JHPR

www.jhpr.birjand.ac.ir

Volume 8, Issue 4, December 2025, E-ISSN: 2588-6169

Journal of Horticulture and Postharvest Research

An International Journal

JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH
2025, VOL. 8(4), 427-450

Journal of Horticulture and Postharvest Research

Journal homepage: www.jhpr.birjand.ac.ir

University of Birjand

Efficacy of peppermint oil-incorporated tragacanth gum coating on postharvest quality and antioxidant enzyme activity in banana fruits

Oluwagbenga Oluwasola Adeogun[1,*], Adeniyi Adetola Sanyaolu[2], Temitope Samuel[1], Aliyu Abubakar[3], Mosqurat Abisola Agbabiaka[1] and Adedotun Adeyinka Adekunle[1]

1, Department of Botany, Faculty of Science, University of Lagos, Lagos, Nigeria
2, Department of Botany and Ecological Studies, University of Uyo, Akwa Ibom, Nigeria
3, Department of Botany, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Nigeria

ARTICLE INFO

Original Article

Article history:
Received 26 December 2024
Revised 29 May 2025
Accepted 6 June 2025

Keywords:
Aspergillus fumigatus
Colletotricum musae
Edible coating
Peroxidase
Musa sapientum

DOI: 10.22077/jhpr.2025.8413.1448
P-ISSN: 2588-4883
E-ISSN: 2588-6169

***Corresponding author:**
Department of Botany, Faculty of Science, University of Lagos, Lagos, Nigeria.

Email: adeogunoluwagbenga@gmail.com

ABSTRACT

Purpose: This study investigated the effectiveness of tragacanth gum (TG) coatings integrated with peppermint oil (PO) in controlling pathogenic fungi in banana fruits. **Research Method**: The research specifically evaluated the *in-vitro* and *in-vivo* responses of these coating agents on fungal pathogens and assessed their impact on peroxidase (POD) and catalase (CAT) activities in banana fruits. **Findings**: The study revealed that tragacanth gum integrated with peppermint oil (TGPO) effectively inhibited the growth of *Colletotrichum musae* and *Aspergillus fumigatus* in bananas. TGPO-treated fruits exhibited significantly lower disease incidence (32.67±1.00 % for *C. musae* and 28±1.00 % for *A. fumigatus*) and severity compared to the controls after 14 days. The treatment also maintained higher catalase and peroxidase enzymatic activities, indicating enhanced disease resistance. Furthermore, TGPO-treated bananas retained better quality parameters during the 15-day storage period, including optimal pH levels, lower total soluble solids, reduced water activity, greater firmness, and decreased electrical conductivity. **Research limitations**: No limitations were identified during the course of carrying out this study. **Originality/Value**: The originality and value of this work lie in being the first study to investigate TGPO as a natural preservative coating for bananas. While previous research has examined various edible coatings and essential oils separately, this study uniquely demonstrates how integrating tragacanth gum and peppermint oil creates an effective, eco-friendly solution for extending banana shelf life by controlling fungal pathogens (particularly *C. musae* and *A. fumigatus*) while maintaining fruit quality. This novel approach addresses growing consumer demand for natural food preservation methods while potentially reducing post-harvest losses in the banana industry.

INTRODUCTION

Fruits and vegetables are among the most widely consumed plant-based foods, recognized for their high concentrations of essential vitamins, minerals, fibres, and phytochemicals, which contribute to their numerous health benefits (Samtiya et al., 2021; Slavin & Lloyd, 2012). Consumer preferences for fresh produce are greatly influenced by visual qualities such as size, freshness, appearance, and shelf life, which play a significant role in purchasing decisions (Slavin & Lloyd, 2012; Török et al., 2023). Increasing consumer demand for fresh, natural, minimally processed, and additive-free foods has heightened the importance of maintaining post-harvest quality (Mesías et al., 2021).

However, substantial post-harvest losses of fruits can occur due to a range of factors, including premature harvesting, improper handling, and substandard materials during processing, inappropriate transportation, and poor retail practices (Rajapaksha et al., 2021). In particular, pest infestations, fungal infections, and inadequate storage conditions accelerate spoilage, leading to significant reductions in both fruit quality and marketability (Jiao et al., 2022; Wang et al., 2022).

