Chitosan (1%) (Table 2). The present result was similar to the findings of Krishna and Rao (2014), they found that the total weight loss increased gradually in all the treatments with advancement of storage period. Islam et al. (2018) also found that the application of 2% chitosan showed the lowest weight loss 6.58% in banana compared to the control samples. Edible coating closed the opening of stomata and lenticels thereby, reducing

the rate of transpiration and respiration, which increases retention of moisture in the fruit. During storage period, weight loss increased with the advancement of storage which might be due to increase in respiration rate of fruits (Azam et al., 2020).

pH

Significant variation between two varieties was seen in cases of fruits pulp pH during total storage period except 6 DAS. However, from 2 DAS to 10 DAS, an increasing pH trend was seen. At 2 DAS, the highest pH was 3.78 which increased to 6.13 at 10 DAS in Swarupkathi while in Thai Piara, at 2 DAS and 10 DAS, the pH was 3.52 and 5.70, respectively. At 4, 6, 8 and 10 DAS, the higher pH (4.29, 4.63, 5.58 and 6.13 respectively) was recorded in Swarupkathi whereas the lower pH was recorded (3.96, 4.60, 5.58, and 5.70) respectively in Thai Piara (Fig. 4a).

Significant difference among the postharvest guava treatments in respect pH of fruit pulp was recorded during storage. Only the control treatment showed a higher rate of pH increment, while other treatments, particularly chitosan (1%), showed a slower rate of pH increment. At 2 DAS, the highest pH (4.02) was found in control treatment and the lowest pH (3.38) was marked in the fruits in chitosan (1%) treatment followed by 3.45 in aloe vera gel (25%) + chitosan (1%), 3.60 in carboxy methyl cellulose (Fig. 4b).

The combined effect of varieties and edible coating on pH was significant at 2 and 10 DAS but non-significant at 4, 6, and 8 DAS (Table 3). At 2 DAS, Uncoated Thai piara exhibited the greatest pH (4.23) and Swarupkathi piara coated with chitosan (1%) showed the lowest pH (3.34). For all treatment combinations, an increasing trend in pH was observed as storage duration increased. At 4, 6, 8 and 10 DAS, the maximum pH (4.56, 5.10, 6.27, and 6.74, respectively) was observed in uncoated Thai piara, whereas the minimum pH (4.09, 4.77, 5.10, and 5.30, respectively) was recorded in Swarupkathi piara treated with chitosan (1%). Similar results were also observed by Azam et al. (2020). They found that pH values increased slightly during storage periods. With the progression of the storage period, pH was found to be higher in untreated fruits and lower in acetic acid treated fruits (He et al., 2018).

Table 1. Effect of variety and postharvest treatments on firmness of guava during storage.

	Firmness at different DAS				
Variety × Treatments	2	4	6	8	10
V_1T_1	3.81	3.43	3.06	2.61e	2.01 e
V_1T_2	4.25	4.16	4.08	3.95 de	3.81 cd
V_1T_3	4.65	4.50	4.45	4.35 ab	4.15 a-c
V_1T_4	4.51	4.46	4.30	4.18 b-d	4.00 a-d
V_1T_5	4.40	4.38	4.20	4.05 c-e	3.91 b-d
V_1T_6	4.20	4.10	4.00	3.90 e	3.75 d
V_2T_1	3.90	3.55	3.15	2.85 f	2.20 e
V_2T_2	4.36	4.26	4.20	4.05 c-e	3.86 b-d
V_2T_3	4.68	4.61	4.56	4.46 a	4.28 a
V_2T_4	4.63	4.55	4.43	4.33 ab	4.2 ab
V_2T_5	4.53	4.41	4.28	4.21 a-c	4.15 a-c
V_2T_6	4.26	4.18	4.06	3.93 de	3.85 cd
Level of significance	NS	NS	NS	*	*
LSD at 1%	0.22	0.2	0.19	0.21	0.27
CV (%)	2.2	3.08	2.13	2.28	3.19

In a column, means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly as per 0.01 level of probability. * & ** Significant at 5 & 1% level of provability, NS = Non-significant, CV = Coefficient of variation, DAS = Days after storage, V_1: Swarupkathi, V_2: Thai Piara, T_1: Control, T_2: Aloe vera gel (25%), T_3: Carboxy methyl cellulose (CMC) (1%), T_4: Chitosan (1%), T_5: Aloe vera gel (25%) + Chitosan (1%), T_6: Tea leaf extract.

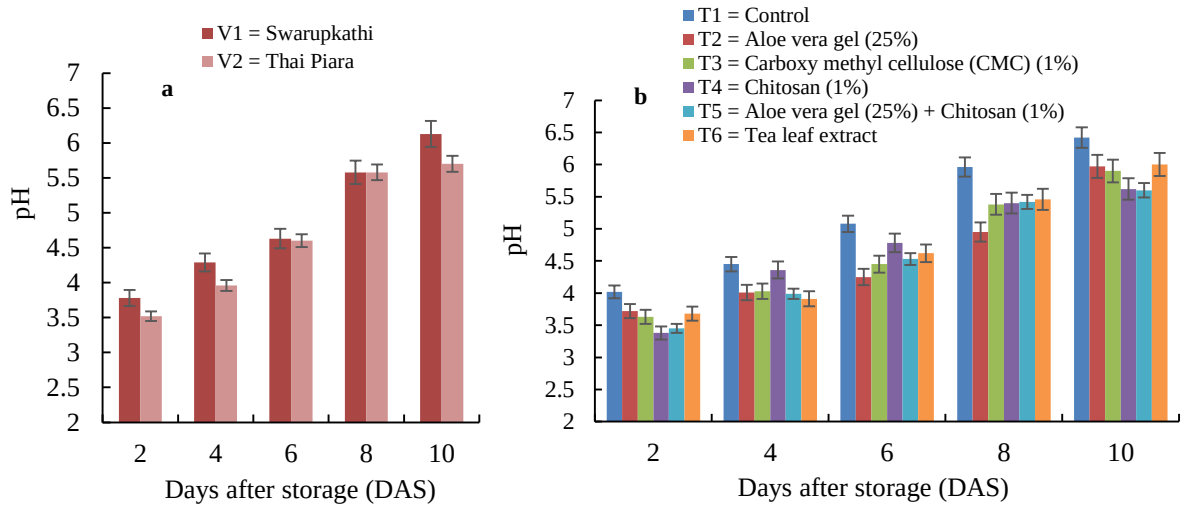

Fig. 4. Effect of variety (a) and treatments (b) on pH of guava at different days after storage. Vertical bars represent standard error.

Table 2. Combined effect of variety and postharvest treatments on total weight loss of guava during storage.

Variety × Treatments	Total weight loss (%) at different DAS				
	2	4	6	8	10
V_1T_1	7.18	8.13	8.55 a	9.48 a	10.16 a
V_1T_2	6.65	6.91	7.53 b	8.69 b	9.02 c
V_1T_3	5.03	5.29	6.25 cd	6.73 e	7.07 e
V_1T_4	4.42	4.87	5.35 e	6.03 fg	6.37 fg
V_1T_5	4.72	5.47	5.78 de	6.74 e	6.88 ef
V_1T_6	6.91	7.25	7.86 b	8.62 b	9.20 bc
V_2T_1	6.79	7.24	7.80 b	8.67 b	9.26 b
V_2T_2	5.74	6.28	6.72 c	7.23 d	8.27 d
V_2T_3	4.84	5.28	5.73 de	6.29 f	7.01 e
V_2T_4	4.21	4.63	5.47 e	5.93 g	6.28 g
V_2T_5	4.28	4.81	5.29 e	5.76 g	6.27 g
V_2T_6	6.26	6.61	7.60 b	8.03 c	8.32 d
Level of significance	NS	NS	*	**	*
LSD at 1%	0.7	0.56	0.57	0.29	0.51
CV (%)	5.46	4.06	3.73	1.7	2.82

In a column, means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly as per 0.01 level of probability.

Table 3. Combined effect of variety and postharvest treatments on pH of guava during storage.

Variety × Treatment	pH at different DAS				
	2	4	6	8	10
V_1T_1	3.82 bc	4.34	5.10	6.27	6.1 bc
V_1T_2	3.46 e	3.77	4.12	4.74	5.61 d-f
V_1T_3	3.40 e	3.84	4.52	5.20	5.76 de
V_1T_4	3.34 e	4.09	4.77	5.10	5.30 f
V_1T_5	3.43 e	3.93	4.43	5.07	5.48 ef
V_1T_6	3.67 cd	3.78	4.67	5.25	5.97 c
V_2T_1	4.23 a	4.56	5.06	5.66	6.74 a
V_2T_2	3.98 b	4.25	4.40	5.16	6.34 b
V_2T_3	3.86 bc	4.23	4.37	5.57	6.04 bc
V_2T_4	3.43 e	4.63	4.78	5.68	5.77 c-e
V_2T_5	3.48 de	4.06	4.64	5.74	5.91 cd
V_2T_6	3.69 c	4.03	4.57	5.68	6.04 bc
Level of significance	**	NS	NS	NS	**
LSD at 1%	0.15	0.48	0.52	0.83	0.28
CV (%)	1.86	5.14	4.86	6.63	2.02

In a column, means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly as per 0.01 level of probability.

Titratable acidity (TA)

In respect of titratable acidity, there was a non-significant variation between two varieties was seen in case of titratable acidity. However, decreasing trend in titratable acidity was found from 2 DAS to 10 DAS. At 2 DAS, the highest titratable acidity was 1.99% which decreased to 1.43% at 10 DAS (DAS) in Swarupkathi while in Thai Piara, at 2 DAS and 10 DAS, the titratable acidity were 1.92% and 1.42% respectively which was lowest compared to Swarupkathi (Fig. 5a). At 4, 6, and 8 DAS, the highest titratable acidity (1.90, 1.68 and 1.50%, respectively) was recorded in Swarupkathi whereas the lowest titratable acidity (1.81, 1.58 and 1.50 %, respectively) was measured in Thai Piara.

The results on titratable acidity of guava showed that there was a highly significant variation among the postharvest treatments in relation to storage duration (Fig. 5b). Higher rate of decreasing trend in titratable acidity was recorded only on control treatment while slow decreased rate on titratable acidity was recorded for other treatments especially in case of chitosan (1%). At 2 DAS, the highest titratable acidity 2.49% was found in chitosan (1%) treatment followed by 2.23 in carboxy methyl cellulose (1%) and the lowest 1.4% in the fruits under control treatment (Fig. 5b). At 4, 6, 8 and 10 DAS, the maximum titratable acidity (2.38, 2.20, 2.04 and 1.98%, respectively) was recorded in chitosan (1%) treatment and the minimum (1.34, 1.08, 1.02 and 0.91%, respectively) was marked in control treatment (Fig. 5b).

The combined effect of varieties and treatments on titratable acidity was significant during storage (Table 4). At 2 DAS, the highest titratable acidity 2.56% was recorded in Thai piara treated with chitosan (1%) followed by 2.43 and 2.31% in Swarupkathi piara treated with chitosan (1%) and carboxy methyl cellulose (1%) respectively whereas the lowest titratable acidity 1.53% was recorded in untreated Swarupkathi piara. Decreasing trend of titratable acidity was recorded for increasing of storage duration for all the treatment combinations. At 4, 6, 8 and 10 DAS, the maximum titratable acidity (2.30, 2.24, 2.17 and 2.04%, respectively) was also recorded in Chitosan (1%) treated Thai piara whereas the minimum titratable acidity (1.27, 1.02, 1.02 and 0.77 %, respectively) was found in untreated Swarupkathi piara (Table 4). That is similar to the findings of (Silva et al., 2018), they demonstrated that in the treatment with 2% and 3% of chitosan in the solid soluble content and ascorbic acid were reduced; retarded the loss of titratable acidity during 96 h after treatment. The maximum utilization of acid in the metabolism of organic acid during respiratory process might be the reason for minimum acidity in control and in advancement of storage period (Silva et al., 2018).

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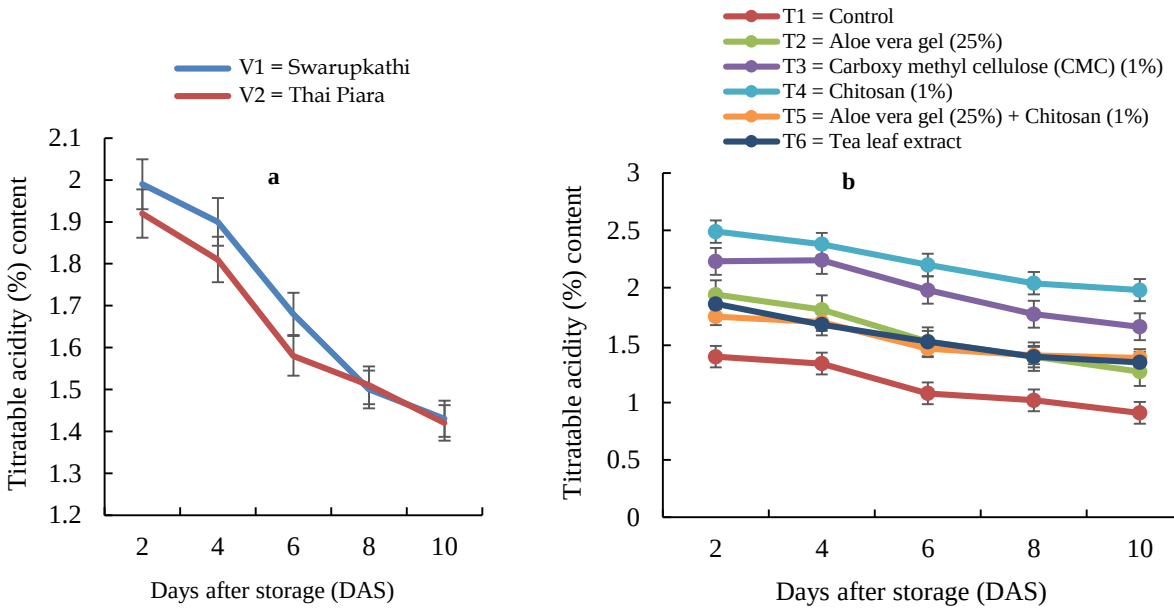

Fig. 5. Effect of variety (a) and treatments (b) on titratable acidity of guava at different days after storage. Vertical bars represent standard error.

Table 4. Combined effect of variety and postharvest treatments on titratable acidity of guava during storage.

Variety× Treatments	Titratable acidity (%) at different DAS				
	2	4	6	8	10
V_1T_1	1.40 e	1.27 g	1.02 h	1.02 g	0.77 g
V_1T_2	2.26 a-c	2.04 c	1.79 b-e	1.66 b-d	1.4 cd
V_1T_3	2.31 a-d	2.21 b	2.04 a-c	1.74 b-d	1.79 b
V_1T_4	2.43 ab	2.46 a	2.17 ab	1.91 ab	1.92 ab
V_1T_5	1.58 de	1.53 ef	1.40 e-h	1.27 e-g	1.29 de
V_1T_6	1.91 a-e	1.79 d	1.66 c-f	1.40 d-f	1.41 cd
V_2T_1	1.53 e	1.40 fg	1.15 gh	1.02 g	1.05 f
V_2T_2	1.62 c-e	1.57 e	1.27 f-h	1.15 fg	1.15 ef
V_2T_3	2.25 a-c	2.17 bc	1.91 a-d	1.79 bc	1.53 c
V_2T_4	2.56 a	2.3 b	2.24 a	2.17 a	2.04 a
V_2T_5	1.92 a-e	1.87 d	1.53 d-g	1.53 c-e	1.49 cd
V_2T_6	1.79 b-e	1.57 e	1.40 e-h	1.40 d-f	1.28 def
Level of significance	*	*	**	**	**
LSD at 1%	0.52	0.12	0.32	0.29	0.18
CV (%)	11.47	2.89	8.40	8.37	5.50

In a column, means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly as per 0.01 & 0.05 level of probability.

Total soluble solids (TSS)

The different varieties used in the investigation showed statistically significant variation in case of total soluble solid content of guava at 2, 4, and 10 DAS but non-significant variation at 6 and 8 DAS. At 2, 4, 6, 8 and 10 DAS, Thai Piara had higher TSS content (5.32, 5.92, 6.73, 7.79 and 8.66%) and the variety Swarupkathi had lower TSS content (4.93, 5.86, 6.70 7.70 8.55 % at 2, 4, 6, 8 and 10 DAS, respectively) (Fig. 6a).

The different treatments used in the investigation showed statistically significant variation in relation to TSS during storage period. Control treatment showed the highest TSS content 6.36, 6.93, 7.76, 9.05 and 9.76% at 2, 4, 6, 8 and 10 DAS, respectively. But under the treated terms, aloe vera gel (25%) showed the highest TSS content followed by tea leaf extract where the lowest TSS content was achieved by chitosan (1%) treatment (3.60, 4.37, 5.05, 5.73 and

6.60%) at 2, 4, 6, 8 and 10 DAS, respectively followed by aloe vera gel (25%) + chitosan (1%) (Fig. 6b).

It was found that the combined effects of variety and postharvest treatments were statistically significant during entire storage period (Table 5). It was found that untreated Thai piara (6.80, 7.23, 7.87, 9.10 and 9.81%) showed the highest TSS content compared to Swarupkathi piara (6.60, 6.90, 7.80, 8.87 and 9.73%) and at 2, 4, 6, 8 and 10 DAS, respectively. Results also revealed that the lowest TSS content was noted from chitosan (1%) treated Swarupkathi (3.50, 4.30, 4.96, 5.53 and 6.43%) followed by Thai piara treated with same treatment (3.70, 4.41, 5.13, 5.93 and 6.77%) at 2, 4, 6, 8 and 10 DAS, respectively. (Table 5). This observation is somewhat similar to Kumar et al. (2017). Kumar et al. (2020), reported that that fruits treated with chitosan (0.25% and 0.50%) were better in maintaining all physico-chemical characteristics (pH-4.60, TSS-9.40, Acidity-0.34, Ascorbic acid-208, Weight loss-14.87 and Moisture-73.37) than control throughout the storage period. The highest TSS in aloe vera gel coated fruits might be due to more concentration of juice resulting higher content of sugars, while minimum acidity may be due to more utilization of acids in biochemical activities leading to depletion of organic acids. The increasing trend of percent total soluble solids contents of fruit during storage could be attributed mainly to the breakdown of starch into simple sugars Mondal et al. (2023).

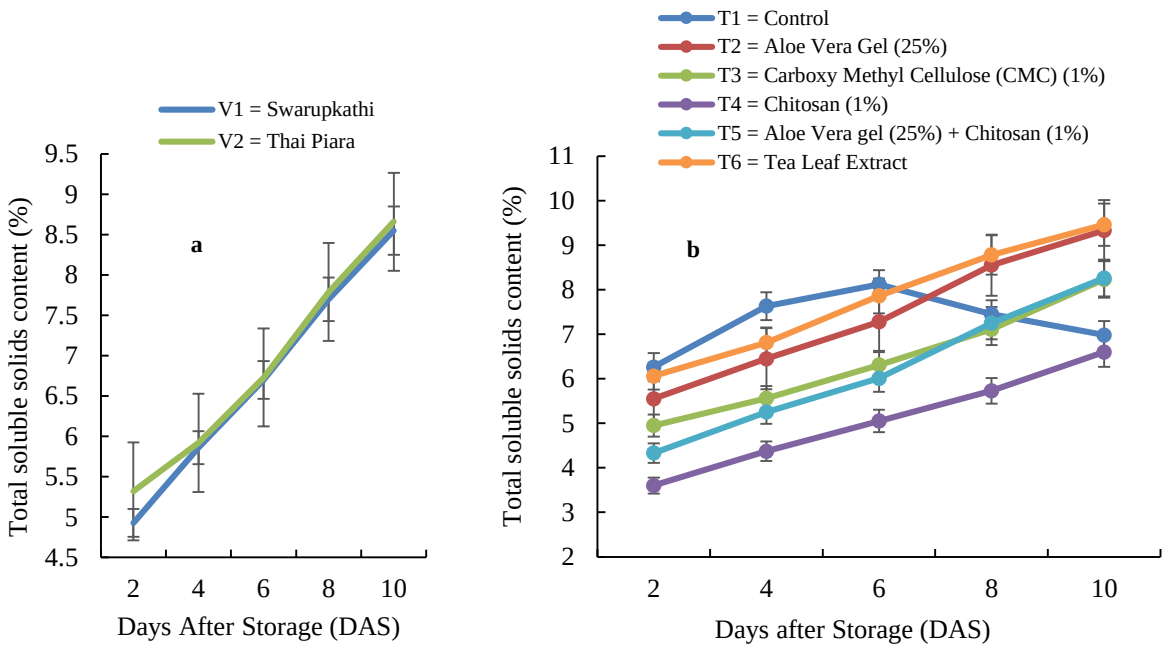

Fig. 6. Effect of variety (a) and treatments (b) on TSS of guava at different days after storage. Vertical bars represent standard error.

Table 5. Combined effect of variety and postharvest treatments on TSS of guava during storage.

	TSS (° Brix) at different DAS				
Variety × Treatments	2	4	6	8	10
V_1T_1	6.60 a	6.9 ab	7.80 a	6.62 de	6.43 e
V_1T_2	5.40 cd	6.36 c	7.06 b	8.60 c	9.26 b
V_1T_3	4.60 e	5.30 e	6.20 cd	7.13 d	8.16 c
V_1T_4	3.50 g	4.30 f	4.96 e	5.53 f	6.43 e
V_1T_5	4.20 f	5.33 e	6.13 cd	7.26 d	8.2 c
V_1T_6	5.70 b	6.53 bc	7.50 ab	8.51 c	9.40 b
V_2T_1	6.80 a	7.23 a	7.87 a	6.69 de	6.51 de
V_2T_2	5.70 b	6.73 bc	7.86 a	9.00 ab	9.53 ab
V_2T_3	5.23 d	5.83 d	6.43 c	7.10 d	8.31 c
V_2T_4	3.70 g	4.41 f	5.13 e	5.93 e	6.77 d
V_2T_5	4.46 ef	5.16 e	5.91 d	7.23 d	8.33 c
V T_6	5.53 bc	6.63 bc	7.73 a	8.70 bc	9.41 b
Level of significance	**	**	**	*	*
LSD at 1%	0.21	0.38	0.39	0.29	0.26
CV (%)	1.81	2.84	2.54	1.65	1.29

In a column, means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly as per 0.01 & 0.05 level of probability.