To address these challenges and extend the shelf life of fresh produce, edible coatings derived from natural sources, including plants and animals, have emerged as an eco-friendly solution. Edible coatings composed primarily of polysaccharides, proteins, and lipids, form a protective barrier around food products, helping to control moisture loss, gas exchange, and microbial contamination (Mendy et al., 2019; Thakur et al., 2018). Such coatings, applied via dipping, brushing, or spraying, create a semi-permeable membrane that suppresses respiration and enhances food preservation (Raghav et al., 2016). Protein-based coatings typically exhibit superior mechanical properties, while polysaccharide-based coatings are more effective in reducing gas permeability and water transfer (Purewal et al., 2024). Understanding the water activity (${}^{a}{}_{w}$) of coated fruits and vegetables is essential for determining the effectiveness of these coatings, as ${}^{a}{}_{w}$ directly influences microbial growth, enzymatic reactions, and overall food stability (Perez-Vazquez et al., 2023; Tapia et al., 2008). Lowering ${}^{a}{}_{w}$ can significantly reduce spoilage rates by restricting the availability of free water, which is necessary for microbial proliferation (Tapia et al., 2008).

Several studies have explored the effectiveness of edible coatings in extending the shelf life of bananas (Iacovino et al., 2024). Chitosan-based coatings, for instance, have been widely studied for their antifungal properties and ability to reduce weight loss and delay ripening in bananas (Hossain & Iqbal, 2016). Similarly, pectin-based coatings have demonstrated potential in maintaining fruit firmness and minimising microbial decay (Moalemiyan et al., 2012; Sanchís et al., 2017). In addition to polysaccharide-based coatings, research has also investigated the incorporation of natural compounds such as essential oils to enhance the antimicrobial and antioxidant properties of edible coatings (Anis et al., 2021; de Souza et al., 2019; López et al., 2015). Essential oils, including thyme, peppermint and cinnamon oil, have been shown to improve post-harvest quality and reduce fungal infections in various fruits, including bananas (Mohammadi et al., 2020; Pawar et al., 2024). The established literature highlights the potential of integrating natural bioactive compounds into edible coatings to improve shelf life and postharvest management, particularly for fruits like bananas.

Bananas (*Musa sapientium*), a major staple in tropical and subtropical regions, are particularly susceptible to post-harvest spoilage (Alhassan & Ndomakaah, 2024). Rich in essential nutrients such as vitamins A and C, potassium, and fibres, bananas play a crucial role in global food security (Rajapaksha et al., 2021). Tragacanth gum (TG), a natural polysaccharide derived from various *Astragalus* species, has gained attention for its potential in edible coatings due to its emulsifying properties and water-soluble components (Azarikia &

Abbasi, 2016; Gavlighi et al., 2013). When combined with essential oils, such as peppermint oil (*Mentha piperita* L.), tragacanth gum-based coatings may offer both preservative and antioxidant benefits, potentially extending the shelf life of perishable fruits like bananas (Singh et al., 2015).

Given the growing interest in natural alternatives instead of chemical preservatives, this study aims to evaluate the efficacy of tragacanth gum coatings impregnated with peppermint oil in assessing the *in-vitro* and *in-vivo* responses of these coating agents on pathogenic fungi associated with banana fruits. In addition, the study also investigates the impact of tragacanth gum coatings impregnated with peppermint oil on peroxidase (POD) and catalase (CAT) activities in banana fruit.

MATERIALS AND METHODS

Sources of test samples

Healthy, green *Musa sapientum* fruits, which had not yet fully ripened, were collected from the middle part of the bunch to ensure uniform exposure to sunlight and consistent ripening conditions. These fruits were free from peel damage, insect infestation, and fungal infection, with a maturity index of 22% by dry matter prior to treatment. They were obtained through a fruit vendor at a commercial market in Yaba, Lagos State, Nigeria. Tragacanth gum (TG) and peppermint oil (PO) were sourced from the Mycology Laboratory, Department of Botany, University of Lagos.