Total Sugar

There was a significant difference between the two varieties in terms of total sugar content. The highest total sugar content (8.26%) was recorded in Thai Piara, whereas it was the lowest (7.85%) in Swarupkathi at 10 DAS. However, increasing trend in percent total sugar content was found from 2 DAS to 10 DAS (Fig. 7a).

The results on percent total sugar content showed that there was a highly significant variation among the postharvest treatments of guava pulp in relation to storage duration. Higher rate of increasing trend in percent total sugar content was recorded only on control treatment while lower increased rate on percent total sugar content was recorded for other treatments especially in case of T_5 (25% aloe-vera gel + 1% Chitosan) (Fig. 7b).

The combined effect of varieties and treatments on percent total sugar content was highly significant during entire storage period. At 10 DAS, the maximum percent total sugar content (9.79%) was recorded in untreated Thai piara whereas the minimum percent total sugar content (5.78%) was found in Swarupkathi piara treated with aloe vera gel (25%) + chitosan (1%) (Table 6).

Under the present study total sugar content increased during storage period which is similar to the observation of (Augustin & Osman, 1988) and he reported that storing guava at ambient temperature showed significant increase in total sugar content. Total sugars of fruits are considered one of the basic criteria to evaluate the fruit ripening. From our results it was observed that total sugars were very low at initial stage but it was gradually increased with advancement of storage period. The increase in sugars during storage period might be due to rapid conservation of polysaccharides into sugars. The result is also similar to the findings of Bose et al. (2019).

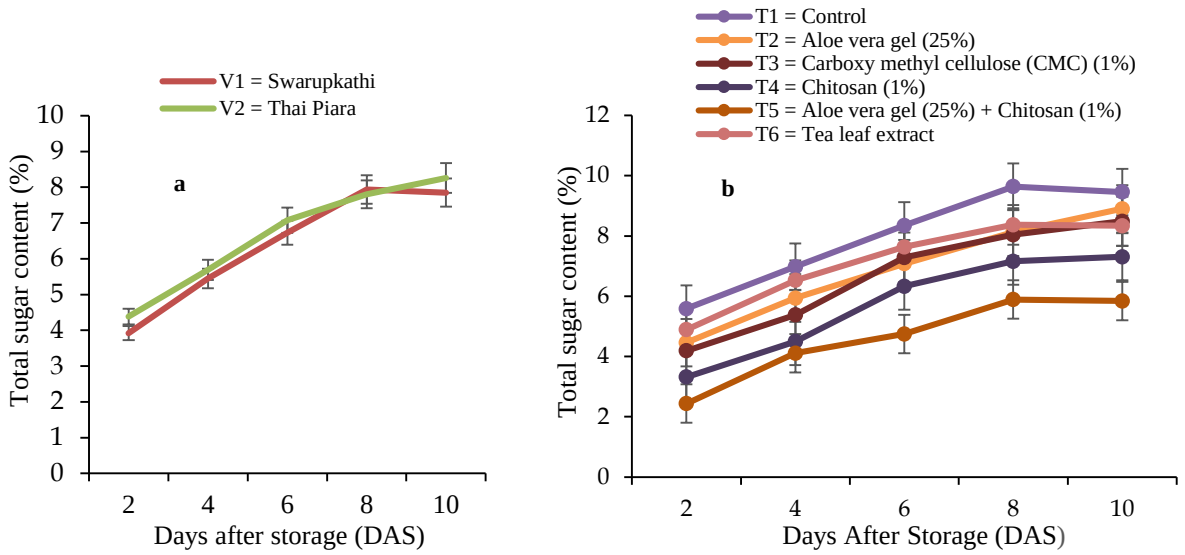

Fig. 7. Effect of variety (a) and treatments (b) on total sugar content of guava at different days after storage. Vertical bars represent standard error.

Table 6. Combined effect of variety and postharvest treatments on total sugar content of guava during storage.

Variety × Treatments	Total sugar content (%) at different DAS				
	2	4	6	8	10
V_1T_1	5.62 a	6.79 b	8.17 b	9.94 a	9.16 b
V_1T_2	4.01 c	5.68 d	6.27 f	7.91 e	9.07 bc
V_1T_3	3.97 c	5.32 e	7.72 cd	8.07 de	8.3 e3
V_1T_4	2.84 d	4.26 g	5.92 g	7.08 f	7 g
V_1T_5	2.34 d	3.77 h	4.49 i	5.96 g	5.78 h
V_1T_6	5.09 b	6.98 ab	7.82 c	8.7 c	7.84 f
V_2T_1	5.67 a	7.19 a	8.52 a	9.28 b	9.79 a
V_2T_2	4.88 ab	6.28 c	7.82 bc	8.3 d	8.83 cd
V_2T_3	4.41 bc	5.42 de	6.86 e	8.06 de	8.64 d
V_2T_4	3.81 c	4.73 f	6.74 e	7.24 f	7.61 f
V_2T_5	2.65 d	4.47 fg	5.02 h	5.81 g	5.9 h
V_2T_6	5.05 ab	6.11 c	7.47 d	7.99 e	8.44 cd
Level of significance	**	**	**	**	**
LSD at 1%	0.51	0.24	0.26	0.25	0.23
CV (%)	5.34	1.88	1.47	1.34	1.22

In a column, means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly as per 0.01 level of probability.

Vitamin C content

Vitamin C content of guava pulp was significantly influenced between two varieties of guava during storage period. The higher vitamin C content (199.32, 194.00, 187.59, 181.58 and 177.19 mg/100g) was found in Thai Piara and the lower vitamin C content (194.42, 191.49, 184.87, 178.24 and 171.79 mg/100g) was observed in Swarupkathi at 2, 4, 6, 8 and 10 DAS, respectively (Fig. 8a).

Effects of different postharvest treatments in respect of vitamin C content were statistically significant at different days of storage. There was a decreasing trend in relation to vitamin C content of fruit pulp during storage. The higher vitamin C content (191.18 mg/100g) was recorded in chitosan (1%) treatment followed by treatment aloe vera gel (25%) + chitosan (1%) and lower vitamin C content (159.64 mg/100g) was found in control followed by aloe vera gel (25%) at 10 DAS (Fig. 8b).

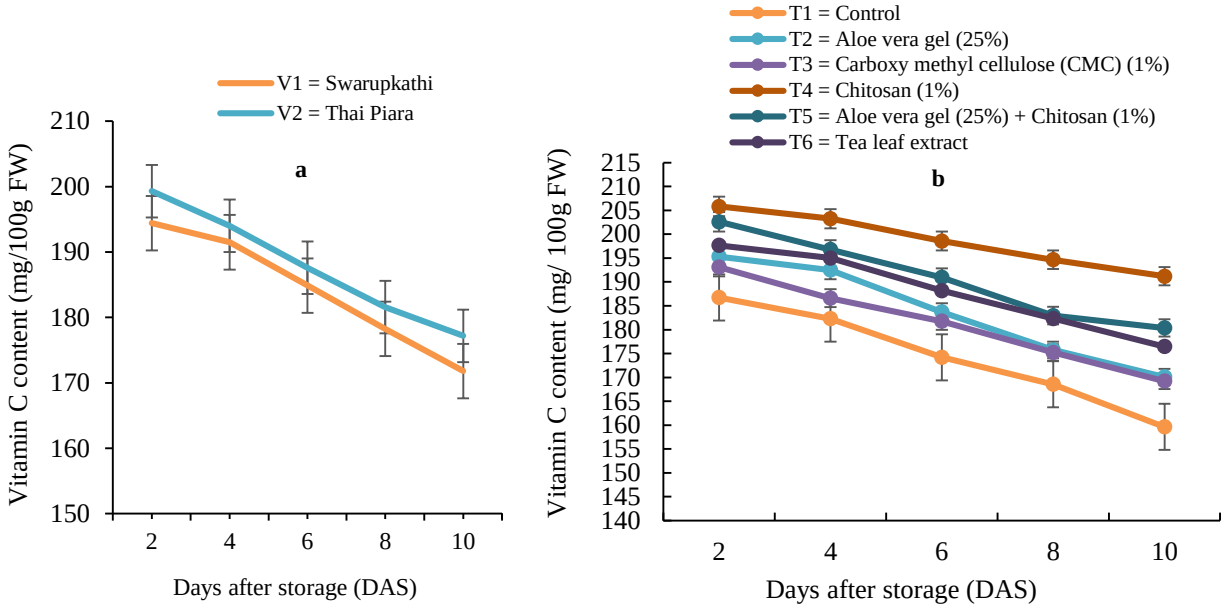

Fig. 8. Effect of variety (a) and treatments (b) on vitamin C content of guava at different days after storage. Vertical bars represent standard error.

Table 7. Combined effect of variety and postharvest treatments on vitamin C content of guava during storage.

	Vitamin C content (mg/100g FW) at different DAS				
Variety × Treatments	2	4	6	8	10
V_1T_1	185.21 j	180.97 k	172.60 k	168.99 de	157.3 h
V_1T_2	193.27 g	190.45 g	182.65 h	175.81 c	168.59 ef
V_1T_3	191.60 h	187.64 h	185.26 fg	174.17 cd	167.55 f
V_1T_4	201.52 c	200.94 b	196.78 b	194.08 a	190.92 ab
V_1T_5	198.41 de	195.36 de	186.29 e	177.67 c	173.43 d
V_1T_6	196.52 f	193.58 f	185.62 ef	178.67 c	172.41 de
V_2T_1	188.23 i	183.63 j	175.83 j	168.13 e	161.98 g
V_2T_2	197.38 ef	194.55 ef	184.77 g	175.67 c	171. 58 d-f
V_2T_3	194.58 g	185.56 i	178.26 i	176.25 c	170.90 d-f
V_2T_4	210.12 a	205.58 a	200.38 a	195.17 a	191.44 a
V_2T_5	206.79 b	198.20 c	195.64 c	188.22 b	187.26 b
V_2T_6	198.84 d	196.46 d	190.68 d	186.02 b	180.50 c
Level of significance	**	**	**	**	**
LSD at 1%	1.04	0.95	0.6	4.2	3.19
CV (%)	0.23	0.21	0.14	1.01	0.8

In a column, means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly as per 0.01 level of probability.

Combined effects of variety and postharvest treatments on vitamin C content were significant during storage period. The higher vitamin C content (210.12, 205.58, 200.38, 195.17 and 190.92 mg/100g) was found in chitosan (1%) treated Thai piara followed by Swarupkathi piara treated with same treatment at 2, 4, 6, 8 and 10 DAS, respectively. The lower vitamin C content (185.21, 180.97, 172.60, 168.99 and 157.3 mg/100g) was observed in untreated Swarupkathi piara followed by untreated Thai piara at 2, 4, 6, 8 and 10 DAS, respectively (Table 7).

The result is also similar to the findings of Silva et al. (2018). They demonstrated that in the treatment with 2% and 3% of chitosan in the solid soluble content and ascorbic acid were reduced; retarded the loss of titratable acidity during 96 h after treatment. Vitamin C is one of the powerful antioxidant and scavenger of the reactive oxygen species (ROS) produced in the body thus helps to save the human from many serious diseases (Patel, Naik, & Arbat, 2011). The fruits coated with 1% chitosan maintained the higher levels of vitamin C compared to other coated materials. It might possibly be due to retardation of oxidation process and consequently slow rate of conversion of L-ascorbic acid into dehydroascorbic acid by ascorbic acid oxidase. Similar observation have also been recorded in mango (Jain & Mukherjee, 2011) and mandarin orange (Yadav, Kumar, Singh, & Singh, 2010).

Shelf life

In the current investigation, a highly significant difference in shelf life between the two guava varieties was found. Thai Piara had longer shelf life (9.94 days) than Swarupkathi's (7.94 days) (Fig. 9a).

The shelf life of guava was significantly varied by postharvest treatments (Fig. 9b). The study's findings showed that guava fruits had a shelf life of between 6 and 11.67 days. The longest shelf life (11.67 days) was found in aloe vera gel (25%) + chitosan (1%) followed by (11.6 days) in chitosan (1%), whereas the shortest shelf life (6.00 days) was recorded in Control (Fig. 9b).

The combined effects of variety and postharvest treatments were significant in extending shelf life (Table 8). The longest shelf life (13.00 days) was observed in Thai piara treated with aloe vera gel 25% + chitosan 1% followed by chitosan (1%) (12.67 days). On the other hand, the shortest shelf life (5.00 days) was observed in untreated Swarupkathi piara followed by untreated Thai piara (6.67 days), (Table 8). Fruits degrade to the simpler inorganic compound (CO_2, HO_2, and NH_3), decreased in free energy and increase in respiration, consequently reduce the shelf life as well as other qualities of fruits (Mondal et al., 2023).

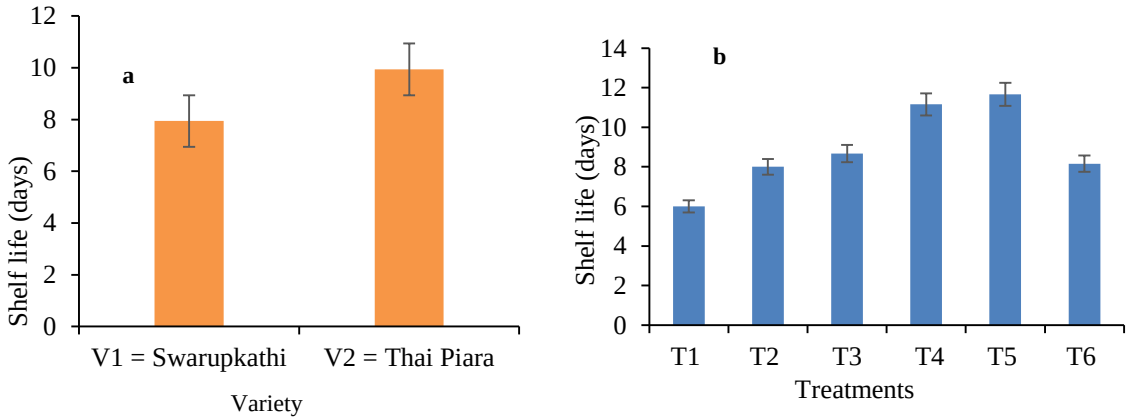

Fig. 9. Effect of variety (a) and treatments (b) on shelf life of guava at different days after storage. Vertical bars represent standard error.

Table 8. Combined effect of variety and postharvest treatments on shelf life of two variety of guava.

Variety × Treatments	Shelf life (days)
V_1T_1	5.33 g
V_1T_2	7.33 def
V_1T_3	7.67 e
V_1T_4	10.33 b
V_1T_5	9.67 bc
V_1T_6	7.00 ef
V_2T_1	6.67 f
V_2T_2	8.67 d
V_2T_3	9.67 bc
V_2T_4	12.67 a
V_2T_5	13.00 a
V_2T_6	9.00 cd
Level of significance	*
LSD at 1%	1.19
CV (%)	5.81

Means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly as per 0.05 level of probability.

CONCLUSION

It can be concluded that between the two tested variety, Thai Piara performed better than Swarupkathi. Thai Piara with edible coating aloe vera gel 25% + chitosan 1% exhibited longest shelf life (13 days) compare to control with Swarupkathi. chitosan 1% with Thai Piara also showed promising results. Finally, this study recommends that guava treated with chitosan 1%, followed by aloe vera gel 25% + chitosan 1% solution as edible coating is promising for long term storage and maintaining overall quality of guava fruits.

This study recommends chitosan as the best edible coating material that is very effective in improving the overall quality of mango fruits.

Conflict of interest

Author declared no conflict of interests.

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The effect of some edible coating treatments on shelf life of pomegranate arils cultivar "Malas-e Saveh"

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ABSTRACT

Purpose: The purpose of this research was to evaluate various coatings for preserving the quality attributes of "Malas-e Saveh" pomegranate arils during storage. **Research method**: A bi-factorial experiment in frame of completely randomized design was conducted to compare eight coating treatments at two storage times (two and four weeks) with three replications. **Findings**: The highest pH and acidity was observed in the ascorbic acid treatment after four and two weeks of storage, respectively. The control after two weeks had the highest TSS and the nanosilicate container after four weeks had the highest taste index. The zero-day control had the highest vitamin C, while *Aloe vera* gel + chitosan and nanosilicate container after four weeks had the lowest. The zero-day control had the lowest total phenols but the highest total flavonoids and anthocyanins. *Aloe vera* gel, ascorbic acid and nanosilicate container after two weeks had the highest antioxidant activity, which first increased and then decreased with storage. The coatings did not significantly affect maintaining the L index or brightness of pomegranate arils. However, *Aloe vera* gel better maintained the (redness) and b (yellowness) indices. *Aloe vera* gel + ascorbic acid best preserved the sensory values closest to the zero-day control. **Research limitations**: None were found to report. **Originality/Value**: After comparing conventional coating materials with emerging options, this study revealed that *Aloe vera* gel alone or in combination with other coating materials was effective in preserving the quality of pomegranate arils during storage.

INTRODUCTION

Marketing is the final stage of the production cycle for horticultural products, including pomegranates. Improved storage and distribution techniques are needed to optimize pomegranate fruit marketing in Iran. Developing pomegranate packaging, especially for pomegranate arils, is crucial given growing global demand due to reported health benefits (Singh, 2010). Efficient aril separation techniques could generate interest by providing ready-to-eat fresh arils. Recent attention has focused on isolating and selling arils (Oz & Ulukanli, 2012). Accordingly, aril separation devices have been designed and utilized. However, extending the shelf life of arils via postharvest methods remains necessary to maintain their quality during storage and transportation.

The pomegranate (*Punica granatum* L.) is an ancient and popular fruit characterized by rounded, shiny red or yellow-green fruit that turns purple when ripe. Arils, which are separated by thin membranes within the fruit (Nikdel et al., 2016), contain juicy flesh that can be red, white, or pink depending on the cultivar. Various plant biochemicals have been extracted from pomegranate parts (peel, arils, spongy tissue, and seeds). Around 52% of the weight of a pomegranate is made up of arils, which contain 80% juice and 20% seed. The arils are composed mostly of water (85%) and contain sugars, carbohydrates, organic acids, anthocyanins, vitamins, polysaccharides, polyphenols, antioxidative phytochemicals, antimicrobial compounds, and essential minerals (Yang et al., 2022). Since the tough outer layer of the pomegranate cannot be eaten and is typically discarded, removing the fruit's arils and separating them becomes a laborious and difficult process. This can be a deterrent for people who want to consume or choose this fruit (Akhtar et al., 2015).

When pomegranate arils were packaged in a modified atmosphere, they only had a shelf life of 10 days, and the quality of their flavor and aroma could only be maintained for 7 of those days (Caleb et al., 2013). Therefore, it is necessary to find new solutions that can help decrease the number of microorganisms on pomegranate arils and slow down the deterioration of their quality. Coating treatments can be applied to fresh pomegranate arils to extend their shelf life and maintain their quality. This coating also helps to prevent moisture loss and protect the arils from damage during transport (Kawhena et al., 2020). Using biodegradable edible coatings to maintain the quality of minimally processed products after harvest is becoming an increasingly popular option. This may be because these coatings are made from natural sources and are therefore biodegradable. Additionally, consumers now expect safer and healthier food products that have excellent sensory qualities (Ncama et al., 2018).

A range of biopolymers obtained from both plants and animals are utilized to create coating materials that can be applied to food products (Singla et al., 2022). After cellulose, chitin is the second most abundant biopolymer found in nature. A study was conducted using a combination of chitosan and ascorbic acid as an edible coating to determine its effect on the shelf life of pomegranate arils. The study revealed that this coating helped to preserve the visual quality of the arils during storage and prevented bacterial and fungal growth on them (Özdemir & Gökmen, 2017). *Aloe vera* gel is rich in vitamins, minerals, and antioxidants, which can be used as a natural food preservative and coating material due to its ability to prevent the growth of microorganisms and extend the shelf life of food products. Application of coatings such as *Aloe vera* gel on pomegranate arils resulted in lower respiration rate (Martínez-Romero et al., 2013). Honey has natural antimicrobial properties and has been used as a food preservative for centuries. As far as we know, there is little available literature regarding the use of honey to maintain pomegranate arils. Polymer nanotechnology involves the manipulation of materials at the nanoscale level to create new materials with improved properties. In the food industry, this technology can be used to develop new packaging

materials that offer better barrier properties, are more resistant to punctures and tears, and can extend the shelf life of food products (Silvestre et al., 2011).

Ready-to-eat fruits are uncommon in Iran. Expanding this sector, at least for export, could increase product value. Despite promising Iranian pomegranate cultivars, insufficient information exists on coating treatments' effects on quality attributes and shelf life of "Malas-e Saveh" pomegranate arils, an important Iranian export cultivar. While arils' nutritional importance is recognized, limited research has evaluated maintaining aril quality during storage. Given concerns over chemical overuse in postharvest technology and consumer demand for healthy products, research on postharvest edible coatings is warranted. This research presents a comparison between conventional coating materials, such as honey and *Aloe vera* gel, and emerging options, such as chitosan and nanosilicate containers, for maintaining the quality and shelf life of "Malas-e Saveh" pomegranate arils.

MATERIALS AND METHODS

This research was conducted at the Gorgan University of Agricultural Sciences and Natural Resources.

Fruit material

Pomegranate fruits of the "Malas-e Saveh" cultivar were harvested at the stage of commercial maturity according to native growers' experience from an orchard in Golestan province, Iran, with an altitude of 86m above sea level, latitude 37.12 N, and longitude 54.85 E. Healthy, undamaged fruit of almost uniform size were selected for the experiment and immediately transported to the laboratory.

Experimental treatments

A factorial experiment in the form of a completely randomized design was applied with two factors: coating treatment and storage time. There were eight coating treatments: *Aloe vera* gel (100%), ascorbic acid (1%), *Aloe vera* gel + ascorbic acid (1%), *Aloe vera* gel + honey (20%), *Aloe vera* gel + chitosan (1%), polypropylene nanosilicate container, control (uncoated fruits), and zero-day control (fresh untreated arils separated from fruits before storage). The fruit was stored for either two or four weeks at three replications per treatment.

Coating preparation and application

The fruits were first surface-sanitized by soaking them in 0.5% sodium hypochlorite for 5 minutes and then rinsing them with boiled water at room temperature. The fruits were then left to air dry before being cut with a sharp knife and the arils carefully separated and placed on clean paper towels. The arils were immersed in the desired treatment for 10 minutes at room temperature for coating. After coating, the arils were separated from the solution using laboratory sieves and dried on paper towels for 30 minutes. Finally, 100 grams of treated arils per treatment were placed in plastic containers with lids and stored at 4°C. The polypropylene nanosilicate containers (thickness 0.02 mm) were obtained from Nano Company (Baspar Aitech) in Tehran, Iran. The *Aloe vera* gel was prepared by washing fresh leaves with distilled water and removing the flesh with a clean knife before blending it for 5 minutes to crush it (Emamifar, 2015).