Media preparation and isolation of fungal pathogens

Potato Dextrose Agar (PDA) was prepared by dissolving 40 g of PDA powder in 1,000 mL of distilled water. The mixture was sterilized at 121°C for 15 minutes under 15 lbs of pressure in an autoclave. Once cooled to 45–50°C, 0.5 g/L of chloramphenicol was added to inhibit bacterial growth. The medium was poured into Petri dishes (15–20 mL per plate) and allowed to solidify. Small sections (5 mm) of banana tissue showing infection symptoms were surface-sterilized with 40% sodium hypochlorite for 3 minutes, rinsed three times with sterile distilled water, and air-dried on sterile filter paper. The tissue sections were then aseptically placed on PDA plates and incubated at room temperature (25 ± 2°C). Pure fungal isolates were obtained by repeated subculturing on fresh PDA plates (Matche & Adeogun, 2022). Thereafter, a pathogenicity assay was conducted to ascertain the pathogenicity of the isolated fungi on the fruits. The symptomless and surface-sterilized banana fruits were wounded and inoculated with fungal cultures. The fruits were incubated under high humidity for six days then examined for disease symptoms. Morphological and microscopic analyses confirmed the pathogenicity of the reisolated fungi (Matche & Adeogun, 2022).

Preparation of tragacanth gum (TG) and peppermint oil (PO) coating

The coating formulation was prepared by dissolving 10 g (10% $^{w}/_{v}$) of Tragacanth gum (TG) into 90 mL of deionized water, followed by equilibration at 70°C for 25 minutes (Adekunle et al., 2021; Adeogun et al., 2023). The mixture was then stirred using a magnetic stirrer, and 2 mL of glycerol was introduced as a plasticizer to improve the mechanical properties of the coating. The pH was adjusted to 5.6 using 1N sodium hydroxide (NaOH) while homogenizing the solution for 5 minutes. Thereafter, the solution was filtered using Mira-cloth and 0.5 mL (0.5%) of peppermint oil (PO), pre-dissolved in 99.5 mL of ethanol, and incorporated into the filtered Tragacanth gum solution (Adeogun et al., 2023; Nasiri et al., 2018). The same preparation was made for the control sample without the addition of the oil.

Preparation of spore suspension

Fungal cultures aged 8–10 days, used for spore preparation, were used based on a modified method of Hojnik et al. (2019). Individual cultures were flooded with 10 mL of an aqueous Tween 80 solution (1 drop in 1000 mL sterile water) and gently scraped with a sterile loop to dislodge the spores. The resulting crude suspension was filtered through a layer of Miracloth to remove mycelial fragments. One gram of fungal mycelium was suspended in 9 mL of sterile distilled water. The spore suspension was serially diluted seven times with sterile distilled water and then stored for subsequent use.

***In vitro* antifungal assay**

The antifungal efficacy of TG and TG-PO was tested against isolated pathogenic fungi from banana fruit. PDA was amended with: TG alone (10%, w/v), and TG (10%, w/v) incorporated with PO (0.5%, v/v). Fungal discs (5 mm) from 14-day-old cultures of the pathogenic fungi were placed at the centre of Petri dishes containing the treated PDA. Control plates containing only PDA were also prepared. The plates were incubated at 25 ± 2°C, and radial mycelial growth was measured daily for 6 days. Antifungal activity was assessed by measuring the colony diameter (cm) using a ruler (Matche & Adeogun, 2022).

In vivo antifungal assay on banana fruits using poisoned food technique

The *in-vivo* antifungal effects of tragacanth gum (TG) and TG incorporated with peppermint oil (TGPO) were evaluated on the fruit of *Musa sapientum* (banana) using the poisoned food technique. Bananas that were not yet fully ripe, with a 22% maturity index based on dry matter and exhibiting yellow colouration, were washed with 20% sodium hypochlorite, rinsed three times with distilled water, and air-dried. Some of the fruit were dipped in TG alone, while others were dipped in the TGPO solutions for 2–3 minutes. The last set, acting as a control, was dipped in sterile distilled water for 2–3 minutes, and all treatments were then air-dried. After treatment, the fruit was inoculated with spore suspensions of the test pathogens using a sterile needle. All treated fruit was wrapped in aluminum foil, packed in commercial cartons, and stored at room temperature. Disease incidence and severity were monitored at 2-day intervals for up to 14 days.