Physicochemical analyses

The weight of the arils was measured using a scale with an accuracy of 0.01 grams. To determine the percentage of weight loss, the arils for each treatment were weighed and

marked. After two and four weeks of storage, the percentage of fruit weight loss was calculated. Following two and four weeks of storage, the juice was extracted from the pomegranate arils and centrifuged for 10 minutes at 2500 rpm before measuring the desired traits. The pH of the fruit juice was measured using a pH meter (pH 110 meter, EUTECH/OAKTON Instruments, USA) and the electrical conductivity was measured using a conductivity meter (Cond315iSET, made in Germany).

The amount of total soluble solids (TSS) in pomegranate juice was determined using a digital refractometer (Digital Abbe refractometer, model Quartz, Ceti, Belgium) (Feyzi et al., 2018). The acidity rate was determined by titration with sodium hydroxide (Selcuk & Erkan, 2014). The ratio of TSS to titratable acidity was calculated as a taste index. The antioxidant activity was measured according to Sun and Ho (2005) using a spectrophotometer (SQ 2800 UV/VIS, UNICO, USA). After 30 min of incubation of the prepared solution, the absorbance was measured against a blank (methanol) at 517 nm and the inhibition of free radical DPPH was calculated using the appropriate formula. The total phenolic content of the juice was measured using the Folin-Ciocalteu phenol indicator and the spectrophotometer. Total flavonoid content was measured using the method introduced by Fawole and Opara (2012) and vitamin C content was measured according to Kashyap and Gautam (2012). The differential pH method (Giusti & Wrolestad, 2001) was employed to determine the total anthocyanin content. The method involved measuring the absorbance of each sample at 510 and 700 nm in buffers at pH 1.0 and 4.5 and then calculating the difference in absorbance between the two pH values. During storage, the decay of arils is assessed through visual observations. After the emergence of symptoms such as browning and mold growth, the number of infected arils was counted and the percentage of decay was determined (Moradinezhad et al., 2023).

Color and sensory evaluation

The color of the pomegranate arils was measured using a colorimeter (Lovibond CAM-System 500), which utilizes three indices: a*, b* and L*. Sensory evaluation was conducted to assess the appearance, smell intensity, taste, and freshness of the pomegranate arils. A five-point test was used where a score of 5 represented the highest value and a score of 1 indicated the lowest value. Six trained panelists participated in the evaluation (Emamifar, 2014). Color and sensory data were collected only before storage (zero-day control) and at the end of storage (after four weeks), and analyses were conducted using a completely randomized design.

Statistical analysis

All data from this research were analyzed using SAS software. Comparison of means was performed using the least significant difference (LSD) test.

RESULTS AND DISCUSSION

Physicochemical properties

The analysis of variance showed significant interaction effects between coating treatment and storage period for various traits. Significant interaction effects ($P<0.001$) were observed for acidity, flavor index, vitamin C, total phenols, total flavonoids, total anthocyanins, antioxidant activity, and decay rate. Additionally, there were significant interaction effects ($P<0.05$) for pH and TSS. Consequently, mean comparisons were conducted for these traits based on the interaction effects. In contrast, the interaction effect for weight loss and electrical conductivity was not significant. The coating treatment had a significant ($P<0.001$) impact on weight loss

and electrical conductivity, while the storage period did not. Therefore, simple effects were compared for these two traits.

The highest pH value was observed for the ascorbic acid treatment after four weeks of storage (3.443), although it was not significantly different from some other treatments. Conversely, the lowest pH value was observed after two weeks of storage for the zero-day control and some other treatments (Table 1). For most treatments, pH increased after four weeks of storage compared to two weeks, although the difference was not significant for some. Previous study by Shahi et al. (2022) also found that pH was initially low and increased over time. Ghorbani et al. (2017) showed that pH decreased from day 10 to 16 with increasing storage time of pomegranate arils. The varying results could be attributed to differences in storage periods. As fruit storage time increases, respiration and consumption of organic acids rise, causing acids to convert to sugars and higher pH (Singh, 2010). Furthermore, the relative growth of molds and yeasts during storage also leads to higher pH due to consumption of organic acids (Zivanovic et al., 2007).

The ascorbic acid treatment after two weeks of storage gave the highest acidity (0.642%), while the nanosilicate container after four weeks resulted in the lowest acidity. The control acidity increased during storage compared to the zero-day control but then decreased slightly after four weeks, though for most treatments the value was slightly lower after four weeks. The present study's findings are consistent with those of Singh et al. (2022) and Singla et al. (2022), who observed an increase in aril acidity from the first day of storage to day 15. However in this study, it followed by a further decrease due to degradation after extended storage. The reduction in fruit organic acids is attributed to their consumption during respiration, which is closely linked to metabolism (Rahemi, 2006).

The control had the highest TSS (18.03 °Brix) after two weeks; while, the *Aloe vera* gel and *Aloe vera* gel + ascorbic acid after two and four weeks and the *Aloe vera* gel + honey and ascorbic acid treatments after four weeks showed the lowest TSS values. These treatments were not statistically different from some others. These results agree with Shahi et al. (2022) and Moradinezhad (2021), who found that the control had the highest TSS. The amount of TSS remained almost constant during storage, which is consistent with the findings of Barzegar et al. (2019). However, some researchers showed that TSS decrease during storage (Hajivand-Ghasemabadi et al., 2022; Shahi et al., 2022). The nanosilicate container after four weeks and the ascorbic acid treatment after two weeks of storage had the highest and lowest taste indices, respectively. Moradinezhad et al. (2019) also reported that packaging can improve the taste index in pomegranate.

The highest amount of vitamin C was in the zero-day control (4.312 mg/100 ml) and the lowest in the *Aloe vera* gel + chitosan and nanosilicate container after four weeks. This observation is consistent with the findings of Mandegari et al. (2020), who reported a reduction in vitamin C during storage. Vitamin C damage increases with longer storage, higher temperature, lower humidity, physical damage and frost. The main reason for vitamin C reduction is oxidation in the environment; higher pH from enzymatic activity also destroys it.

The lowest amount of total phenols was observed in zero-day control, though it had the highest values for total flavonoids and total anthocyanins. The *Aloe vera* gel + ascorbic acid treatment showed the highest total phenols (22.92 mg GAE/100 ml) after four weeks of storage. Overall, total phenols increased during storage for most treatments. In another study, Ayhan and Eştürk (2009) showed that the phenolic compounds of pomegranate arils decreased initially and then increased, which is consistent with the present results, although their storage period was much shorter. Changes in phenolic compounds are likely due to changes in acidity and TSS, impacting antioxidant activity.

Table 1. The Interaction effect of coating treatments and storage period on some physicochemical characteristics of edible arils of pomegranate cultivar "Malas-e Saveh".

Coating treatments	Storage time (week)	pH	Acidity (%)	TSS (°Brix)	Taste index	Vitamin C (mg/100 ml)
		P=0.039	P<0.001	P=0.029	P<0.001	P<0.001
Zero-day control	-	3.303 b	0.338 f	17.43 ab	51.59 bcd	4.312 a
Control	2	3.323 b	0.560 b	18.03 a	32.54 ij	2.288 d
	4	3.410 ab	0.453 d	17.56 ab	42.06 efgh	1.798 e
Aloe vera gel	2	3.363 ab	0.350 ef	15.60 c	44.88 ef	2.552 c
	4	3.430 ab	0.336 f	15.77 c	46.92 cde	1.672 ef
Aloe vera gel + honey	2	3.287 b	0.455 cd	17.07 b	37.66 hi	2.288 d
	4	3.413 ab	0.359 ef	15.80 c	44.12 efg	1.584 ef
Aloe vera gel + chitosan	2	3.373 ab	0.513 bc	16.80 bc	32.76 ij	1.731 e
	4	3.440 ab	0.313 fg	16.77 bc	53.77 ab	1.320 f
Aloe vera gel + ascorbic acid	2	3.273b	0.408 de	16.13 c	39.56 fgh	2.141 d
	4	3.360 ab	0.336 f	16.00 c	47.67 bcde	1.672 e
Ascorbic acid	2	3.223 b	0.642 a	16.90 bc	26.40 j	2.053 d
	4	3.443 a	0.350 ef	16.10 c	46.00 def	1.760 e
Nanosilicate container	2	3.297 b	0.327 fg	17.33 ab	53.23 ab	2.875 b
	4	3.420 ab	0.294 fg	17.33 ab	59.10 a	1.525 f

In each column, different letters indicate significant difference (at 1 or 5% probability level, LSD).

Table 1. (*Continued*).

Coating treatments	Storage time (week)	Total phenols (mg GAE/100 ml)	Total flavonoids (mg QE/100 ml)	Total anthocyanins (mg C_3GE /100 ml)	Antioxidant activity (%)	Decay rate (%)
		P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
Zero-day control	-	10.58 g	6.593 a	25.84 a	78.25 c	0.00 e
Control	2	11.69 defg	3.796 de	18.57 c	88.33 ab	4.70 e
	4	13.49 c	2.587 fg	11.34 de	59.30 e	90.48 a
Aloe vera gel	2	11.96 def	3.518 def	18.05 c	90.64 a	0.00 e
	4	11.86 def	2.724 efg	9.59 e	49.47 fg	31.29 c
Aloe vera gel + honey	2	12.44 cde	5.053 bc	21.27 bc	71.05 d	0.00 e
	4	18.95 b	2.916 efg	11.68 de	48.67 fg	29.80 cd
Aloe vera gel + chitosan	2	11.90 def	4.209 cd	18.26 c	83.73 b	0.00 e
	4	12.18 de	2.043 g	9.68 e	46.72 g	20.57 d
Aloe vera gel + ascorbic acid	2	12.55 cde	6.072 ab	25.10 a	89.10 ab	0.00 e
	4	22.92 a	2.939 efg	13.02 de	48.05 fg	41.17 b
Ascorbic acid	2	12.52 cde	5.055 bc	23.58 ab	90.46 a	0.00 e
	4	10.58 fg	3.385 def	13.49 d	50.89 fg	9.25 e
Nanosilicate container	2	12.64 cd	4.530 cd	23.78 ab	89.93 a	0.00 e
	4	10.87 fg	3.669 def	12.49 de	52.22 f	35.39 bc

In each column, different letters indicate significant difference (at 1 or 5% probability level, LSD).

Aloe vera gel + chitosan had the lowest amount total flavonoid content after four weeks of storage which was in agreement with the results of Singla et al. (2022). For total anthocyanins, both *Aloe vera* gel and *Aloe vera* gel + chitosan showed the lowest values after four weeks of storage. *Aloe vera* gel + ascorbic acid after two weeks of storage (25.10 mg cyanidin 3-glycoside per 100 ml) and the zero-day control (25.84 mg cyanidin 3-glycoside per 100 ml) had the highest anthocyanin content, although no statistical difference was observed compared to some other treatments. Factors like temperature, oxygen, storage time, moisture loss, and fruit type can reduce anthocyanins (Oz & Ulukanli, 2012). The decrease in total anthocyanins during storage is consistent with previous studies (Ghorbani et al., 2017; Mandegari et al., 2020; Shahi et al., 2022).

Table 2. Simple effect of coating treatments and storage period on weight loss and electrical conductivity (EC) of edible arils of pomegranate cultivar "Malas-e Saveh".

	Weight loss (%)	EC (mmohs/cm)
Coating treatments	P<0.001	P<0.001
Zero-day control	0.000 b	4.253 a
Control	3.000 a	4.204 a
Aloe vera gel	0.426 b	4.115 b
Aloe vera gel + honey	0.157 b	3.992 d
Aloe vera gel + chitosan	0.795 b	4.128 b
Aloe vera gel + ascorbic acid	0.393 b	4.083 bc
Ascorbic acid	0.287 b	4.042 cd
Nanosilicate container	0.412 b	4.120 b
Storage time (week)	P=0.119	P=0.453
2	0.564	4.112
4	0.804	4.122

In each column, different letters indicate significant difference (at 1% probability level, LSD).

The highest antioxidant activity was observed for *Aloe vera* gel, ascorbic acid and nanosilicate container after two weeks of storage (90.64%, 90.64% and 89.93%, respectively). The lowest activity was seen in *Aloe vera* gel + chitosan after four weeks of storage. The results showed that antioxidant activity initially increased and then decreased over time. These findings are consistent with those of with Zahran et al. (2015), who also reported that the antioxidant activity of pomegranate arils increased over time. In pomegranates, antioxidant activity is more influenced by phenolic compounds like elagitannins, and less by anthocyanins and ascorbic acid (Tehranifar et al., 2014). Some edible coatings can enhance antioxidant properties by reducing respiration and ethylene production (Ghasemnezhad et al., 2013).

The results of the present study revealed that, after 2 weeks of storage, no decay was observed in treatments except for the control, which had a decay rate of 4.70% (Table 1). The highest decay rate was observed in control after four weeks (90.48%). Among the other treatments, *Aloe vera* gel + chitosan exhibited the lowest decay rate after four weeks of storage (20.57%). Moradinezhad et al. (2023) also demonstrated that packaging treatments led to reduced decay in pomegranate arils during storage.

Table 2 presents the simple effect of coating treatment and storage period on the weight loss of edible pomegranate arils ("Malas-e Saveh" cultivar). The zero-day control had the lowest value without weight loss, while the control treatment had the highest weight loss (3%). Other coating treatments showed slight, statistically similar weight loss to the zero-day control. No significant difference in weight loss was observed between two and four weeks of storage. These results agree with Mandegari et al. (2020) and El-Beltagi et al. (2023), who also reported decreased aril weight during storage and reduced weight loss with coatings. Weight loss during storage is caused by water evaporation, membrane damage, and increased senescence. The zero-day control and control also showed the highest electrical conductivity values (4.253 and 4.204 decisiemens/meter, respectively), while the *Aloe vera* gel + honey had the lowest value, although not significantly different from the ascorbic acid treatment. No statistical difference in electrical conductivity was observed between two and four weeks of storage.

Color properties

The results of the analysis of variance showed that the coating treatments had a significant effect on the color indices a (red color) and b (yellow color) of the pomegranate arils. However, they did not significantly affect the maintenance of the L index or brightness. This

finding is consistent with that of Mandegari et al. (2020), who also showed that 5 µM nitric oxide did not affect the L index.

According to the results, the zero-day control and *Aloe vera* gel produced the highest values for index a (50.11 and 46.87, respectively), while the control and *Aloe vera* gel + ascorbic acid produced the lowest values (Table 3). In other words, the intensity of the arils' redness (an index) decreased over time, but the *Aloe vera* gel coating helped maintain this color. The other treatments were less effective, although there were minor differences. These results agree with Mandegari et al. (2020) and Moradinezhad et al. (2023), who demonstrated that this index decreases over time and that 5 and 10 µM nitric oxide best preserved it. The reduction in red color at the end of storage may be due to anthocyanin degradation (Varasteh et al., 2012). Additionally, the zero-day control had the highest b index value (38.27), while the control and ascorbic acid treatments produced the lowest. During storage, the intensity of the arils' yellowness (b index) decreased which was in agreement with Moradinezhad et al. (2023). As with redness (an index), the *Aloe vera* gel coating better maintained this index compared to other treatments, which were less effective. Fruit color is an important quality characteristic for consumers, as appropriate color makes the fruit appear appetizing and directly impacts marketability. Chemical changes during storage often reduce fruit pigments (Varasteh et al., 2012).

Sensory properties

The results of the analysis of variance indicated that there were statistically significant differences between the coating treatments for the sensory evaluation attributes, including appearance, smell intensity, taste, and freshness.

The sensory evaluation results revealed that consumers preferred the natural taste of the fresh pomegranate arils in the zero-day control, which received the highest scores (Table 4). However, the control had the lowest appearance value (2.3), while the control and *Aloe vera* gel + chitosan had the lowest smell intensity values (both 3.3). The *Aloe vera* gel and *Aloe vera* gel + chitosan had the lowest taste values (both 2.5), and the control and nanosilicate container received the lowest freshness values (1.8 and 2.5, respectively). Appearance is the most important indicator in sensory evaluation (de Resende et al., 2008), as any signs of contamination or decay will reduce marketability. Thus any factor that slows aging and prevents decay signs will better preserve appearance and sensory appeal. In this study, most coating treatments better maintained the arils' appearance compared to the control, which is consistent with the findings of Mandegari et al. (2020).

Table 3. The effect of coating treatments on color properties in edible arils of pomegranate cultivar "Malas-e Saveh".

Coating treatments	*L*	*a*	*b*
	P=0.065	P<0.001	P<0.001
Zero-day control	35.39	50.11 a	38.27 a
Control	41.36	36.42 c	8.86 c
Aloe vera gel	40.09	46.87 a	27.34 ab
Aloe vera gel + honey	36.22	41.06 b	20.75 bc
Aloe vera gel + chitosan	35.24	39.54 bc	18.62 bc
Aloe vera gel + ascorbic acid	32.18	36.48 c	16.85 bc
Ascorbic acid	36.98	38.52 bc	13.56 c
Nanosilicate container	36.85	39.88 bc	21.14 bc

In each column, different letters indicate significant difference (at 1% probability level, LSD).

Table 4. The effect of coating treatments on sensorial properties in edible arils of pomegranate cultivar "Malas-e Saveh".

Coating treatments	Appearance	Aroma	Taste	Freshness
	P<0.001	P<0.001	P<0.001	P<0.001
Zero-day control	5.0 a	5.0 a	5.0 a	5.0 a
Control	2.3 e	3.3 c	3.2 bc	1.8 d
Aloe vera gel	3.0 cd	4.0 b	2.5 c	3.0 cd
Aloe vera gel + honey	3.5 bc	4.0 b	3.0 bc	3.0 cd
Aloe vera gel + chitosan	2.7 de	3.3 c	2.5 c	3.2 bcd
Aloe vera gel + ascorbic acid	3.7 b	4.0 b	3.5 b	3.8 b
Ascorbic acid	3.0 cd	4.5 ab	3.2 bc	3.5 bc
Nanosilicate container	2.5 de	4.0 b	3.3 b	2.5 d

In each column, different letters indicate significant difference (at 1% probability level, LSD).

CONCLUSION

In recent years, there has been a growing trend to separate and sell fresh pomegranate arils in the market. However, after harvesting and separation from the fruit, pomegranate arils undergo a series of enzymatic and biochemical reactions that may lead to quality deterioration during storage. Therefore, certain precautions should be taken to minimize these changes. This study aimed to investigate the effectiveness of various food coatings in preserving the quality of "Malas-e Saveh" pomegranate arils during storage. The results showed that the coating treatments and storage time had a significant interaction or simple effect on acidity, flavor index, vitamin C, total phenols, total flavonoids, total anthocyanins, antioxidant activity, TSS, and weight loss. All coatings were effective in preventing weight loss. Nanosilicate containers largely maintained TSS content during storage. *Aloe vera* gel + ascorbic acid were effective in preserving total phenols, total flavonoids, total anthocyanins, and antioxidant activity. *Aloe vera* gel was most effective at maintaining aril color. *Aloe vera* gel + ascorbic acid had the greatest impact on maintaining sensory evaluation attributes including appearance, smell intensity, taste, and freshness. Further research is suggested to investigate the effects of edible coatings and nano packaging containers on the storage of edible pomegranate arils across different cultivars.

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Conflict of interest
The authors have no conflict of interests to declare.

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In vitro antifungal activity of barberry fruit extract (*Berberis* spp.) against *Fusarium* spp.

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ABSTRACT

Purpose: *Berberis integerrima* Bunge and *Berberis vulgaris* L. are traditional plants known for their many health benefits. The aim of this study was to investigate the antifungal potential of *B. vulgaris* and *B. integerrima* fruit extracts against *Fusarium* spp. pathogens as an environmentally compatible natural antifungal compound. **Research methods**: The antifungal activity of methanolic fruit extracts of *B. vulgaris* and *B. integerrima* against *Fusarium solani*, and *Fusarium graminearum* was investigated using the microdilution method, growth area measurement, and morphological Changes were studied using scanning electron microscopy analysis. **Findings:** The methanolic fruit extracts of *B. vulgaris* and *B. integerrima* had significant antifungal activity against the studied plant pathogens, with *B. integerrima* exhibiting a stronger effect. The MIC values of *B. vulgaris* fruit extract against *F. graminearum and F. solani* were 150 and 75 mg mL^{-1}, and *B. integerrima* fruit extract had 100 and 75mg mL^{-1}, respectively. *F. graminearum* was the most resistant fungal species. Scanning electron microscopy analysis showed that the extracts of both medicinal plants changed the structure and morphology of mycelia and, dose-dependently, inhibited conidia formation. **Research limitations:** There were no limitations. **Originality/Value**: The study showed that fruit extracts of *B. vulgaris* and *B. integerrima* have the potential to be used as natural and environmentally friendly agents against *Fusarium* species.

INTRODUCTION

Fusarium spp., a widespread filamentous fungus found in soil, plants, and organic substrates, is a significant plant pathogen responsible for various diseases, economic losses on crops, and food spoilage (Nehra et al., 2021), with over 300 species in 22 species complexes (Nosratabadi et al., 2022), and 24 toxic species that have a significant impact on both human and animal health (Adeyeye, 2016). Among the most important ones, which are more destructive, can be pointed out: the *F. graminearum* species complex, responsible for Fusarium head blight in wheat and barley; and the *F. solani* species complex, the cause of destructive foot and root rot (Aoki et al., 2014). Heavy reliance on synthetic pesticides to manage plant pathogens has become an important concern due to their negative effects on human health, the environment, and the emergence of resistant pests and disease-causing species (Lengai et al., 2020). Therefore, new antifungal strategies aim to create fungicides with low production costs, high efficacy, and safe for people, animals, host plants and ecosystems. Biological control is one of the strategies that, due to its effectiveness on target organisms and its biodegradability, has gained global popularity (Pârvu & Pârvu, 2011). Plant extracts and plant-derived compounds have received much attention as a potential alternative to synthetic fungicides for biological control. Plant tissues produce secondary metabolites that are highly active against pathogens and have been tested against various fungal pathogens (Bhandari et al., 2021). Berberis is a genus of plants with 650 species and 15 genera, found in Asia, North Africa, and Europe (Goodarzi et al., 2018). Barberry species, including *B. vulgaris*, are produced worldwide for medicinal purposes. In addition to the pharmaceutical industry, they are also used in the food sector, and ornamental species are used for decoration in different places (Rahimi-Madiseh et al., 2017). The diverse and contentious nature of barberry species identification has prompted numerous studies (Ghahramanlu et al., 2023; Rezaei et al., 2011). Species including *B. vulgaris*, *B. orthobotrys*, *B. khorasanica*, *B. integerrima*, *B. crataegina*, *B. lycium*, and *B. aristata* are frequently utilized in traditional medicine in Iran and other regions (Rahimi-Madiseh et al., 2017; Rezaei et al., 2011). *Berberis vulgaris* L., a variety in Khorasan Province, Iran, is a special fruit with high economic value for farmers and a rich history in folk medicine. Its high antioxidant capacity may increase its popularity. In vitro and in vivo studies have shown barberry's pharmacological activities, making it a valuable addition to the country's diet (Goodarzi et al., 2018). It is known for its health benefits, including fat reduction, anti-cancer, anti-diabetes, liver protection, antioxidant, and anti-inflammatory properties (Ardestani et al., 2015). *Berberis integerrima* Bunge, a wild barberry species, is used in Iran for its antioxidant, anti-diabetic and renal prevention properties. Its pharmacological activity includes antinociceptive, anticonvulsant, antiinflammation, antioxidant, anticancer, antihyperglycemic, antihypertensive, and antibacterial effects (Moein et al., 2020). In order to identify a natural and environmentally friendly anti-fusarium agent, this study investigated the antifungal properties of *B. vulgaris* L. and *B. integerrima* Bunge fruit extracts against *F. graminearum* and *F. solani*, as well as their effects on the morphology of these pathogens.

MATERIALS AND METHODS

Chemicals and media

Potato dextrose agar (PDA), sabouraud dextrose agar (SDA), sabouraud dextrose broth (SDB), and absolute methanol were all obtained from Merck (Germany).

Plant material and preparation of berberis extracts

Berberis vulgaris L. from Qain city in South Khorasan province and *Berberis integerrima* Bunge species from Shahr-e-Babak city in Kerman province were collected in 2022 and deposited in the herbarium of the Faculty of Natural Resources and Environment of Birjand University with voucher numbers 2670 and 2911, respectively. The barberry fruits from Birjand were seedless, while those from Kerman had one to three small spindle-shaped seeds. The fruits were washed, dried in an oven (CE.FH.151.4, Germany) for two days at 50 °C, milled to a fine powder, and stored at -20 °C until extraction. The *Berberis* spp. powders were added in a ratio of 1:10 with 80% methanol (methanol: water, 80:20 vv^{-1}) at 50 °C for 24 h with stirring at 150 rpm in a shaking incubator (Lab Tech, South Korea). The extracts were filtered twice using Whatman No. 1 filter paper. A rotary evaporator (IKA, RV 10, DS 99. Germany) was used to evaporate the solvent from the extracts at 50 °C until the thick syrup was collected. The syrups were entirely dried using a freeze-drying device (VaCo 5-D, Zirbus Technology, Germany), and the dried extracts were kept at -20 °C to do tests.

Preparation of fungal spore suspension

*Fusarium solan*i and *Fusarium graminearum* were obtained from the microorganism collection of the Department of Plant Medicine, Faculty of Agriculture, Shiraz University, Shiraz, Iran. Spores were prepared by soft scraping and pipetting sterile normal saline solution or sterile distilled water onto a seven-day PDA culture at 25–28 °C. The spore number was measured using a hemocytometer and adjusted to 2×10^6 spores per millilitre.

Assessment of antifungal activity of berberis fruit extracts against *Fusarium spp.*

Antifungal activity of *B. vulgaris* and *B. integerrima* fruit extracts on the growth of *F. solani, F. graminearum*, was determined by the micro-well dilution technique, and growth area in agar media.

Micro-well dilution technique

The minimum inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC) of *Berberis vulgaris* and *Berberis integerrima* fruit extracts were determined by the micro-well dilution applied by Kumar et al. (2016) with a slight modification. A volume of 100 µL of concentrations of 400, 300, 200, 150, 100, 75 mg mL^{-1} of extracts were added to the wells of a 96-well plate containing 100 µL of SDB and 10 µl of spore suspension (2×10^6 spores mL^{-1}), incubating at 25°C for 5-7 days. Wells containing 200 µl of SDB and 10 µl of spore suspension were considered positive controls. The lowest concentration of extracts that caused complete inhibition of fungal growth after seven days was considered the MIC. To distinguish fungistatic and fungicidal activity and determine the MFC, after reading the MIC, 20 µl of culture wells with no growth of fungal cells and also a positive control was subcultured onto SDA (incubation at 25°C for five days). The lowest concentration without a fungal colony was considered MFC. The experiments were performed twice, with three replications for each treatment.

Determination of the fungal growth zone

The inhibitory effect of the extracts by the method of Salem et al. (2021) with minor modifications in the 70 mg mL^{-1} concentration of both plant fruit extracts on agar culture medium on the growth of *F. solani* and *F. graminearum* spores was investigated by the spotting method in SDA medium in three replicates for each treatment inoculated with 10 µl spores (2×10^6 spores mL^{-1}) and incubated at 25°C). The growth area was calculated on various days, including 3, 5, 7, 9, 12, 16, 20, 25 and 30. The percentage of growth inhibition

(PGI) (%) was calculated by the formula: PGI (%) = [C-T] × 100/C, where C is the diameter of the control colony and T is the diameter of the treated colony. Three replicates were carried out for all of the treatments.

Scanning electron microscopy (SEM) analysis of the effect of berberis fruit extracts on mycelial morphology

The effect of the *B. integerrima* and *B. vulgaris* extracts on the mycelial structure of *F. solani* and *F. graminearum* was investigated by SEM with some modifications to the Sellamani et al. (2016) method. A volume of 20 µ of spores (2×10^6 spores mL^{-1}) was added to the SDA culture at MIC50 amounts of methanolic extracts and incubated at 25 °C. With the appearance of mycelium on the culture medium, the blocks of mycelium (1×1), were separated and dried with a freeze-dryer to stabilize and prepare for SEM imaging. The mycelia were sputter coated with gold (Q150R ES, Quorum Technologies, United Kingdom), and the morphological feature was observed by SEM (TESCAN-Vega3, Czech Republic) at 20.0 kV in environmental mode. Mycelia grown in cultures without extract were considered as control. (Sellamani et al., 2016)

Statistical analysis

The study utilized a generalized linear model (GLM) for ANOVA, the Statistical Analysis System (SAS), Version 9.3 for examining the significant differences between species using the least significant difference (LSD) test, and Graphpad Prism 8.2.1 for creating graphs.

RESULTS

Assessment of antifungal activity of berberis fruit extracts against *Fusarium spp.*

Microdilution method

The antifungal activity of *B. integerrima* and *B. vulgaris* fruit extracts against *Fusarium* spp. was studied using the microdilution method. The results showed that *B. vulgaris* fruit extract had MIC values against *F. graminearum* and *F. solani* of 150 and 75mg mL^{-1}, respectively, and MFC values of 400 and 300 mg mL^{-1}, respectively. At MIC values of 100 and 75mg mL^{-1} and MFC values of 200 and 100 mg mL^{-1}, respectively, *B. integerrima* fruit extract effectively inhibited *F. graminearum* and *F. solani* (Fig. 1). The results showed that *F. graminearum* was more resistant fungus and *B. integerrima* fruit extract had a stronger anti-fusarium effect.

Determination of the fungal growth zone

The results showed that *B. integerrima* fruit extract had a stronger antifungal effect than *B. vulgaris* on the growth percentage of *F. solani* and *F. graminearum* spores in agar medium (Fig. 2B). The fruit extract of *B. integerrima* effectively inhibited the growth of *F. solani* until the end of the incubation period (day 30) (Fig. 2A). *B. vulgaris* fruit extract had the highest inhibitory effect on *F. solani* (Fig. 2C). In general, *F. graminearum* was more resistant fungal species (Fig. 2B). The results of the ANOVA analysis of the impact of plant and fungal species on the percentage of fungal growth inhibition are presented in Table 1.

SEM analysis of mycelial morphology

Changes in the structure of hyphae were easily visible without the use of a microscope. In contrast to the mycelium mass in the control sample, which was spread out throughout the plate, the mycelium mass in the culture medium treated with the extract grew in the center of the plate (Fig. 3).

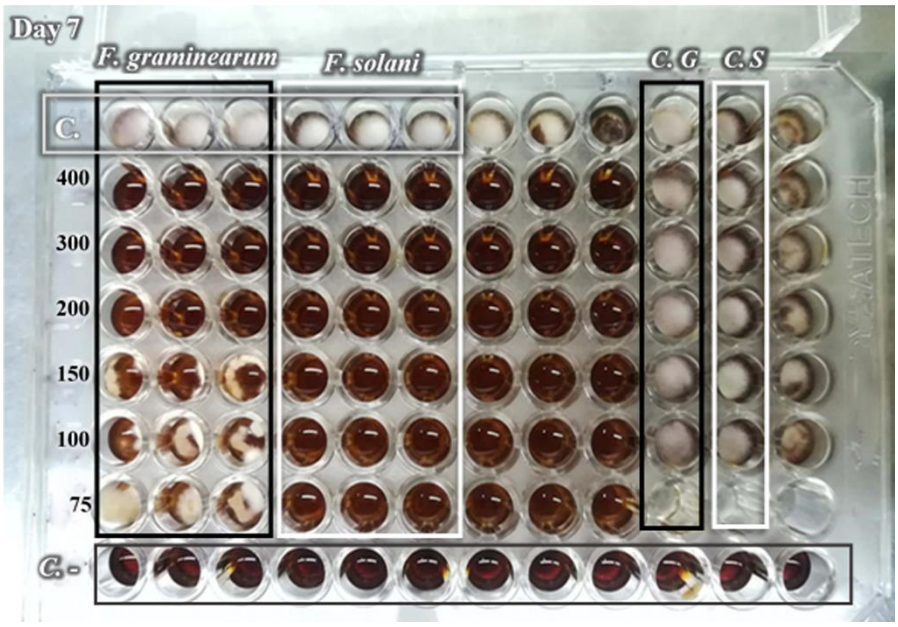

Fig. 1. Seven-old day culture of *F. solani* and *F. graminearum* spores (2×10^6 spores mL^{-1}) in SDB medium at 25°C with 400, 300, 200, 150, and 75 $mgmL^{-1}$ concentrations of methanolic fruit extracts of *B. integerrima*. Control (C.) positive control of *F. graminearum* (C. G); positive control of *F. solani* (C. S); negative control (SDB medium without extract and spores) (C. -).

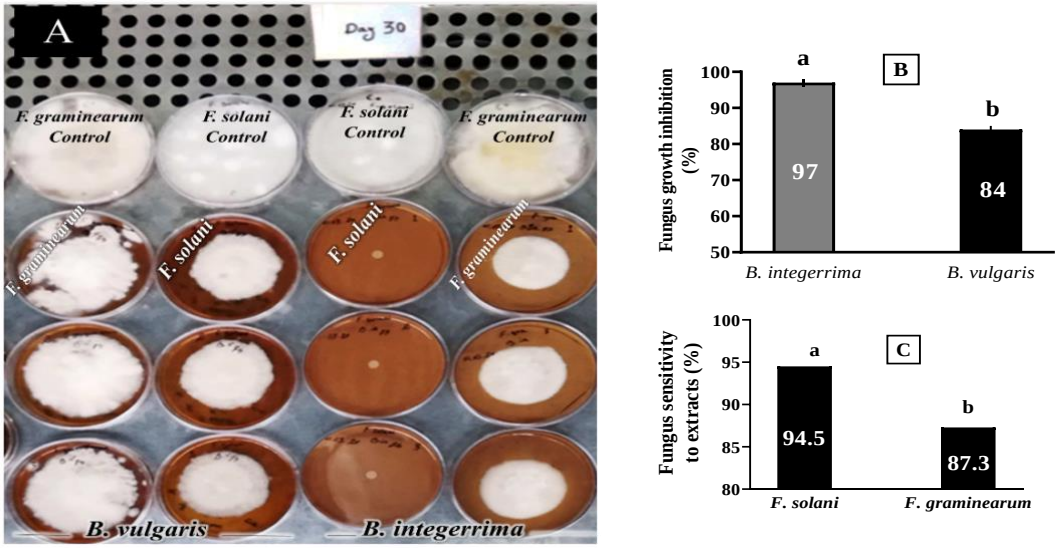

Fig. 2. 30-old-day cultures of spore culture of *F. solani* and *F. graminearum* at 25 °C in SDA medium containing 70 mg mL^{-1} of *B. vulgaris* and *B. integerrima* fruit extracts in three replicates, (A). Comparison of the *B. vulgaris* and *B. integerrima* fruit extracts' ability to inhibit *Fusarium* spp. (B). The sensitivity percentage of *F. solani* and *F. graminearum* to *B. vulgaris* and *B. integerrima* fruit extracts, (C). Controls (no extract); Bars with different letters differ from each other significantly ($P < 0.05$).

Table 1. ANOVA analysis of the impact of plant (*B. vulgaris* and *B. integerrima*) and fungal species (*F. solani* and *F. graminearum)* on the percentage of fungal growth inhibition.

S. O. V	df	Mean Square
Plant	1	5148.3***
Fungi	2	1569.6***
Plant × Fungi	1	142.2 ***
CV (%)		1.92

S.O.V: Sources of variations; df: degrees of freedom;
CV (%): Coefficient of variation. *** Significance at the level of <0.0001 probability.

SEM images of the effect of B. vulgaris on F. graminearum mycelia

The growth rate of *F. graminearum* mycelia in media containing *B. vulgaris* fruit extract decreased significantly compared to the control sample, and the fungal mass was concentrated at the spore inoculation site, unlike in the control sample, which had grown all over the plate (Fig. 3B). Unlike the long, slender and smooth mycelium in the control sample (Fig. 4, A1–A3), the mycelium grown in the extract media was thick, dense, and deformed (Fig. 4, B1–B3). No spores were observed in the extract-containing culture, while many conidia were present in the control sample.

SEM images of the effect of B. vulgaris on F. solani mycelia

In the medium containing *B. vulgaris* fruit extract, thick mycelia with denser texture and structure could be seen (Fig. 5, B1–B3) in contrast to the long, narrow, and smooth mycelia of *F. solani* grown in control cultures (Fig. 5, A1–A3). In the cultures that contained the extract, fewer spores were seen. In comparison to the control sample, the growth rate significantly decreased (Fig. 3B).

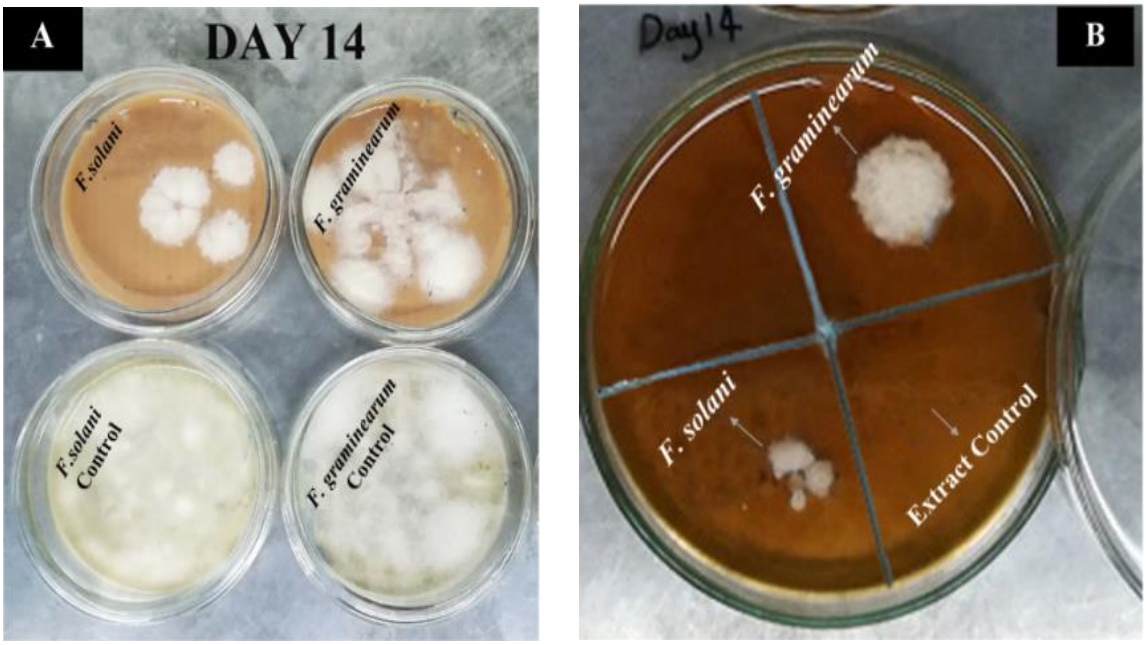

Fig. 3. 14 old-day cultures of *F. solani* and *F. graminearum* in SDA at 25 °C; medium containing the MIC50 value of *B. integerrima* fruit extract with fungus controls, (A); medium containing the MIC50 value of *B. vulgaris* fruit extract, (B).

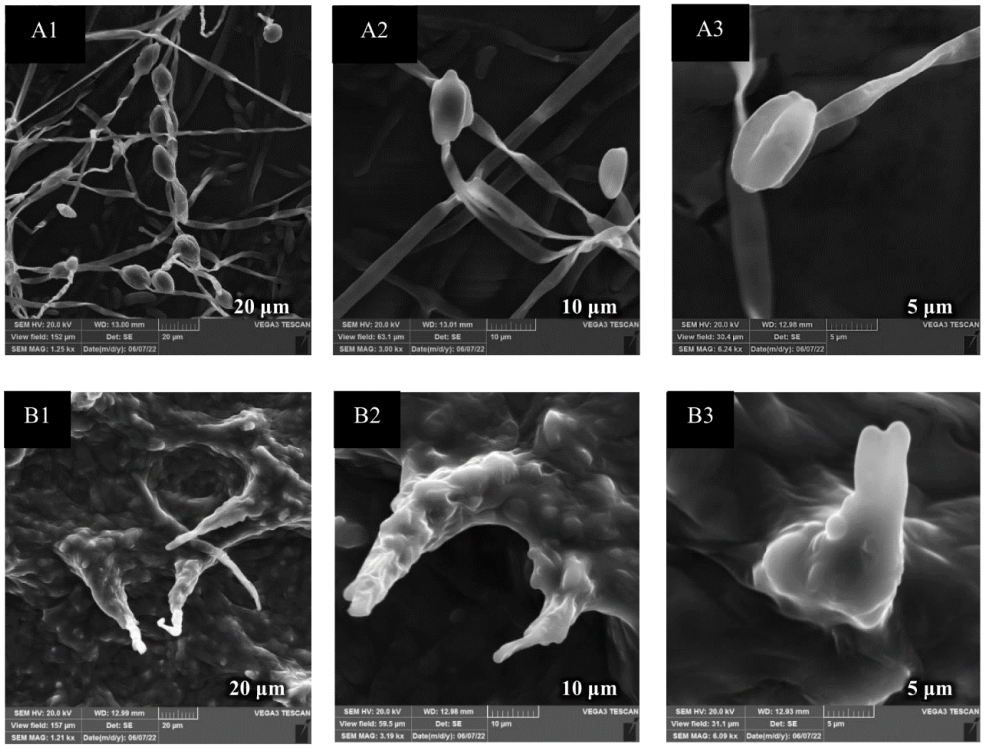

Fig. 4. SEM image of the effect of *B. vulgaris* fruit extract on the structure of *F. graminearum* mycelium in SDA. Control sample (without extract), (A1–A3); MIC50 value of *B. vulgaris* fruit extract, (B1–B3).

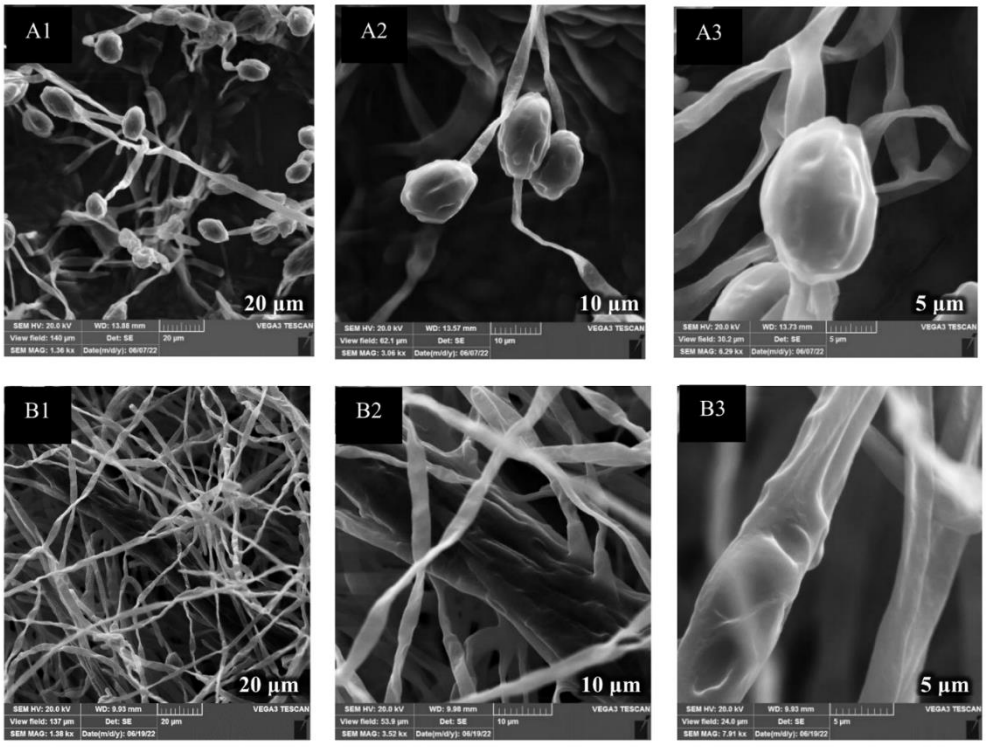

Fig. 5. SEM image of the effect of *B. vulgaris* fruit extract on the structure of *F. solani* mycelium in SDA. Control sample (without extract), (A1–A3). MIC50 value of *B. vulgaris* fruit extract, (B1–B3).