Disease severity was assessed on a scale of 1 to 5, with 1 indicating that 0% of the fruit surface was rotted, and 5 indicating that 76–100% of the fruit surfaces were rotted. Disease incidence was recorded as the percentage of fruits showing symptoms of rot (Kumar et al., 2021; Mohammed Idris et al., 2015).

Measurement of antioxidant enzymatic activities

The extraction of tissue samples for the determination of antioxidant enzyme activities follows the methods described by Chen et al. (2015). Tissue samples (1 g) were collected from an area 2 mm away from the inoculation site on 20 bananas. The samples were homogenized in appropriate buffer solutions and then centrifuged at 15,000 g for 30 minutes at 4°C. Supernatants were used to assess peroxidase (POD) and catalase (CAT) activities, using a sodium phosphate buffer (100 mM, pH 7).

Peroxidase (POD) activity assay

POD activity was determined following the method of Sellamuthu et al. (2013), with modifications. The reaction mixture contained 144 µL of buffered substrate (100 mM sodium phosphate, pH 7.0, and 20 mM guaiacol) and 36 µL of tissue extract. After adding 72 µL of H_2O_2 (100 mM), the increase in absorbance at 460 nm was measured over 120 seconds. Enzyme activity was expressed as ΔA_{460} (min^{-1} mg $protein^{-1}$).

Catalase (CAT) activity assay

CAT activity was measured according to Beers and Sizer (1952), with slight modifications. The reaction mixture included 150 µL of sodium phosphate buffer (pH 7.0, 100 mM), 50 µL of H_2O_2 (100 mM), and 50 µL of enzyme extract. The breakdown of H_2O_2 was monitored at 240 nm, and enzyme activity was expressed as units per mg of protein, where one unit is defined as the conversion of 1 µmol of H_2O_2 per minute.

Quality assessment of treated banana fruits

The banana fruits were coated with Tragacanth gum, either incorporated with peppermint oil (TGPO), alone (TG), or treated separately with double-distilled water (CTRL). They were then stored and analyzed at intervals of Days 0, 5, 10, and 15, at a temperature of 29.63±0.072°C and a humidity of 67.33±0.272%.

pH measurement

The pH of the samples was determined using a pH probe (Hanna 37030, Germany) at ambient temperature with consistent stirring. The hydrogen ion concentration in the solution was represented as the negative logarithm of its activity (Adeogun et al., 2020).

Total soluble solids (TSS)

Total soluble solids were assessed following the procedure outlined by Adeogun et al. (2023). Measurements were taken using a handheld refractometer (Erma, Japan) at 20°C. The refractive index was recorded and expressed in degrees Brix (°Brix).

Water activity

Water activity of the samples was evaluated with an Amtast Water Activity Analyzer (WA-60A, USA) set at 20°C. Calibration of the device was performed using two reference standards: 6.0 Molal NaCl in water (aw = 0.760) and 0.5 Molal KCl in water (a_w = 0.984). Samples were cut into pieces, placed in sealed plastic containers, and introduced into the calibrated instrument's chamber (Parreidt, 2018).

Firmness

The firmness of banana fruits was measured using a manual firmness tester (Graigar, China). For each fruit, two firmness readings were taken at its equatorial section, rotating the fruit by 180° between measurements (Tesfay et al., 2017).

Electrical conductivity analysis

Five discs, each with a 1 cm diameter, were cut from the banana fruits and submerged in separate test tubes containing 25 mL of deionized water under continuous agitation. After 5 hours of incubation, the solution's electrical conductivity was recorded using a Hanna HI 98192 conductivity meter. Subsequently, the discs were boiled in the same water for 20 minutes, and the conductivity was re-measured. The electrical conductivity (EC) was determined by calculating the electrical conductivity values through subtracting the initial electrical conductivity reading from the final electrical conductivity reading and thereafter divided by the number of samples evaluated (Tesfay et al., 2017; Venkatarayappa et al., 1984).