SEM images of the effect of B. integerrima on F. graminearum mycelia

Unlike the control sample's long, thin, and smooth mycelia (Fig. 6, A1–A3), *F. graminearum* mycelia grown in medium containing *B. integerrima* extract were thick, dense, and deformed. The mycelium in the extract-containing medium was networked and interconnected, unlike the control sample's filamentous and separate mycelium (Figs. 6, B1–B3, and C1, C2). The growth rate was significantly reduced, and the mycelium's mass was concentrated at the spore inoculation site (Fig. 3A). No spores were observed in cultures containing the MIC50 value extract and very few in the 25 mg mL^{-1} extract sample, whereas many conidia were present in the control sample (Fig. 6).

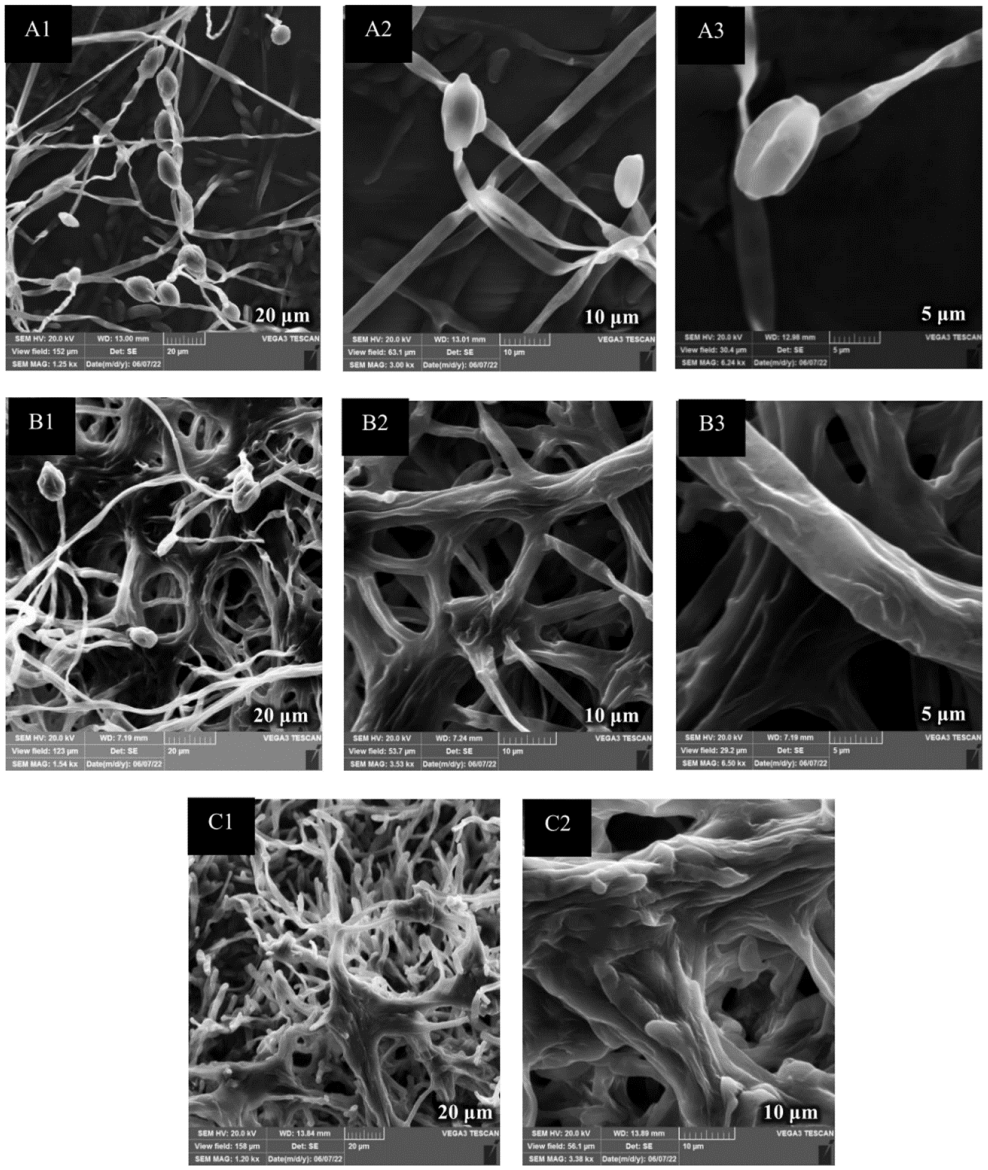

Fig. 6. SEM image of the effect of *B. integerrima* fruit extract on the structure of *F. graminearum* mycelium. Control sample (without extract), (A1–A3); volume of 25 and 50 mg mL^{-1} of *B. integerrima* fruit extract, respectively, (B1-B3 and C1-C2).

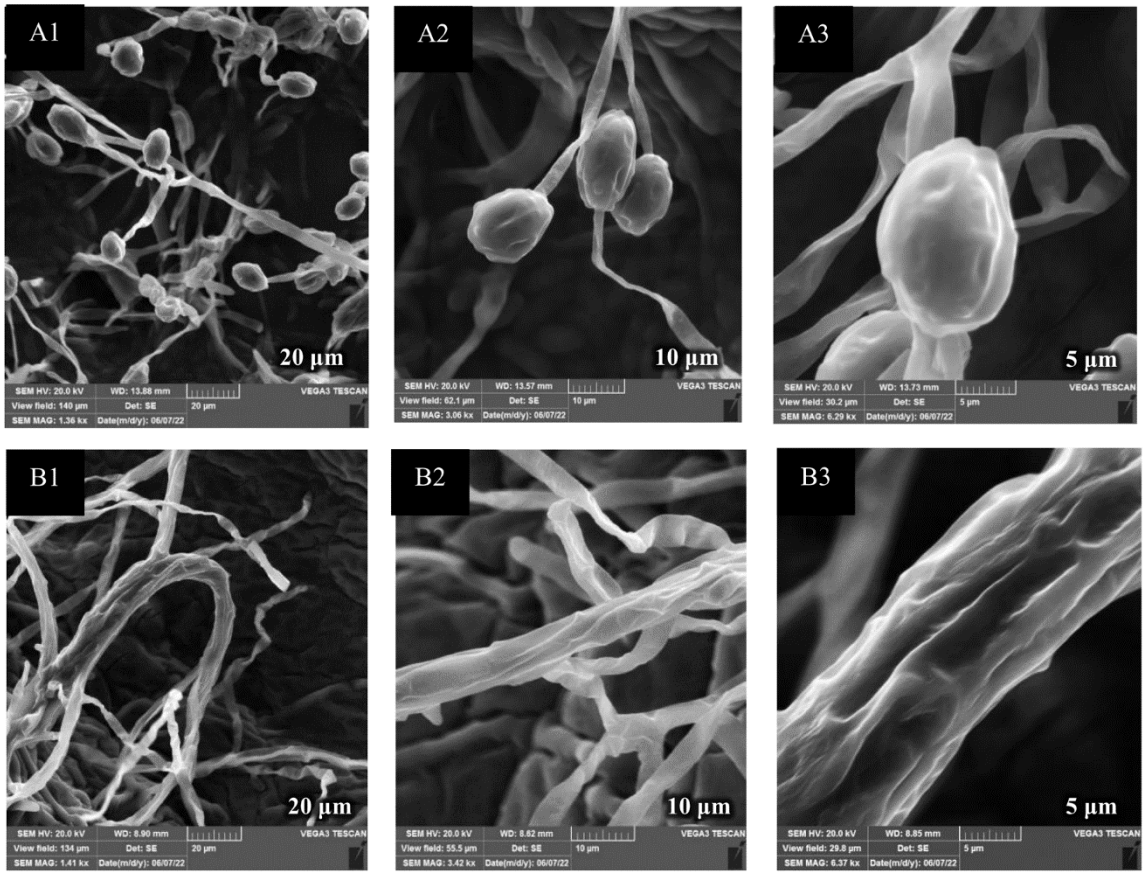

Fig. 7. SEM image of the effect of *B. integerrima* fruit extract on the structure of *F. solani* mycelium. Control sample (without extract), (A1–A3); MIC 50 value of *B. integerrima* fruit extract, (B1-B3).

SEM images of the effect of B. integerrima on F. solani mycelia

The *F. solani* mycelium grown in a medium containing *B. integerrima* extract was thicker, irregular, and dense than the filamentous, thin, and delicate mycelium of control (Fig. 7). Spores were not observed in extract samples. The fungal colony appeared convex and dense, in contact with the culture medium. The growth rate was slower than in the control sample (Fig. 3A).

DISCUSSION

Plant pathogenic fungi, with over 10,000 species, are the most dangerous plant pathogens that cause significant damage to economically important crops (Nazarov et al., 2020). The increasing number of *Fusarium* species exposed to whole-genome sequencing, underscores the significant threat *Fusarium* poses to agriculture and human health (Munkvold, 2017). Over the past decades, numerous research studies have focused on developing an efficient and eco-friendly method for managing phytopathogens (Seo et al., 2013). Several plant families have shown fungicidal activity against *Fusarium* species, such as *Asteraceae* (Rongai et al., 2012), *Oleaceae* (Korukluoglu et al., 2008), and *Lamiaceae* (Yazgi et al., 2015). No reports of anti-*Fusarium* effects were found from barberry fruits of the *Berberidaceae* family. The study demonstrated that *B. vulgaris* and *B. integerrima* fruit extracts effectively inhibited the growth of the studied *Fusarium* spp., with *B. integerrima* exhibiting a stronger inhibitory effect. The phytochemical analysis of the *Berberis* fruit revealed the presence of alkaloids, tannins, carotenoid, vitamin, protein, lipid, anthocyanin, and phenolic compounds (Salehi et al., 2019).

The greater potency of *B. integerrima* fruit extract compared to *B. vulgaris* may be related to the bioactive compounds present in its seeds. Fatty acids (linolenic, linoleic, and oleic acids, as well as omega-3 and omega-6 fatty acids) and phytosterols are also present in the oil found in *B. integerrima* seeds (Tavakoli et al., 2017). This plant is often used in pharmacological studies as a rich source of bioactive substances (Moein et al., 2020). In the present study, *F. graminearum* was the most resistant species both in liquid culture and in agar medium. In the study of Samie and Mashau (2013), *F. graminearum* was more resistant to most plant extracts than other *Fusarium* species. Our results agreed with them. *B. integerrima* fruit extract exhibited MIC values of 75–100 mg mL^{-1}, and *B. vulgaris* fruit extract had MIC values of 100-150 mg mL^{-1} for the fungi that were being examined in this study. *Piper sarmentosum* extract at 1-2 mg mL^{-1} against *F. graminearum* (Zhou et al., 2023), and *Taxus wallichiana* Zucc extract showed inhibitory effects against *F. solani* at MIC values of 0.08–200 mg mL^{-1} (Nisar et al., 2008). Phytochemicals play a crucial role in plant defense against fungal pathogens, either directly by affecting pathogen physiology and morphology (Dang-Minh-Chanh et al., 2013) or indirectly by inducing plant systemic resistance (Al-Wakeel et al., 2013). Studies have linked various bioactive compounds in plants, including alkaloids, organic acids, flavonoids, etc., with antifungal activity (Daradka et al., 2021). Berberine alkaloid, a bioactive compound found in barberry fruit, has been found to have antifungal properties due to its ability to inhibit sterol and cell wall biosynthesis and cell damage by increasing reactive oxygen species production (Xie et al., 2020).The most separated substance from the different parts of *B. integerrima* is also alkaloids (Moein et al., 2020). Both fruit extracts demonstrated inhibitory effects against test microbes, with berberine possibly being an effective compound in this activity. SEM and transmission electron microscopy (TEM) images have shown that the MIC or MFC of some plant extracts caused fungal ultrastructural changes (Dang-Minh-Chanh et al., 2013; Pârvu and Pârvu, 2011). Our study's SEM images revealed significant changes in mycelium structure and inhibition of microconidia production at the MIC50 value of the extract. The mycelia of *F. solani* and *F. graminearum* grown in a culture containing *B. vulgaris* and *B. integerrima* fruit extracts had thick, amorphous, altered, and bulky structures compared to the control mycelium. Bioactive compounds could be responsible for the changes in morphology (Perveen et al., 2022). The presence of various organic acids in barberry fruit extract, including oxalic, tartaric, ascorbic, acetic, malic, and fumaric acids (Ardestani et al., 2015), leads to acidification and lowers the extract's pH. This creates unfavorable environmental conditions for microorganisms and increases the antimicrobial properties of the extract (Khan et al., 2022). The fungal cell wall is a dynamic structure that shields cells from osmotic pressure changes and environmental stress (Gow & Lenardon, 2023). Environmental pH fluctuations and antifungal drug treatments impact gene expression, alter cell wall enzyme expression, stimulate regeneration mechanisms, and induce new cell wall structure changes. A stiffer cell wall, influenced by growth conditions, reduces the risk of cell damage, ultimately increasing cell survival and integrity during hyperosmotic stress. Less-elastic cell walls protect the plasma membrane from rupture during acute osmotic shocks (Ene et al., 2015). These changes were clearly evident when *Fuarium culmorum* was exposed to tebuconazole (a systemic fungicide). Tebuconazole inhibited fungal growth and caused swelling, excessive branching, and cell wall thickening (Kang et al., 2001). Our findings were in line with Kang et al.'s (2001) study. Along with glucan and various glycoproteins, chitin is a vital component in filamentous fungi that contributes to the stiffness, mechanical strength, and structural integrity of the cell wall (Hasim & Coleman, 2019). It has been reported that a reduction in overall chitin synthesis leads to excessive swelling of hyphae and changes in conidiation. The chitin synthase enzyme (ChsE enzyme) is involved in the synthesis of bulky chitin (Bowman & Free, 2006). Bulky mycelia and a lack of conidia

formation in MIC50 extracts observed in our SEM images can be affected by the change in chitin synthesis. The reduction in fungal conidia in the presence of antifungal compounds was reported in the studies of Al-Nazwani et al. (2021) and Perveen et al. (2022), and our findings are consistent with these studies. Our research added new information to the literature regarding the anti-fusarium activity of the fruits of *B. vulgaris* and *B. integerrima* and the effects of their extract on the morphological changes of *F. graminearum* and *F. solani* hyphae. (Al-Nazwani et al., 2021) (Samie & Mashau, 2013)

CONCLUSION

The study explored the potential of *B. integerrima* and *B. vulgaris* fruit extracts as antifungal agents against *F. graminearum* and *F. solani*. Comparative analysis revealed effective inhibitory activities on the mycelial growth of *Fusarium* spp., particularly for *B. integerrima*. SEM analysis of the studied *Fusarium* species showed the ability of the methanolic extracts of *B. integerrima* and *B. vulgaris* fruit to change the structure of mycelia and supported their potential as effective agents to control plant pathogenic fungi. The study emphasizes the need for exploring natural plant compounds as a sustainable and environmentally friendly alternative to synthetic chemicals for disease control. Further research is needed on the antimicrobial components of *B. integerrima* and *B. vulgaris*, their mechanisms of action, and their development into more effective and environmentally friendly solutions.

Declaration of interest

The authors declare that there is no conflict of interest.

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A systematic review on the various biochemical treatments for preservation of fresh tomato

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ABSTRACT

Purpose: Despite the worldwide rise in annual tomato production, approximately 15-50% of harvested tomatoes are lost each year, posing a significant challenge to global food security. This review seeks to assess the efficacy of biochemical treatments in preserving tomatoes to mitigate post-harvest losses. A machine-based search mapped articles on "Chitosan coating and tomato preservation," "Calcium chloride and tomato preservation," and "Potassium permanganate and tomato preservation" using Google Scholar. Seventy relevant articles published between 1995 and 2024 were included in the systematic literature review. **Findings:** Calcium chloride, Chitosan coating, and Potassium permanganate exhibit promise in enhancing tomato shelf life, yet their efficacy is contingent upon variables like tomato variety and storage conditions. Achieving a universally effective treatment proves challenging due to variations in study outcomes, highlighting the complexity of preserving tomatoes optimally. **Limitations:** The variability observed in reported outcomes poses significant challenges when it comes to discerning the most effective and optimal treatment. This inherent inconsistency in results not only complicates the identification of a universally applicable solution but also underscores the intricate nature of the factors influencing treatment effectiveness. **Directions for Future Research:** Future research should examine treatment combinations, consider responses to tomato cultivars, assess ecological impacts, implement safety protocols, and utilize advanced analytical techniques to refine tomato preservation methods.

INTRODUCTION

The global demand for tomatoes has been steadily increasing due to growing awareness of their health and medicinal benefits. This, along with the increase in human population, has led to an increase in global tomato production. Global tomato production in 2018 was estimated at 182.3 million tons (FAOSTAT 2022 as cited in Schreinemachers et al., 2022; Vats et al., 2022) and is expected to increase by 50% by 2050 (Stratton et al., 2021). However, despite this growth, excessive post-harvest losses in tomatoes continue to pose a significant challenge to food security. Post-harvest losses in fresh fruits and vegetables, including tomatoes, are estimated at 15-50% with developing countries recording higher post-harvest losses (35-50%) (Adeola, 2020; Shehu et al., 2014; Stratton et al., 2021) compared to developed countries (less than 15%) (Arah et al., 2016; Farooq et al., 2023). Post-harvest losses in tomatoes directly impact the income of farmers and traders (Kitinoja, 2013). Farmers lose money when their tomatoes are lost after harvest, and retailers lose money when tomatoes are lost in the supply chain. This can lead to financial difficulties and difficulty in repaying loans (Abdullahi et al., 2021; Dandago et al., 2021). Post-harvest losses in tomatoes can also impact the national economy by reducing food availability and leading to higher prices for consumers (Onwualu & Olife, 2013; Stratton et al., 2021). Consequently, post-harvest loss of tomatoes may also reduce the availability of jobs in the agricultural and food processing sector (Adeola, 2020). Furthermore, post-harvest loss of tomatoes has been documented to have negative environmental impacts; as tomatoes decompose, they release methane, a greenhouse gas that contributes to climate change as well as the waste of water, fertilizer, and other resources in tomato production (Ali et al., 2021; Jones et al., 2012; Martínez-Blanco et al., 2011).

In recent decades there has been a growing interest in exploring the causes of these losses and implementing remedial measures (Dandago et al., 2021; Kitinoja, 2013). Many studies have suggested a wide range of remedies for post-harvest loss. One of the commonly used approaches was the use of hot water for a short period (Ghaouth et al., 2019; Tonna et al., 2016). Another approach involved the use of chemical treatments such as organic acids (e.g. citric acid and lactic acid) (Al-Obeed, 2012; López–Gómez et al., 2020) as well as essential oils (Romanazzi et al., 2017) as well as biological coatings (Chitosan, Aloe vera and Moringa) (Athmaselvi et al., 2013; Ragab et al., 2019; Rayees et al., 2013; Tesfay & Magwaza, 2017) to reduce the impact of microorganisms on tomatoes.

Current research extensively addresses the causes and mitigation strategies for postharvest losses in tomatoes, with a particular focus on the widespread use of calcium chloride, chitosan and potassium permanganate (Betchem et al., 2019; Chepngeno et al., 2016; Salgado-Cruz et al., 2021; Silva et al., 2009; Sohail et al., 2015; Xing et al., 2015). However, despite the popularity of these methods, there is a significant gap in existing studies regarding sufficient data on their suitability and potential risks. Furthermore, identifying the most effective biochemical treatment remains an ongoing challenge. This review aims to fill this gap by addressing commonly used biochemical treatments, assessing their relative effectiveness, and examining the associated benefits and challenges.

MATERIALS AND METHODS

The selection criteria were based on the documented PRISMA guidelines (Moher et al., 2009). Articles published between 1995 and 2024 were searched based on predefined search terms. Considering that much may have changed over the years, at the end, only articles published between 1999 - 2024 were used in the review. The procedures for article selection and data extraction are presented as follows.

The systematic search was carried out using Google Scholar Search Engine on two dates and for both searches, the "Keywords Search" was used. The first search was conducted on July 14, 2023 with the following search terms "Chitosan coating" OR "Calcium chloride" OR "Potassium permanganate" AND "Tomato preservation". Documents that were wholly or partially related to the Search terms matched and 274 documents were returned based on the initial search. The search was further refined by year of publication that was narrowed to publications between 1995 and 2023; this resulted in 105 documents (conference papers). These included published articles and reports as well as theses and dissertations.

The second search was conducted with the same Search Engine and procedure on January 05, 2024. The search terms used in the second search are: "Chitosan coating" OR "Calcium chloride" OR "Potassium permanganate" AND "Tomato preservation" OR "Tomato storage". A total of 74 records (articles).

The articles from the first and the second search were combined on an EXCEL spread sheet for easy identification of duplicate records. A total of 52 articles were identified as duplicates and excluded from the records. The articles were again refined by the Year of Publication. Seventeen (17) articles were published between 1995 and 1998 and were discarded for being relatively too old. Twenty-four (24) articles were incomplete (Abstract only) as access to full article could not be obtained. Both the older and incomplete articles were discarded leaving a total of 86 articles. The 86 articles were checked for suitablity, from which 60 articles were used for the review.

To simplify the analysis, the review was carried out and reported under the following subtopics: i) calcium chloride, ii) chitosan coating, iii) potassium permanganate and the related implementation of biochemical treatments of tomato qualities

RESULTS AND DISCUSSION

Calcium chloride

There is an extensive discussion in the literature about the use of calcium chloride to preserve fresh fruits and vegetables. Table 1 provides a summary of the different treatments used in previous research to investigate the effects of calcium chloride concentration on the postharvest quality of tomato fruits. Prakash et al. (2018) examined the efficacy of calcium dip (1% $CaCl_2$) and irradiation (1 and 1.5 kGy) treatments on the postharvest qualities of tomatoes. Mujtaba et al. (2014) examined the effects of calcium chloride (1%, 2%, and 3%); Sati and Qubbaj (2021) calcium chloride ($CaCl_2$) 6% (w/v), gum arabic solution 10% (w/v), and cactus mucilage extract (2/1) (w/w). Other researchers have even looked at the effects of pre-harvest spraying of calcium chloride (3 and 5% w/v) in single and multiple applications on the postharvest qualities and shelf life of tomatoes. Other studies have examined the effects of similar levels of calcium chloride (0-6% $CaCl_2$) but for a dipping duration of between 10-30 minutes (Arthur et al., 2015; Coolong et al., 2014). Some of the studies have even examined the effects of combining calcium chloride (1% $CaCl_2$) and chitosan (0.5%) as well as hydrogen peroxide (0.12% H_2O_2) and 1% ozonated water. Furthermore, some previous studies have examined the combination effects of Calcium chloride (0.1%, 0.2%, and 0.4%) and potassium thiosulphate (0.0%, 0.2%, and 0.4%) under cold storage conditions (Semida et al., 2019) and that of the combination of gibberellic acid (0.075%, 0.1% and 0.125%) and Calcium chloride (1%, 1.5% and 2%) (Demes et al., 2021). Nonetheless, others have even examined the effect of hot water treatment (40-50 $^{\circ}C$) besides calcium chloride (2% $CaCl_2$) on the postharvest qualities of tomatoes (Hao et al., 2020).

Table 1. Effects of calcium chloride on the postharvest qualities of tomato fruits.

Fruit	Treatment	Effects	Reference
Tomato	Calcium dip treatment (1% $CaCl_2$) Irradiation treatment (1 and 1.5 kGy) Combination treatment of Calcium dip and irradiation	Calcium chloride stimulated ethylene production. Combination treatment showed the best effects of maintaining tomato quality, limiting softening and reducing microbial population.	Prakash et al. (2007)
Tomato	Calcium chloride, $CaCl_2$ (1%, 2% and 3%)	2% Calcium chloride was most effective in preserving tomato qualities	Mujtaba and Masud (2014)
Tomato	$CaCl_2$ (0.0%, 1.0%, 1.5%, and 2.0% (w/v)); Maturity stages (Mature green, breaker, and half-ripe stage)	Mature green stage treated with 2% $CaCl_2$ indicated the best qualities	Mazumder et al. (2021)
Tomato	Calcium chloride ($CaCl_2$) 6% (w/v), Gum arabic solution 10% (w/v), Cactus mucilage extract (2/1) (w/w)	Dipping tomato fruits in 6% $CaCl_2$ for 10 minutes + coating treatments using either 10% Gum arabic or 50% cactus mucilage was most effective in preserving tomato quality.	Sati and Qubbaj (2021)
Tomato (cultivar Rajitha)	Calcium chloride (3 and 5 %w/v), Two spraying protocols: single application at 7 days after full bloom (7 DAFB) or Weekly application from 7 DAFB to harvest	Pre-harvest calcium chloride treatment, particularly at concentrations of 3% and 5% w/v with both single and multiple applications, significantly extended the shelf life of the tomato cultivar by 2.3 to 3.8-fold, increased firmness, Calcium content, and total soluble solids, while showing a lower fresh weight at harvest and increased weight loss during storage, with no consistent impact on titratable acidity	Daundasekera et al. (2015)
Tomato	Calcium (Ca) nutrient solution (60, 180, and 360 mg/L); Foliar application of Calcium chloride (0%, 1%, and 2% $CaCl_2$)	Postharvest disease incidence was not affected by calcium treatment, but weight loss during storage was negatively impacted by calcium chloride sprays.	Coolong et al. (2014)
Tomato	Different concentrations of $CaCl_2$ (2%, 6%, 0%) by dipping for 10, 20, and 30 minutes	Tomato fruit dipped in 6% $CaCl_2$ was more effective than 2% $CaCl_2$ and 0% in maintaining quality. Dipping for 20 -30 was significantly more effective than 10 min. dip, but up to 40 minutes indicated tomato skin injuries.	Arthur et al. (2015)
Tomato (Solanum lycopersicum L. cv. 448)	Chitosan (0.5%); Calcium chloride (CaCl2) (1%); Hydrogen peroxide (H2O2) (0.12%); Ozonated water (1%)	Chitosan (0.5%) and calcium chloride ($CaCl_2$) (1%) were the most effective treatments in maintaining attributes of tomato fruit	Shehata et al. (2021)
Tomato fruit (hybrid 65010)	Calcium chloride (0.0%, 0.2%, 0.4%); Potassium thiosulfate (0.0%, 0.2%, 0.4%) Cold storage	0.4% + Potassium thiosulfate at 0.4% + cold storage showed best preservation effect	Semida et al. (2019)
Tomato (Kochoro variety)	Gibberellic acid (GA3) (0.075%, 0.1%, 0.125%); Calcium chloride (1%, 1.5%, 2%)	1.5% $CaCl_2$ and 0.125% GA3 were effective in maintaining quality and shelf life.	Demes et al. (2021)
Tomato	40°C hot water + 2% $CaCl_2$;40°C hot water treatment alone; 50°C hot water + 2% CaCl2; 50°C hot water treatment alone	40°C hot water + 2% $CaCl_2$ showed lowest weight loss; 50°C hot water + 2% $CaCl_2$ showed the highest firmness level; lycopene content was not explicitly affected by treatments	Hao et al. (2020)

Most studies have consistently agreed that calcium chloride affects the qualities of harvested tomatoes (Mujtaba & Masud, 2014; Prakash et al., 2007; Sati & Qubbaj, 2021) however; the concentrations that were considered as being effective differed between studies. Arthur et al. (2015) found that tomatoes dipped in 6% $CaCl_2$ were more effective in preserving tomato qualities than 2% and the control group. Their results further showed that while 10 -30 minutes of the dip was beneficial to tomato qualities, dipping tomatoes up to 40 minutes was damaging to tomato skin. Sati and Qubbaj (2021) reported that dipping tomato

fruits in 6% $CaCl_2$ was effective especially when dipped for 10 minutes + coating treatments using either 10% gum arabic or 50% cactus mucilage was most effective in preserving tomato quality. In a related study, however, Mujtaba and Masud (2014) found that 2% $CaCl_2$ was most effective on tomato postharvest qualities. Yet, when Prakash et al. (2007) revealed that 1% $CaCl_2$ in the presence of irradiation (1- 1.5 kGy) was most effective in preserving tomato qualities. While the aforementioned studies focused on the postharvest application of Calcium chloride treatments, other studies have investigated the effects of the pre-harvest application of Calcium chloride on its postharvest qualities. For example, a study by Daundasekera et al. (2015) on a local cultivar of tomato ('Rajitha' cultivar) in Sri Lanka found that pre-harvest Calcium chloride treatment, particularly at concentrations of 3% and 5% w/v with both single and multiple applications, significantly extended the shelf life of tomato fruits as well as increased fruit firmness, calcium content, and total soluble solids.

However, despite agreeing with the fact that post-harvest application of calcium chloride is important in preserving the postharvest qualities of tomatoes, a study by Coolong et al. (2014) reported the beneficial effects of the pre-harvest foliar application of Calcium chloride in the concentrations of up to 2% $CaCl_2$ on tomato weight loss after harvest, postharvest disease incidence on tomatoes was not affected by the calcium chloride treatment. From this review, it is clear that the influence of the concentration of calcium chloride in preserving the qualities of tomatoes after harvest depends on several factors such as the concentration and duration of the treatment (Arthur et al., 2015), when the treatment was applied (pre-harvest or postharvest) (Daundasekera et al., 2015), presence of other additives/treatments such as coating with Gum arabic or cactus mucilage or irradiation (Prakash et al., 2007; Sati & Qubbaj, 2021) as well as by the application method (Coolong et al., 2014). There is a need for future research to optimize calcium chloride treatments for tomatoes, investigating concentrations, durations, and coatings. Understanding mechanisms, considering environmental factors, and offering practical guidelines for farmers will enhance postharvest preservation strategies.

Chitosan coatings

Table 2 displays the different levels of chitosan used in previous research to examine its effects on harvested tomato fruits. Sucharintha et al. (2018), in their study on the effect of chitosan at concentrations between 0.25 and 0.5%, found that a lower chitosan concentration (0.25%) resulted in better maintenance of physicochemical parameters (pH, TSS, ascorbic acid, weight loss and moisture) showed this further reduced microbial growth and improved sensory properties compared to the control and the higher concentration (0.5%). Parvin et al. (2018) showed that a lower chitosan concentration (0.15%) had greater effects on protecting tomato quality. Nonetheless, vitamin C content decreased with increasing chitosan concentration after 3 weeks of storage, while acid levels increased with higher chitosan concentration, potentially affecting acceptance. Findings from Romanazzi et al. (2017), however, showed that a higher chitosan coating at a concentration of 2.0% (2000 ppm) was most effective in inhibiting the loss of firmness and colour change as well as the decline in titratable acidity and fruit weight. In their study on the effects of a chitosan concentration between zero and 1500 ppm, Sakif et al. (Islam Sakif et al., 2016) found that both 500 ppm and 1000 ppm chitosan equally protected tomatoes from decay for up to 8 days.

Table 2. Effects of chitosan on the postharvest qualities of tomato fruits.

Fruit	Treatment	Effects	Reference
Cherry tomatoes	Chitosan colloid (1% [w/w]); Grapefruit seed extract (GSE) concentrations: 0.0%, 0.5%, 0.7%, 1.0%, and 1.2% [w/w].	Coating with GSE at 1.0% [w/w] was most effective on tomato qualities except for fruit colour. Efficacy was stronger at 25 °C for Chitosan-GSE coating compared to Chitosan coating without GSE	Won et al. (2018)
Tomato	Aloe vera (0, 15, 30, 45 and 60%), Chitosan (0, 0.5, 1, 1.5 and 2%)	Coating formulation with Aloe vera gel (60%) + Chitosan (2%) performed the best results. It reduced weight loss, maintained total soluble solids, pH, ascorbic acid value, acidity, and reduced microbial load. More so, it maintained the firmness and colour of tomatoes during the 12-day storage.	Farooq et al. (2023)
Tomato	Chitosan coating at concentrations (0.25% and 0.5%)	Lower Chitosan concentration (0.25%) showed better maintenance of physicochemical parameters (pH, TSS, Acidity, Ascorbic acid, Weight loss, Moisture), reduced microbial growth, and improved sensory attributes compared to the control throughout the storage period	Sucharintha et al. (2018)
Tomato	Aloevera gel (0, 20, 40, 60 and 80%)	A higher concentration of Aloe vera gel (80%) was more effective in reducing weight loss, colour changes, and maintaining firmness	Firdous et al. (2021)
Tomato	Chitosan-based films with 40% glycerol (Formulation 60/40-TM i.e. Tomato/Moringa extracts).	Coated films exhibited lower water loss (0.892 g) compared to uncoated films (1.132 g); no microbial growth in coated samples, while uncoated samples exhibited bacterial growth. Both coated and uncoated groups indicated good overall acceptability.	Canche-Lopez et al. (2023)
Tomato	Chitosan (1.0%, 1.5% and 2.0%), cinnamon extract	Combining Chitosan with cinnamon extract indicated decreased effectiveness in controlling fruit decay.	Romanazzi et al. (2017)
Tomato (Solanum lycopersicum L.)	Chitosan solutions at concentrations of 0.5%, 1.0%, and 2.0%.	2.0% Chitosan coating was most effective in inhibiting loss of firmness and colour change and in inhibiting the decline in titratable acidity and fruit weight.	Meenu et al. (2023)
Tomato	Chitosan (0.5% and 1%) applied either by dipping or spraying	Chitosan coatings applied by spraying were more effective in all analyses.	Tafi et al. (2023)
Tomato	Chitosan coating with a solution of 1.5% (wt/vol) Chitosan in 1% (vol/vol) lactic acid.	Chitosan-coated tomatoes exhibited less weight loss (216%) and increased firmness (140%) compared to the control group.	Pagno et al. (2018)
Tomato	Irradiated Chitosan solution with concentrations of 500 ppm, 750 ppm, 1000 ppm, 1500 ppm, and 2000 ppm.	1500 ppm Chitosan solution was most effective in preserving tomato qualities. However, vitamin C content decreased with increasing Chitosan concentration after a 3-week storage period. The acidity values increased with higher Chitosan concentration, potentially affecting acceptability.	Parvin et al. (2018)
Cherry tomatoes	Chitosan (CS)-based chickpea hull polysaccharides (CHPS) edible coating CHPS (0.25%, 0.50%, 0.75%, and 1.00% based on CS weight)	CS-incorporated CHPS coatings successfully lowered respiratory activity, total soluble solids, total polyphenols, firmness, weight loss, lycopene content, and vitamin C compared to the control. Results showed a correlation between coating concentration and observed effects.	Akhtar et al. (2024)
Tomato, banana (cvs. Shabri and Champa), strawberry, and oranges	Chitosan solutions (0 ppm (control), 500ppm and 1000 ppm)	Both 500 ppm and 1000 ppm of Chitosan equally protected tomatoes from decay until 8 days. Higher doses of Chitosan (1000 ppm) resulted in faster decay in strawberries.	Sakif et al. (2016)

Tomato	Chitosan 1% ; Chitosan 1% + Tomato plant extract 0.1%; Chitosan 1% + Tomato plant extract 0.3%.	Chitosan 1% + Tomato plant extract 0.1% exhibited the highest antioxidant capacity, total phenolic content, and overall acceptance.	Ruiz-Cruz et al. (2018)
Tomato	Blending edible coatings with essential oils and active compounds using nanotechnologies to overcome limitations.	Edible coatings were noted to have poor barrier properties. Some coatings impart undesirable flavours to produce.	Duguma (2022)

While other studies have suggested that chitosan concentrations as high as 2% was effective in preserving the postharvest qualities of tomato fruits (Meenu et al., 2023). However, some previous studies have shown that chitosan concentration as low as 0.25% is sufficient for the preservation of the total soluble solids, pH, ascorbic acid, weight loss and reduced microbial growth, it also improved sensory attributes compared to the control group (Succharintha et al., 2018). The study conducted by Tafi et al. (2023) shed light on a different perspective regarding the effects of varying levels of chitosan on the postharvest qualities of tomatoes. They compared the effect of Chitosan application methods (dipping or spraying) for concentrations of 0.1 and 1% on the postharvest qualities of tomato fruits (Table 2). Their results showed that spray-applied chitosan coatings were more effective in all analyses. The findings from the study conducted by Jianglian and Shaoyin (2013) indicate that a single application of chitosan may not fully inhibit certain microorganisms across a broad spectrum of fruits and vegetables, potentially resulting in fruit decay. Although their review encompassed various fruits and vegetables, it remains unclear how valid their assertion is specifically regarding tomato fruits. Some studies advocated the use of a combination of chitosan with other biochemical additives to improve the protective effect of these treatments on tomato qualities (Dobrucka et al., 2017; Semida et al., 2019; Zakriya et al., 2023). A study by Farooq et al. (2023) found that a coating formulation containing aloe vera gel (60%) + chitosan (2%) achieved the best results. It reduced weight loss, maintained total soluble solids, pH, ascorbic acid and acidity, and reduced microbial load. In addition, it maintained the firmness and colour of the tomatoes during 12 days of storage. Nevertheless, a study by Romananzzi et al. (2017) found that the combination of chitosan with cinnamon extract indicated reduced effectiveness in combating fruit decay. Some previous studies have shown that chitosan concentration as low as 0.25% is sufficient for the preservation of the total soluble solids, pH, ascorbic acid, weight loss and reduced microbial growth, it also improved sensory attributes compared to the control group (Succharintha et al., 2018). In a related study by Duguma (2022) examined the effects of blending edible coatings with essential oils and active ingredients using nanotechnologies to overcome limitations. His results showed that edible coatings had poor barrier properties. Some coatings impart undesirable flavours to products.

Potassium permanganate

The application of potassium permanganate in the preservation of tomatoes and other fruits and vegetables has been evaluated for tomato fruits (Mujtaba et al., 2014; Wabali and Esiri, 2021), as well as other fruits and vegetables (Dobrucka et al., 2017; Kapsiya et al., 2015; Sanches et al., 2019) (Table 3). A study by Alvarez-Hemandez et al. (2019) revealed that commercial-scale deployment of $KMnO_4$-based technology remains limited due to uncertainty about its potential as an effective post-harvest tool and health, environmental and safety concerns, but positive effects of potassium permanganate have been documented.

Potassium permanganate is used as an ethylene scavenger in fresh fruit and vegetable packaging, Dobrucka et al. (2017) examined the effect of potassium permanganate (6.4 g/100

ml) at 20 °C for different periods (between 3 minutes and 6 hours). The bags in the package contained 1 and 2 grams of the prepared ethylene absorber. They found that the group with ethylene absorbers had delayed mould growth compared to the group without absorbers. In a study examining the effects of Potassium permanganate concentration (2.5 ppm, 5.0 ppm, 7.5 ppm, 10.0 ppm, 12.5 ppm, and 15.0 ppm), Wabali and Esiri (2021) found that concentrations as low as 5.0 ppm were more effective in preserving tomato texture and colour under ambient conditions. In a study by Mohammed et al. (2022). The combination of 400 ppm $KMnO_4$ with a negative pressure of 50 kPa was the most effective in maintaining tomato quality (Muhammad et al., 2023). However, the results of Wabali and Esiri (2021) showed that only 5 ppm $KMnO_4$ (i.e. 0.0005% potassium permanagante) resulted in an acceptable quality in terms of colour and texture under ambient condition. Although several previous studies (Arthur et al., 2015; Semida et al., 2019; Romanazzi et al., 2017) have shown promising results for the effects of potssaium permanganate in preserving the qualities of tomato fruits, there is a need for future research to focus on assessing the toxicity and risk concerns raised on potassium permangante in some studies that examined their effects on fruit flavour (Wabali et al., 2017).

Table 3. Effects of potassium permanganate on the postharvest qualities of tomato fruits.

Fruit (s)	Treatment	Effects	Reference
Tomato	Potassium permanganate concentration (saturated)	Titratable acidity decreased over time, with 400 ppm Potassium permanganate exhibiting the highest acidity.	Mujtaba and Masud (2014)
Tomato	KMnO4-based ethylene scavenger	The use of KMnO4-based technology remains limited at a commercial scale due to uncertainty about its potential as an effective postharvest tool and concerns related to health, environment, and safety.	Alvarez-Hernandez et al. (2019)
Tomato	KMnO4 (2.5 ppm, 5.0 ppm, 7.5 ppm, 10.0 ppm, 12.5 ppm and 15.0 ppm)	5.0 ppm was more effective in preserving tomato texture and colour under ambient condition	Wabali and Esiri (2021)
Tomato	400 ppm of KMnO4; Hypobaric pressures (40 kPa or 50 kPa); A combination of KMnO4 with 40 kPa or 50 kPa hypobaric pressure	The combination of 400 ppm KMnO4 with 50 kPa hypobaric pressure was most effective in preserving tomato quality.	Muhammad et al. (2023)
Tomato (hybrid 65010)	pre-harvest foliar application of Calcium chloride levels (0.0%, 0.2%, 0.4%); Potassium thiosulfate levels (0.0%, 0.2%, 0.4%) on storage qualities of tomato	Calcium chloride at 0.4% × Potassium thiosulfate at 0.4% had the best effect on the storage qualities of tomato	Semida et al. (2019)
Tomato	Potassium permanganate (6.4 g/100 mL) at 20°C for varying times (between 3 min and 6 h). The sachets in the packaging contained 1 and 2 grams of the prepared ethylene absorber.	the group with ethylene absorbers experienced delayed mould growth compared to the group without an absorber	Dobrucka et al. (2017)

Relative performance of biochemical treatments on tomato qualities

Various studies have examined the relative influence of different treatments on maintaining the quality of harvested tomatoes (Table 4). A study by Bal et al. (2018) focused on the effect of chitosan and calcium chloride, while other researchers (Shalini et al., 2018; Shehata et al., 2021) examined chitosan and potassium permanganate. Mujtaba and Masud (2014) aimed to improve the post-harvest storage life of tomato fruit by using treatments with Calcium chloride ($CaCl_2$). They compared various concentrations of $CaCl_2$ (1%, 2%, and 3%) to assess their impact on the quality of tomato fruit during storage. The findings showed that using a 2% $CaCl_2$ solution, packed with a ventilated 0.6 mm polyethylene cover, was highly effective in minimizing storage losses and preserving the quality of the produce. Additionally, the study revealed that both storage intervals and treatments significantly influenced the quality parameters of the tomato fruits. In conclusion, the study suggested that $CaCl_2$ treatments could mitigate economic losses of perishable fruits and promote sustainable agriculture practices. Furthermore, it observed that storage duration generally led to increases in pH, titratable acidity, weight loss, ascorbic acid, total sugar, and lycopene content, while the total soluble solids (TSS) remained constant throughout the storage period.

Semida et al. (2019) found that preharvest calcium chloride as well as potassium thiosulfate at 0.2% and/or 0.4% increased total titratable acidity, vitamin C, total soluble sugar, lycopene, and firmness content of the fruit. However, there were limitations with potassium permanganate ($KMnO_4$). Due to high toxicity and insufficient long-term effectiveness at high humidity (Gaikwad et al., 2020). In a related study on tomato fruits, 2% $CaCl_2$ + $KMnO_4$ was the most effective and the shelf life of tomatoes was up to 40 days without quality and phytochemical deterioration (Zakriya et al., 2023). But in another study on tomato fruits, the following treatments were used: Palladium-enhanced nano-zeolite (0%, 1%, 2.5%, 5%); $KMnO_4$-promoted Nano zeolite (0%, 10%, 15%, 20%); 1-MCP (1-methylcyclopropene) at 30 ppm; $CaCl_2$ (Calcium chloride) (0%, 1%, 1.5%, 2%); Salicylic acid (SA): 0% (control), 0.1%, 0.5%, 1% 6. UV-C (ultraviolet-C): 0 min (control), 5 min, 10 min, 15 Min., Mansourbahmani et al. (2018) found that palladium-promoted nano-zeolite at 5% was the most effective treatment for postharvest qualities of tomatoes. When comparing the effectiveness of chitosan, calcium chloride, potassium permanganate and Boric acid, Mujtaba and Masud (2014) found that a combination of 2% calcium chloride and 800 ppm boric acid was effective in maintaining pH, titratable acidity, lycopene and β-carotene. In a similar study on tomato fruits using the following treatments: 2% $CaCl_2$ and $KMnO_4$, 1 mM salicylic acid and $KMnO_4$, 2% $CaCl_2$ and $K_2Cr_2O_7$, 1 mM salicylic acid and $K_2Cr_2O_7$, Zakriya et al. (2023) found that 2% $CaCl_2$ and 50 g $KMnO_4$ significantly reduced weight loss and titratable acidity and extended the shelf life of tomatoes up to 40 days without deteriorating quality or secondary plant substances.

When generally assessing the performance of ethylene scavengers on fruits and vegetables, Vermeiren et al. (1999) found that C_2H_4 scavengers may not yet be very successful, possibly due to insufficient adsorption capacity. $KMnO_4$-based products are limited to sachets due to the toxicity of $KMnO_4$. A review by Arah et al. (2016) found that chitosan (0.5%) had a positive effect on total soluble solids (TSS), firmness, hue angle, and weight loss. Cinnamic acid (2 mM) influenced firmness, weight loss and TSS value of tomato fruits (Dladla & Workneh, 2023; Mior-Azmai et al., 2019).