Statistical analysis

All results are presented as means ± standard deviations using Statistical Package for the Social Sciences (SPSS) 26.0. A completely randomized design (CRD) was used for all experiments. Pathogenicity tests were conducted on four bananas per treatment. *In vitro* tests were performed in triplicate with five Petri dishes per treatment, while *in vivo* tests involved five bananas per treatment with three replications. Quality assessments of the banana fruits coated with Tragacanth gum containing peppermint oil were conducted. Data were analyzed for significance using ANOVA. Assessment times for the *in vitro* study were days 0, 1, 2, 3, 4, 5, and 6; for the *in vivo* study, they were days 0, 2, 4, 6, 8, 10, 12, and 14; and for antioxidant enzymatic activities, they were days 0, 2, 4, 6, and 8. Quality assessments of the fruits were performed on days 0, 5, 10, and 15. These time points were considered as factors influencing the inhibitory and responsive activities of TGPO.

RESULTS

Fungal isolation and pathogenicity

Colletotrichum musae, Aspergillus niger, and Aspergillus fumigatus were isolated from banana fruits. Figures 1a-c show the micrographs of the isolated fungi from banana fruits. The results of pathogenicity assessment indicated that a significant proportion of the fruits exhibited symptoms and cultural traits similar to those caused by *Colletotrichum musae* and *Aspergillus fumigatus*. This finding was further confirmed through re-inoculation of the two organisms on potato dextrose agar (PDA), which revealed successful re-isolation of *C. musae* and *A. fumigatus*. These findings indicate that *C. musae* and *A. fumigatus* are the causative agents responsible for the rot observed in the tested banana fruits.

The *in-vitro* assessment of peppermint oil integrated with tragacanth gum on the pathogenic fungi

Figure 1 shows the micrographs of the isolated fungi from banana fruits. Figure 2 illustrates significant differences in the efficacy of peppermint oil incorporated with Tragacanth gum, Tragacanth gum solution alone, and the control solution (distilled water) on fungal growth. The inhibitory effects of the Tragacanth gum solution on *C. musae* (TGCM: Day 0, 0.5 ± 0.05 cm; Day 6, 6.3 ± 0.12 cm) and *A. fumigatus* (TGAF: Day 0, 0.58 ± 0.02 cm; Day 6, 3.85 ± 0.03 cm) were greater than those of the control solutions (CTRLCM: Day 0, 1.13 ± 0.06 cm; Day 6, 6.3 ± 0.12 cm and CTRLAF: Day 0, 1.3 ± 0.1 cm; Day 6, 5.30 ± 0.06 cm). Figure 2 also demonstrates that peppermint oil integrated with Tragacanth gum exhibited relatively insignificant inhibition on *C. musae* (TGPOCM: 0.47 ± 0.06 cm on day 1 and 0.87 ± 0.06 cm on day 6). Likewise, peppermint oil integrated with Tragacanth gum had no inhibitory effect against *A. fumigatus* (TGPOAF: 0.54 ± 0.02 cm on day 1 and 0.76 ± 0.02 cm on day 6).

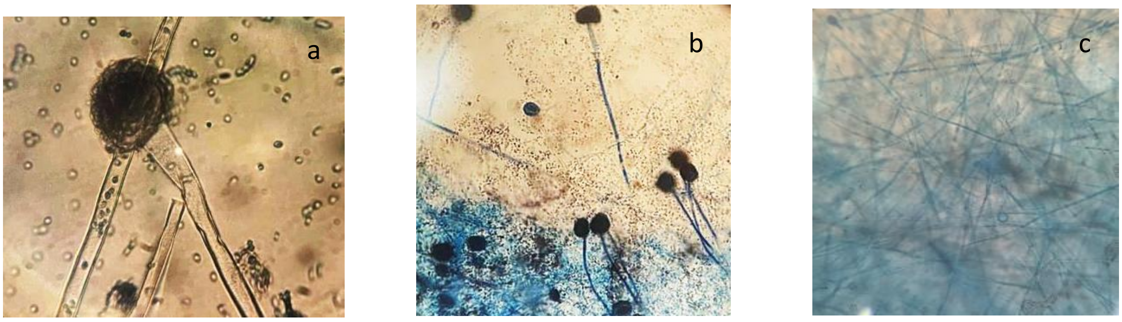


Fig. 1. a: Micrographs of *Aspergillus fumigatus*, b: *Aspergillus niger*, c: *Colletotrichum musae* grown on PDA.