Table 4. Comparison of the efficacy of calcium chloride, potassium permanganate and chitosan on the postharvest qualities of tomato fruits.

Fruit (s)	Treatment	Effects	Reference
Tomato	Potassium permanganate applied as sachets and polymeric films	limitations of Potassium permanganate (KMnO4) due to high toxicity and inadequate long-term effectiveness in high moisture conditions	Gaikwad et al. (2020)
Tomato (hybrid 65010)	Pre-harvest foliar application of Calcium chloride (0.0%, 0.2%, and 0.4%) and Potassium thiosulfate at (0.0%, 0.2%, and 0.4%)	pre-harvest foliar Calcium chloride or Potassium thiosulfate at 0.2% and/or 0.4% increased fruit total titratable acidity, vitamin C, total soluble sugars, lycopene, and firmness contents	Semida et al. (2019)
Tomato	Palladium-promoted nano zeolite (0%, 1%, 2.5%, 5%); KMnO4-promoted nano zeolite (0%, 10%, 15%, 20%); 1-MCP (1-methyl-cyclopropene) @30 ppm; CaCl2 (Calcium chloride) (0%, 1%, 1.5%, 2%); Salicylic acid (SA): 0% (control), 0.1%, 0.5%, 1% 6. UV-C (ultraviolet-C): 0 min (control), 5 min, 10 min, 15 min	Palladium-promoted nano zeolite at 5% was the most effective treatment for the postharvest qualities of tomato.	Mansourbahmani et al. (2017)
Tomato	Calcium chloride concentrations (1%, 2%); Boric acid concentrations(400 ppm, 800 ppm); Potassium permanganate concentration (Saturated)	2% Calcium chloride and 800 ppm boric acid were effective in maintaining pH, titratable acidity, lycopene, and β-carotene.	Mujtaba and Masud (2014)
Tomato	2% CaCl2 and KMnO4 1mM salicylic acid and KMnO4 2% CaCl2 and K2Cr2O7 1 mM salicylic acid and K2Cr2O7	2% CaCl2 and 50 g KMnO4 significantly reduced weight loss and titrable acidity and extended tomato shelf life to up to 40 days with no quality or phytochemicals deterioration.	Zakriya et al. (2023)
Fruits and vegetables	KMnO4 (4 - 6%) Charcoal + PdCl Mineral packaging films (zeolites, clays, and Japanese Oya)	C2H4 scavengers are noted as not yet very successful, potentially due to insufficient adsorbing capacity. Products based on KMnO4 are limited to sachets due to the toxicity of KMnO4	Vermeiren et al. (1999)
Tomato (cv. ‘Ruchi 618’).	500 ml of Aloe vera-based coating with 0.3% antioxidant-rich herb, a thickening agent (20 g), glycerol (2%), oleic acid (3 ml), cinnamaldehyde (0.2 ml)	Coated tomatoes indicated a longer shelf life (39 days) than the control group (19 days).On the 20th day of storage, weight loss was 7.6% and 15.1% for the coated and control groups, while firmness value was 36 N for control and 46.2 N for coated tomatoes.	Athmaselvi et al. (2013)
Tomato	Chitosan coating at 0.5% Cinnamic acid coating at 2mM	Chitosan (0.5%) positively impacted total soluble solids (TSS), firmness, hue angle, and weight loss. Cinnamic acid (2mM) influenced firmness, weight loss, and TSS value.	Tonna et al. (2016)

CONCLUSION AND RECOMMENDATION

This review highlights some biochemical treatments for tomato preservation and recognizes their potential to reduce postharvest losses. Treatments studied include calcium chloride, chitosan coating, and potassium permanganate solution. The review found that the choice of treatment depends on several factors, such as tomato variety, concentration, storage conditions, ripeness and method of application. More so, the selection of treatments should be context-specific and tailored to individual needs and limitations.

There is a need for future research to explore treatment combinations considering the synergies between calcium chloride, chitosan and potassium permanganate. It will be crucial to study how different tomato varieties respond to treatments and to assess the ecological impact of synthetic chemicals such as potassium permanganate. In addition, consumer studies, safety protocols, innovative application methods and advanced analytical techniques should be prioritized to improve preservation methods and deliver high-quality tomatoes to consumers.

Declaration of interest

The authors declare that there is no conflict of interest.

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The effect of nitrogen and selenium on some phytochemical characteristics and allicin of garlic leaf

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ABSTRACT

Purpose: This research has investigated the effect of different levels of nitrogen and selenium on some growth and physiological characteristics of garlic leaves. **Research method:** This research was done as a factorial in the form of randomized complete blocks in 3 replications. The first factor included four levels of nitrogen (0, 50, 100, and 150 kg ha^{-1}) and the second factor included three levels of selenium (0, 5, and 10 mgL^{-1}). **Findings:** In all four nitrogen levels, with increasing selenium concentration, plant height (69.66 cm), fresh weight (10.66 g m^2), and dry weight (51.33 g m^2) of leaf increased. The highest amount of photosynthetic pigments was observed in the treatment of 150 kg ha^{-1} of nitrogen along with 10 mg L^{-1} of sodium selenate. Nitrogen and selenium increased antioxidant capacity (45.69 µmol $g^{-1}FW$), total phenol (295.60 mg 100 $g^{-1}FW$) and ascorbic acid (18.30 mg 100 $g^{-1}FW$). Contrary to selenium, nitrogen increased the amount of allicin in garlic leaf, and the highest amount of allicin (0.33 mgL^{-1}) was in the treatment of 150 kg ha^{-1} of nitrogen along with 0 mgL^{-1} of sodium selenate. The maximum plant height and wet and dry weight of the leaf were observed in the treatments of selenium and nitrogen, which shows the positive effect of both elements on increasing the amount of chlorophyll synthesis and, as a result increasing the amount of photosynthesis and carbon fixation, which ultimately will have, increasing the growth rate of garlic plant. **Research limitations:** None were found to report. **Originality/Value:** As a result, the treatment of 150 kg ha^{-1} of nitrogen along with 10 mgL^{-1} of sodium selenate is recommended to increase the antioxidant compounds of garlic leaf, which is a good source of these compounds and selenium in early spring, which also plays an essential role in human health.

INTRODUCTION

Garlic (*Allium sativum* L.) belongs to the Alliaceae family and is the second most widely used *Allium* next to onion (Amarakoon & Jayasekara, 2017). Garlic possesses anti-microbial, anti-carcinogenic and anti-mutagenic properties. Garlic has acquired a reputation in different traditions as a prophylactic as well as therapeutic medicinal plant. Garlic has played important dietary and medicinal roles throughout the history. Allicin ((R, S)-diallyldisulfid-S-oxide), one of the sulfur compounds from garlic, is formed by the action of the enzyme alliinase on alliin. It possesses antioxidant activity and is shown to cause a variety of actions potentially useful for human health. Allicin exhibits hypolipidemic, antiplatelet, and procirculatory effects. Moreover, it demonstrates antibacterial, anticancer, and chemopreventive activities (Efiong et al., 2020). The commonly used edible organ is the clove, while the leaves and stems are discarded during technological processing. Nevertheless, the cultivation of garlic for bunching, using the young leaves of the plant, has become increasingly popular in recent years (Adem & Tadesse, 2014). Garlic leaf consumption is an alternative to broadening the available options in the fresh vegetable market, especially in the early spring. In this case the edible young leaves in the early stage of growth and the undivided cloves are used for food, which have many medicinal properties (Jedrszczyk et al., 2018). According to research, nutritional value, protein content, sulfur compounds related to taste and phenolic compounds found in young leaves are comparable to cloves (Garg & Sekhon, 2016). Considering the many and valuable compounds that exist in garlic and its different parts, the expansion of consumption of different parts of garlic, it is essential to research the amount of chemical fertilizers used (Kumari et al., 2023).

Nitrogen (N) is an essential element for plant growth, and the availability of nitrogen is essential for the vegetative growth of agronomic and horticultural. Nitrate is one of the primary forms of nitrogen that plays an essential role in many plant metabolic processes. After water, nitrogen is one of the most important factors for plant growth. Nitrogen deficiency is associated with a decrease in the biomass of aerial organs and the leaf surface index and leads to a decrease in photosynthesis and dry matter production. At the same time, excessive nitrogen consumption in the long term will cause diseases, environmental problems, destruction of soil structure and decrease in production. Unfortunately, the use of nitrogen fertilizer in agricultural production is increasing. About 50-70% of the nitrogen consumed in the soil is not available and is not absorbed by the plant (Lei et al., 2022). Nitrogen plays a structural role in proteins and nucleic acids and is an essential component of the protein molecule. The amount of nitrogen the plant absorbs depends on the type of soil, environment, and plant species. In the stages of vegetative growth, the nitrogen requirement of garlic is essential and appropriate levels of nitrogen lead to solid growth and leaf development (Kevlani et al., 2023). Nitrogen affects the height, fresh and dry weight of the plant, stem diameter and the number of garlic leaf (Ibraheim, 2022).

Selenium (Se) is an essential microelement in human and animal nutrition, has important roles including regulation of blood lipid metabolism and potent antioxidant activity. It also protects against chronic diseases such as heart disease and cancer. The recommended intake of selenium for adults is 25 to 34 µg day^{-1} and for children 6 to 22 µg day^{-1} (Patnaik et al., 2023). Vegetables are important sources of selenium for humans, but the amount of selenium in the soil of some areas is low, which reduces the amount of selenium in vegetables. Selenium deficiency in the diet is a worldwide problem. As a result, there is an excellent demand for plants and foods enriched with selenium. Vegetables play an essential role in the human diet, so consuming selenium-enriched vegetables is an effective and safe way to compensate for selenium deficiency (Pérez et al., 2019). In plants, using appropriate levels of

selenium improves the antioxidant capacity of the plant, and leads to an increase in the growth rate and yield of the product. Selenium delays senescence and increases the efficiency of nitrogen use and nitrogen metabolism (Zhang et al., 2023), but little research has on the interaction between nitrogen and selenium. In garlic cloves, selenium increased the amount of photosynthetic pigments, nutrient absorption, antioxidant enzyme activity and nitrogen metabolism (Poldma et al., 2011 & Xia et al., 2012). Therefore, the purpose of this research is to investigate the effect of different levels of nitrogen and selenium on some morphological and physiological characteristics of garlic leaf, which has for the first time.

MATERIALS AND METHODS

This study conducted in Campus of Agriculture and Natural Resources, Razi University, Kermanshah, Iran, in year 2023, which has 34.3176°N, 47.0869°E and it is elevation averages about 1,350 meters above sea level. Average rainfall and temperature during the growing season were 36.8 mm and 11.8 ºC, respectively. This research carried out as a factorial experiment based of a randomized complete block design with two factors of different levels of nitrogen and selenium in three replications on landrace garlic of Kermanshah. The first factor included four levels of nitrogen (0, 50, 100 and 150 kg ha^{-1}) (Hashemi Jozani et al., 2020) and the second factor included three levels of selenium (0, 5 and 10 mg L^{-1} of sodium selenate) (Veisialiakbari et al., 2020). Nitrogen fertilizer from the source of urea (46% nitrogen) added to the soil in two stages, one at the same time as cultivation (October) and the other at the stage of 2 leafy garlic (December). The amount of fertilizers related to each treatment added to each plot in a strip form at the designated times and irrigation was done immediately. Selenium foliar spraying in the form of sodium selenate salt done manually in the evening, and simultaneously with nitrogen top-dressing fertilizer. After preparing the land, plots (experimental units) with dimensions of 3 × 2 m^2 were considered. Cloves were planted in rows at a depth of 5 cm in the first half of November. The distance between planting rows was 30 cm, and between plants on the row was 10 cm. immediately after the planting of the cloves, furrow irrigation carried out. The subsequent irrigations were according to the custom of the region and according to the weather conditions, the amount of rainfall, the soil conditions and environment temperature done. Before conducting the experimenting, samples were taken from the field soil up to a depth of 30 cm to analyze the soil and determine its physical and chemical characteristics, and the test results are presented in Table 1.

In early April, leaves were sampled to determine the studied traits after removing marginal effects from the surface of 0.4 m^2. The samples were taken to the laboratory to measure some morphological traits (plant height, fresh and dry weight of leaf and number of leaf) and physiological (photosynthetic pigments, total phenol, ascorbic acid, antioxidant activity, total protein and total carbohydrate) and allicin.

Table 1. Physical and chemical characteristics of experimental field soil at a depth of 30 cm.

Soil texture	pH	Electrical conductivity (dS m^{-1})	Nitrogen (%)	Phosphorus (mg Kg^{-1})	Potassium (mg Kg^{-1})
Silty-Loamy	7.7	0.87	0.11	13	280

Photosynthetic pigments

To measure chlorophylls and carotenoids, one g of garlic leaf tissue was ground in a mortar containing 10 ml of 80% acetone and then passed through filter paper. The filtered solution was used to measure photosynthetic pigments with a spectrophotometric device according to the following equations (1-4) (Lichtenthaler, 1987; Lichtenthaler &Buschmann, 2001).

$$\text{Chl a (mg L}^{-1}) = (12.7 \times A663) - (2.69 \times A645) \quad (1)$$

$$\text{Chl b (mg L}^{-1}) = (25.8 \times A645) - (4.68 \times A663) \quad (2)$$

$$\text{Chl total (mg L}^{-1}) = (20.21 \times A645) + (8.02 \times A633) \quad (3)$$

$$\text{Car (mg L}^{-1}) = \frac{[(100 \times A470 - 2.27 \times \text{Chla} - 81.4\ \text{Chlb}]}{227} \quad (4)$$

Total phenol

To measure the phenolic content of the leaves, first 0.5 g of leaf samples were utterly crushed in 4 ml ethanol (80%) and a homogeneous solution prepared. After 20 min of centrifugation at 9000 rpm, a clear supernatant solution removed. Afterward, 1200 µl of 7% sodium carbonate and 1.0 ml Folin (10%) mixed with homogenate (300 µl) then placed in a dark place for 20 min. After the required time (20 min), the absorption rate measured by a spectrophotometer at 725 nm (Model Kerry 100, Varian, America) (Singleton & Rossi, 1965).

Ascorbic acid

The ascorbic acid concentration of garlic leaf extract measured based on the color reduction of 6,2-dichlorophenol indophenol by ascorbic acid (Kapur et al., 2012). In this method, one gram of fresh garlic leaf tissue homogenized with 3 ml of metaphosphoric acid (1%). After half an hour, the above samples centrifuged a temperature of 4°C and at a speed of 6000 rpm for 15 min and50 µl removed from the solution and 200 µl of 6,2-dichlorophenol indophenol 50 µmol added to it. The absorbance of the samples read at a wavelength of 520 nm with a spectrophotometer (Model Kerry 100, Varian, America).

Total carbohydrate

Half a gram of garlic leaf tissue ground and crushed with liquid nitrogen in a Chinese mortar. Then, 5 ml of 95% ethanol was immediately added to it and shaken vigorously. The upper part of the resulting solution separated and its sediments washed twice with 5 ml of 70% ethanol and their upper phase added to the previously collected supernatant. The obtained solution centrifuged at 3500 rpm for 10 min. After separating the liquid and solid phase, the liquid part kept inside the refrigerator at a temperature of 4°C (Irigoyen et al., 1992). To measure total carbohydrate, 0.1 mL of alcoholic extract added to 3 mL of freshly prepared anthrone (150 mg anthrone + 100 mL 72% sulfuric acid), then placed in a boiling water bath for 10 min. At this time, a colored substance formed and glucose standards prepared from 0 to 0.1 µm ml^{-1}. Finally, the light absorption of standard solutions and samples read with a spectrophotometer (model Kerry 100, Varian, America) a wavelength of 625 nm (Paquin & Lechasser, 1979).

Total soluble protein

Leaf soluble protein measured based on Bradford's (1976) colorimetric method using albumin as a standard. The total soluble proteins absorbance recording was done at 595 nm by spectrophotometer.

Antioxidant capacity

The antioxidant capacity of garlic leaf measured by the DPPH method. In this method, the neutralizing activity of 2-2 diphenyl-1-picrylhydrazyl radicals determined by the methanolic extract by spectrophotometric method at a wavelength of 515 nm, which follows Lambert's law and the reduction of its absorption, has a linear relationship with the amount of antioxidant. The more the antioxidant substance added, the more DPPH consumed and the purple color tends to yellow. The DPPH is a purple compound that quickly becomes a radical due to the presence of phenyl groups in its structure, and is a source of free radicals. This compound changes color from purple to yellow by taking an electron from the antioxidant compound (D'Abrosca et al., 2007). DPPH radical neutralization activity was calculated based on the following formula. DPPH radical neutralization percentage formula, in this formula (5); AC: Absorbed DPPH radical without any antioxidant as a control As: Absorbed DPPH with the extract, and methanol used as a blank.

$$\text{DPPH} = [\frac{(\text{AC}-\text{AS})}{\text{AC}}] \times 100 \qquad (5)$$

Allicin

The outer skin of the garlic cloves peeled and crushed in a garlic press. The pressed garlic was then collected in a beaker, and mixed thoroughly. 700-900 mg of the pressed mash weighed and transferred to a 50 ml centrifuge tube. Using a volumetric pipette, 25 ml of cold water delivered to the sample and immediately capped and shaken vigorously for 30 seconds. Heat transfer from hands avoided by holding the tube cap while shaking. An additional 25 ml of cold water added and shaken for 30 more seconds to dilute and mix the solution. Each sample is filtered through 0.45µm glass filter into High – performance liquid chromatography (HPLC) vial and capped for injection. Allicin content extracted and determined according to (Hoppe et al., 1996).

Statistical analyses

Data analyzed with SAS (9.1) statistical software. Mean comparisons performed with Duncan's multiple range tests at the 1% significance level.

RESULTS

Growth characteristics of garlic plant

Nitrogen and selenium significantly affected the growth characteristics of the garlic (Table 2). The interaction between these two factors had a significant effect ($p \leq 0.05$) on plant height, fresh and dry leaf weight. The interaction between nitrogen and selenium had non-significant effect on leaf number (Table 2).

Table 2. Analysis of variance of nitrogen and selenium treatments on some growth characteristics of garlic plant.

		Mean square			
S.O.V	df	Plant height	Number of leaf	Leaf fresh weight	Leaf dry weight
Block	2	9.25**	0.25*	19.19**	1.25**
Nitrogen	3	277.44**	6.33**	1868.32**	69.18**
Selenium	2	65.33**	0.58**	174.52**	9.28**
Nitrogen×Selenium	6	8.66**	0.13^{ns}	15.82**	1.51**
Error	22	1.61	0.06	1.43	0.16
C.V (%)	-	2.14	3.19	4.20	8.10

ns, * and **: Non-significant, Significant at the 5% and 1% probability levels, respectively.

Table 3. Mean comparison of the effect of different levels of nitrogen and selenium on some growth characteristics of garlic plant.

Nitrogen (Kg ha^{-1})	Selenium (mg L^{-1})	Plant fresh weigh (cm)	Leaf fresh weight (g m^2)	Leaf dry weight (g m^2)
0	0	48.00^{i}	2.00^{i}	11.00^{j}
	5	53.33^{h}	2.50^{hi}	14.66^{j}
	10	55.00^{gh}	2.80^{gh}	16.66^{i}
50	0	56.66f^{g}	3.16^{gh}	19.66^{h}
	5	58.33^{ef}	3.43^{fg}	20.66^{gh}
	10	60.00^{de}	3.96^{f}	22.00^{g}
100	0	60.00^{de}	4.80^{e}	26.00^{f}
	5	61.00^{cd}	5.60^{d}	32.33^{e}
	10	61.33^{cd}	6.43^{c}	38.33^{d}
150	0	62.66^{bc}	6.86^{c}	41.33^{c}
	5	64.00^{b}	8.66^{b}	48.33^{b}
	10	69.66^{a}	10.66^{a}	51.33^{a}

Similar letters in each column indicate non-significant difference at the 5% probability level according to Duncan's multiple range test.

In all four nitrogen levels, plant height, fresh and dry leaf weight increased with increasing selenium concentration. The maximum plant height (69.66 cm), and fresh weight (10.66 g m^2) and dry weight (51.33 g m^2) of leaf observed in the treatment of 150 kg ha^{-1} of nitrogen along with 10 mg L^{-1} of sodium selenate, which had significantly of different with the control treatment (Table 3).

According to the obtained results, nitrogen and selenium had a positive effect on the number of leaf, and the highest number of leaf was observed at the highest levels of nitrogen (9.11) and selenium (8.41), which were significantly different with the control (Fig. 1a and b).

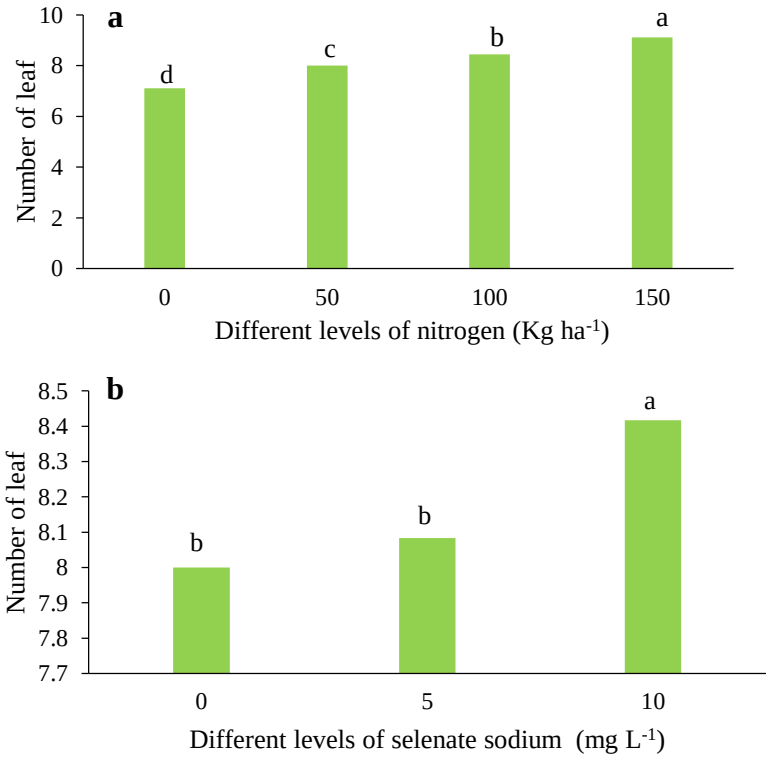

Fig. 1. The effect of different levels of nitrogen (a) and selenium (b) on number of garlic leaf.

Table 4. Analysis of variance of nitrogen and selenium treatments on photosynthetic pigments of garlic leaf.

		Mean square			
S.O.V	df	Chlorophyll a	Chlorophyll b	Total chlorophyll	Carotenoid
Block	2	0.0007**	0.00012**	0.0028**	0.00034*
Nitrogen	3	0.1095**	0.02774**	0.1819**	0.02501**
Selenium	2	0.0094**	0.00107**	0.0199**	0.00203**
Nitrogen× Selenium	6	0.0004**	0.00008**	0.0005*	0.00026*
Error	22	0.00008	0.000020	0.0002	0.00008
C.V (%)	-	1.61	2.50	2.31	3.93

* and **: Significant at the 5% and 1% probability levels respectively.

Photosynthetic pigments

The results showed that nitrogen and selenium significantly affected the photosynthetic pigments in garlic leaves. Also, the interaction between two factors was significant on chlorophyll a, chlorophyll b ($p\leq0.01$), total chlorophyll, and carotenoid ($p\leq0.05$) (Table 4).

Nitrogen and selenium had a positive effect on the photosynthetic pigments. The highest amount of chlorophyll a (0.70 mg g^{-1} FW), chlorophyll b (0.2490 mg g^{-1} FW), and total chlorophyll (0.9113 mg g^{-1} FW) in the treatment of 150 kg ha^{-1} of nitrogen along with 10 mg L^{-1} of sodium selenate observed and the lowest of the photosynthetic pigments were in the control treatment. At the concentration of 50, 100 and 150 kg ha^{-1} of nitrogen, significant of difference was not observed in the carotenoid content of garlic leaf at the levels of 5 and 10 mg L^{-1} of sodium selenate (Table 5).

Physiological traits

According to the results (Table 6), nitrogen, selenium and the interaction between nitrogen and selenium significantly affected at 1% probability level on total phenol, ascorbic acid, antioxidant capacity, total soluble protein and allicin leaf. While the interaction between two factors on total soluble protein and total carbohydrate was non-significant (Table 6).

Table 5. Mean comparison of the effect of different levels of nitrogen and selenium on photosynthetic pigments of garlic leaf.

Nitrogen ($Kgha^{-1}$)	Selenium (mgL^{-1})	Chlorophyll a (mgg^{-1} FW)	Chlorophyll b (mgg^{-1} FW)	Total chlorophyll (mgg^{-1} FW)	Carotenoid (mgg^{-1} FW)
0	0	0.4033^{k}	0.1133^{i}	0.4973^{g}	0.1366^{h}
	5	0.4373^{j}	0.1236^{h}	0.5650^{f}	0.1646^{g}
	10	0.4566^{i}	0.1273^{h}	0.5923^{f}	0.1813^{f}
50	0	0.4786^{h}	0.1360^{g}	0.6300^{e}	0.2063^{e}
	5	0.5000^{g}	0.1466^{f}	0.6566^{e}	0.2326^{d}
	10	0.5600^{f}	0.1670^{e}	0.7200^{d}	0.2380^{d}
100	0	0.5933^{e}	0.2160^{d}	0.7566^{c}	0.2600^{c}
	5	0.6310^{d}	0.2213^{cd}	0.7833^{c}	0.2600^{c}
	10	0.6376^{d}	0.2253^{c}	0.8366^{b}	0.2623^{c}
150	0	0.6550^{c}	0.2276^{b}c	0.8516^{b}	0.2690^{bc}
	5	0.6843^{b}	0.2346^{b}	0.8663^{b}	0.2813^{ab}
	10	0.7000^{a}	0.2490^{a}	0.9113^{a}	0.2930^{a}

Similar letters in each column indicate non-significant difference at the 5% probability level according to Duncan's multiple range test.

With the increase in nitrogen and selenium concentration, the amount of total phenol in garlic leaf increased. A significant difference observed between the high levels of nitrogen and selenium with the control treatment. (Table 7). Selenium and nitrogen had a positive effect on the amount of ascorbic acid and antioxidant capacity of garlic leaf. The highest amount of ascorbic acid (18.30 mg 100 g^{-1} FW) and antioxidant capacity (45.69 µmol g^{-1} FW) observed in the treatment of 150 kg ha^{-1} of nitrogen along with 10 mg L^{-1} of selenium. The lowest amount of ascorbic acid (4.62 mg 100 g^{-1} FW) and antioxidant capacity (12.44 µmol g^{-1} FW) was in the control treatment (Table 7).

Table 6. Analysis of variance of nitrogen and selenium treatments on some antioxidant compounds of garlic leaf.

S.O.V	df	Mean					
		Total phenol	Ascorbic acid	Antioxidant capacity	Total soluble	Total carbohydrate	Allicin
Block	2	251.12^{ns}	2.37**	9.73**	5.50**	9.65**	0.00016**
Nitrogen	3	20839.247**	156.55**	767.80**	514.93**	432.09**	0.01712**
Selenium	2	1148.46**	24.99**	120.86**	29.98**	56.14**	0.00138**
Nitrogen× Selenium	6	379.34**	1.88**	14.51**	0.66^{ns}	2.20^{ns}	0.00002**
Error	22	757.21	0.20	1.42	0.45	0.99	0.000006
C.V (%)	-	12.29	4.33	4.25	2.34	1.72	1.01

ns, * and **: Non-significant, Significant at the 5% and 1% probability levels, respectively.

Table 7. Mean comparison of the effect of different levels of nitrogen and selenium on some antioxidant compounds of garlic leaf

Nitrogen (Kg ha^{-1})	Selenium (mg L^{-1})	Total phenol (mg 100 g^{-1} FW)	Ascorbic acid (mg 100 g^{-1} FW)	Antioxidant capacity (µ mol g^{-1} FW)
0	0	174.98^{ef}	4.62^{i}	12.44^{j}
	5	147.87^{f}	6.00^{h}	18.28^{i}
	10	172.33^{ef}	7.24^{g}	21.64^{h}
50	0	196.00^{def}	7.48^{g}	24.20^{g}
	5	214.33^{cde}	8.00^{g}	25.83^{fg}
	10	220.76^{cde}	9.94^{f}	27.42^{ef}
100	0	231.14^{bcd}	11.36^{e}	28.16^{e}
	5	240.51^{bcd}	12.06^{de}	29.25^{de}
	10	250.27^{abc}	12.78^{cd}	30.59^{d}
150	0	262.00^{abc}	13.46^{c}	35.15^{c}
	5	279.00^{ab}	14.72^{b}	38.42^{b}
	10	295.60^{a}	18.30^{a}	45.69^{a}

Similar letters in each column indicate non-significant difference at the 5% probability level according to Duncan's multiple range test.

Based on the obtained results (Fig. 2), the amount of total soluble protein and total carbohydrate in garlic leaf increased with nitrogen content. So that their highest and lowest amount was observed at the level of 150 kg ha^{-1} of nitrogen and control, respectively. Selenium, like nitrogen, had a positive effect on the amount of total soluble protein and total carbohydrate in garlic leaf. With increasing selenium concentration, the amount of total soluble protein (30.40 g 100 g^{-1} DW) and of total carbohydrate (60.05 g 100 g^{-1} DW) increased in garlic leaf compared to the control treatment (Fig. 2).

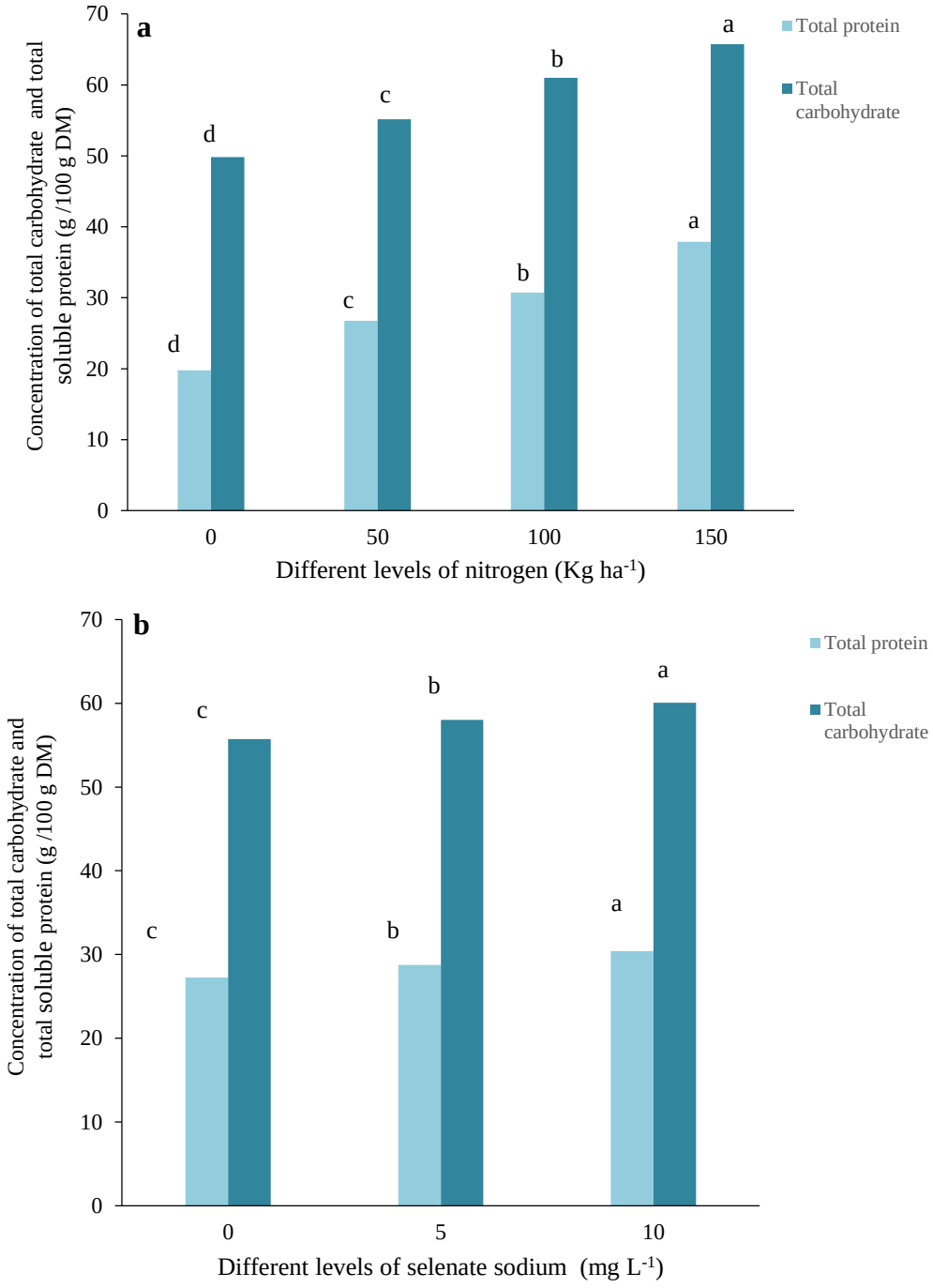

Fig. 2. The effect of different nitrogen levels (a) selenium (b) on total carbohydrate and total soluble protein.

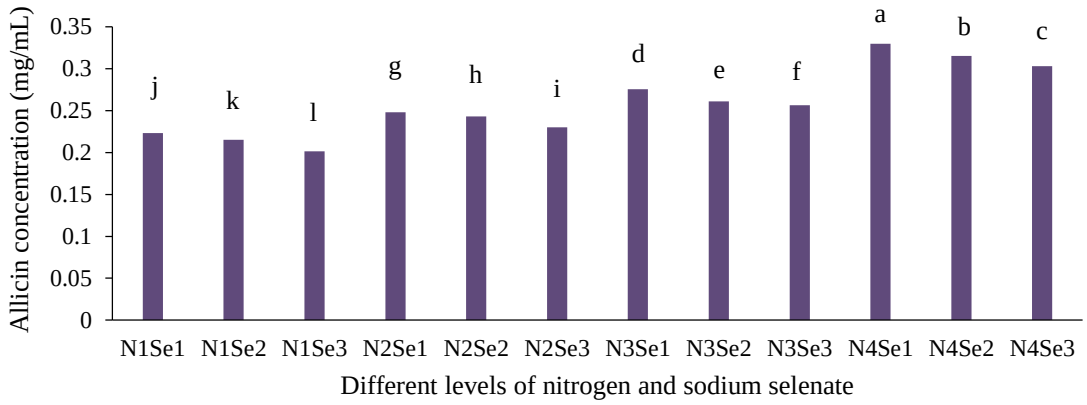

Fig. 3. The effect of different levels of nitrogen and selenium on allicin garlic leaf. N_1, N_2, N_3 and N_4: 0, 50, 100 and 150 Kg ha^{-1} nitrogen, respectively- Se_1, Se_2 and Se_3: 0, 5 and 10 mg L^{-1} sodium selenate, respectively.

Unlike selenium, nitrogen had a positive effect on the amount of allicin in garlic leaf (Fig. 3). In all four levels of nitrogen, the amount of allicin in garlic leaf decreased with increasing selenium concentration. The highest amount of allicin (0.33 mg mL^{-1}) observed in the treatment of 150 kg ha^{-1} of nitrogen along with 0 mg $L^{-1}$of sodium selenate, which was significantly different from the control treatment (Fig. 3).

DISCUSSION

According to the numerous reports on the chemical composition of garlic cloves, the central part of the garlic plant that used, the information on the nutritional and medicinal value in its other organs, especially the leaf, is only available in a few reports. The results have shown that garlic leaves can be an excellent substitute for garlic cloves, especially in early spring when there is a shortage of good-quality garlic cloves and fresh garlic is not yet ready to harvest. Harvesting of young leaves can be started from February 10 and continue until March 11. Also, during the investigations, the amount of phenolic and antioxidant compounds of garlic leaf is comparable to cloves and even higher (Jedrszczyk et al., 2018).

Nitrogen is the most important element required by the plant compared to other commonly used elements and plays a vital role in increasing plant yield (Mahboob et al., 2023). A decrease in soil nitrogen is associated with a decrease in the production of vegetative organs and leaf surface, which ultimately causes a decrease in photosynthesis and the production of dry matter in the plant (Alizadeh Sehzabi et al., 2007; Berenguer et al., 2009). According to the results, the amount of nitrogen used up to the level of 150 kg ha^{-1} during two stages has improved the growth characteristics of the garlic plant. The increase in the height of the garlic plant can be due to the positive effect of nitrogen on stimulating the growth of the plant, the ability of the plant to have more access to nutrients and the improvement of the water holding capacity of the soil. At the same time, applying nitrogen can increase the capacity of photosynthesis and vegetative growth of plant organs (Leesawatwong & Rerkasem, 2003). Nitrogen increases the synthesis of auxin and cytokinin hormones, which is associated with an increase in cell division and cell length, leading to an increase in leaf fresh weight, internode length, and plant height (Wajid et al., 2007). It can be said that the optimal management of fertilizer levels has played an essential role in increasing the growth characteristics of garlic, which is in accordance with the results of the study conducted on

garlic by Singh and Sharma (2023). Selenium increased the growth characteristics of garlic compared to the control treatment (Table 2), which is by the results obtained in onion (*Allium cepa* L.) (Hamed-Far et al., 2022). This, increase can be attributed to the positive effect of selenium on chlorophyll synthesis, carbon fixation, starch synthesis, and stimulation of cell division in meristem cells (Bakhtiari et al., 2023). Selenium in low levels stimulates growth and increases plant growth and photosynthetic activity. However, at high levels, it causes leaf chlorosis, and necrosis, reduced growth and even death before the plant matures. There is also a direct relationship between selenium concentration and glutathione peroxidase activity, which leads to a delay in senescence and increased plant growth (Moulick et al., 2023). Therefore, the concentration of 20 mg L^{-1} of sodium selenate recommended to increase the growth characteristics of the garlic plant. Of course, it is worth noting that garlic is one of the plants that accumulate selenium and is part of the selenophorous plants. As a result, it can convert mineral selenium into an organic form, especially on its sulfur compounds, so the problem of poisoning does not occur (Hamed-Far et al., 2023).

There are few reports on the interaction between nitrogen and selenium. According to the obtained results, the interaction between nitrogen and selenium improved the growth characteristics of the garlic plant, consistent with the results of Amerian et al. (2018) and Veisi-Ali Akbari et al. (2020) in onion. According to the research conducted in lettuce (*Lactuca sativa* L.), selenium increased nitrogen metabolism, which was associated with increased nitrogen efficiency and yield. Selenium at low levels of nitrogen can increase the growth characteristics of lettuce by increasing the amount of chlorophyll and nitrogen metabolism (Bian et al., 2020), which is according to the results obtained from the research. In the present research, with increasing concentrations of nitrogen and selenium, the amount of photosynthetic pigments in garlic leaf increased (Table 5). Chlorophyll is a pigment whose primary responsibility is to receive light energy for photosynthesis. The chlorophyll molecule consists of two parts (a porphyrin head and a long hydrocarbon with a phytol sequence). Porphyrin consists of four nitrogen-containing pyrrole rings arranged in a ring. The complement of the chlorophyll molecule is a magnesium ion that forms a chelate with four nitrogen atoms in the center of the ring (Ahmadi et al., 2004). Chlorophyll as a place to absorb light and synthesize substances necessary for the growth, and development of plants depends on nitrogen. Nitrogen deficiency causes yellowing of old leaves and finally plant growth stops (Gao et al., 2023). In fact, nitrogen plays an essential role in the structure of chlorophyll, which leads to an increase in photosynthetic substances (Rahmani et al., 2008), which is associated with an increase in plant growth (Table 3). Selenium also increases the amount of chlorophyll in leaves by protecting chloroplast enzymes, increasing the biosynthesis of photosynthetic pigments and the efficiency of photosystem II (Safaryazdi et al., 2012). Selenium is involved in the path of chlorophyll biosynthesis and the interaction of selenium with enzymes containing sulfhydryl-SH group, 5-aminolevuinic acid dehydratase and deaminase porfobilinogen is effective in the amount of leaf chlorophyll (Nowaka et al., 2004). By increasing the amount of carotenoid and reducing oxidative stress, selenium increases the amount of sugars in plants, which provides the energy needed by the plant and causes the plant's survival (Khan et al., 2023). Carotenoids are antioxidant pigments that limit chlorophyll peroxidation and chloroplast degradation. Selenium in the appropriate concentration increases the amount of carotenoid, which leads to an increase in chlorophyll and the efficiency of photosynthesis (Tufail et al., 2023). Selenium can increase carotenoids by improving plant metabolism under normal conditions (Feng & Wei, 2012). An increase in the amount of chlorophyll pigments at a concentration of 3 mg L^{-1} has been reported in okra (*Abelmoschus esculentus* L.) (Ali et al., 2023) and pepper (*Capsicum annuum* L.) (Hassan et al., 2023). Nitrogen and selenium had a positive effect on the photosynthetic pigments of

garlic leaf, which could be due to the increase in the synthesis of enzymes related to chlorophyll synthesis (Li et al., 2023).

Nitrogen is one of the most essential elements in the synthesis of proteins, so an increase in nitrogen to a certain extent is associated with an increase in protein, which leads to an increase in plants height and the number of leaves (Chong et al., 2023). Selenium, with its antioxidant properties, increases enzymes and antioxidant compounds and supports all types of proteins by increasing the clearance of free radicals (Cunha et al., 2023). In potato plant (*Solanum tuberosum* L.), different levels of nitrogen and selenium led to an increase in carbohydrates and protein (Li et al., 2023).

Polyphenols are antioxidant compounds and can absorb free radicals. These compounds also play an essential role in the human body, such as protecting the body against various cancers (Torabi-Toran-Pashtoshti et al., 2022). Considering the benefits of these compounds and their presence in plants, it seems necessary to investigate natural sources to find antioxidant and phenolic compounds. In Iran, the consumption of vegetables is high, which, besides providing fiber, vitamins and minerals, can be a good source of phenolic and antioxidant compounds. Garlic has high antioxidant activity due to having phenolic compounds in its structure. Garlic is a rich source of phytochemicals, including antioxidant compounds such as phenols, flavonoids, and allicin, which play an essential role in human health (Pascual-Teresa et al., 2010). Environmental conditions and fertilizer management play an essential role in the compounds found in different organs of garlic, including antioxidants, phenol and flavonoids. The presence of these potent antioxidants in garlic increases human health and resistance to oxidative stress (Rahman et al. 2012). Nitrogen fertilizer management (levels of 150 and 250 kg ha^{-1}) in onion has led to an increase in compounds (total phenol, ascorbic acid, etc.) and antioxidant enzymes (Barrales-Heredia et al., 2023). With the application of selenium, the increase in total phenol related to the increase in the activity of the enzyme phenylalanine ammonia-lyase (Elguera et al., 2013; Abdalla & Mühling, 2023). The increase in the antioxidant activity of the plant under the influence of selenium may be due to the increase in phenolic compounds, the effect of selenium on glutathione redox metabolism. The enzymes involved in this metabolism and, the direct antioxidant the effect of selenium and its organic metabolites (Rad et al., 2019).

Plant nutrition, including medicinal plants, is one in the most essential influencing factors in metabolism, and the amount of secondary metabolites. Allicin is the main component of the biologically active compounds of garlic, which is the result of the degradation of alliine by alliinase. The leaf is the primary site of synthesis of cysteine sulfoxide (alliine), from where it transferred to other parts of the plant such as the clove (Nguyen et al., 2022). According to the obtained results, the amount of allicin in garlic leaf increased with the increase of nitrogen concentration (Fig. 3).

CONCLUSION

The results of this research indicates that the management of nitrogen and selenium fertilizers can play an important role in the growth characteristics, photosynthetic pigments and antioxidant compounds of garlic leaf. Since selenium affects nitrogen metabolism, as a result, simultaneous application of sodium selenate with nitrogen had a vital role in improving some growth and physiological characteristics of garlic plants. The maximum plant height and wet and dry weight of the leaf observed in the treatments of selenium and nitrogen, which shows the positive effect of both elements on increasing the amount of chlorophyll synthesis and, as a result, increasing the amount of photosynthesis and carbon fixation, which ultimately will have increases the growth rate of garlic plant. As a result, the treatment of 150 kg ha^{-1} of

nitrogen along with 10 mg L^{-1} of sodium selenate is recommended to increase the antioxidant compounds of garlic leaf, which is a good source of these compounds and selenium in early spring, which also plays an essential role in human health.

Conflict of interest

The authors have no conflict of interest to report.

Acknowledgments

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