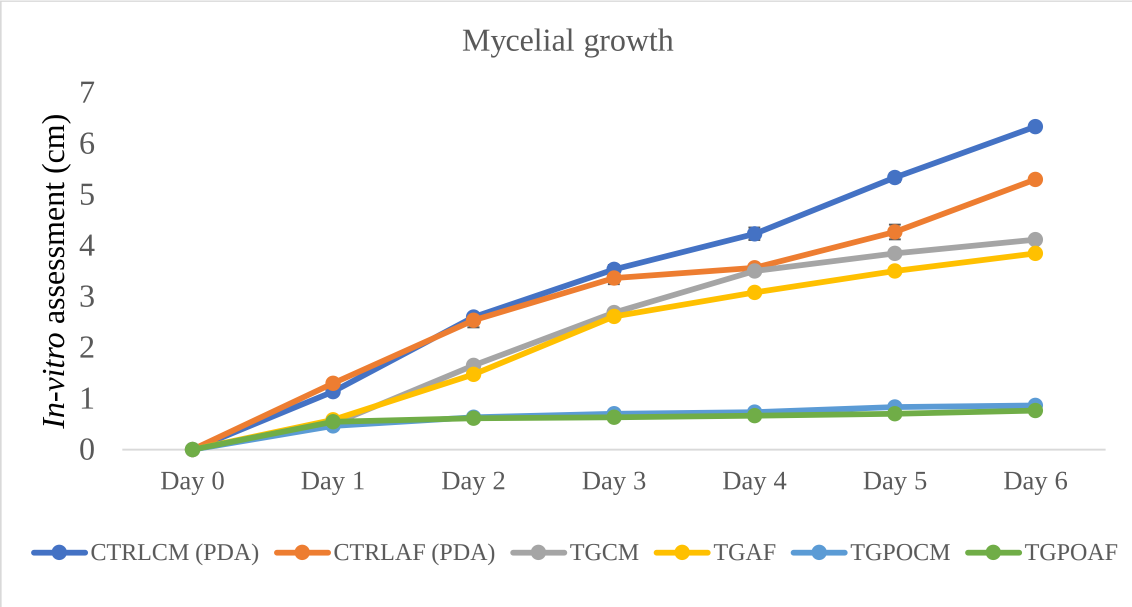


Fig. 2. *In-vitro* activities of peppermint oil incorporated with Tragacanth gum on *Colletotrichum musae* and *Aspergillus flavus*
TGCM: Tragacanth Gum on *Colletotrichum musae*
TGPOCM: Peppermint oil integrated with Tragacanth Gum on *Colletotrichum musae*
CTRLCM: Control solution on *Colletotrichum musae*
TGAF: Tragacanth Gum on *Aspergillus fumigatus*
TGPOAF: Peppermint oil integrated with Tragacanth Gum on *Aspergillus fumigatus*
CTRLAF: Control solution on *Aspergillus fumigatus*

In-vivo assessment of antifungal activity of peppermint oil integrated with tragacanth gum against *Colletotrichum musae* and *Aspergillus fumigatus*

This study, as illustrated in Figure 3a and 3b, showed the *in-vivo* evaluation of the test formulations, including Tragacanth gum alone (TGCM and TGAF), Peppermint oil integrated with Tragacanth gum (TGPOCM and TGPOAF), and control solutions (CTRLCM and CTRLAF), against *Colletotrichum musae* and *Aspergillus fumigatus*. The results demonstrated that the Peppermint oil integrated with Tragacanth gum exhibited significant antifungal activity, markedly reducing the incidence and severity of disease in banana fruits, as shown in Figs 3a and 3b. In contrast, the effects of TGCM and TGAF and the control solutions (CTRLCM and CTRLAF) were notably less effective in mitigating disease occurrence.

Figure 3a shows that Peppermint oil integrated with Tragacanth gum achieved the lowest disease incidences of 32.67±1.00 % for *C. musae* and 28±1.00 % for *A. fumigatus* after 14 days of incubation, with corresponding disease severities of 22.33±1.52 % and 30.67±1.53%, respectively as shown in Figure 3b. These inhibitory effects were significantly more pronounced than Tragacanth gum alone, which resulted in higher disease incidences of 64.33±1.52% for *C. musae* and 57.67±3.51% for *A. fumigatus*. The control solution exhibited the highest incidences at 84.33±2.51 % and 75.67±0.57 % for *C. musae* and *A. fumigatus*, respectively.

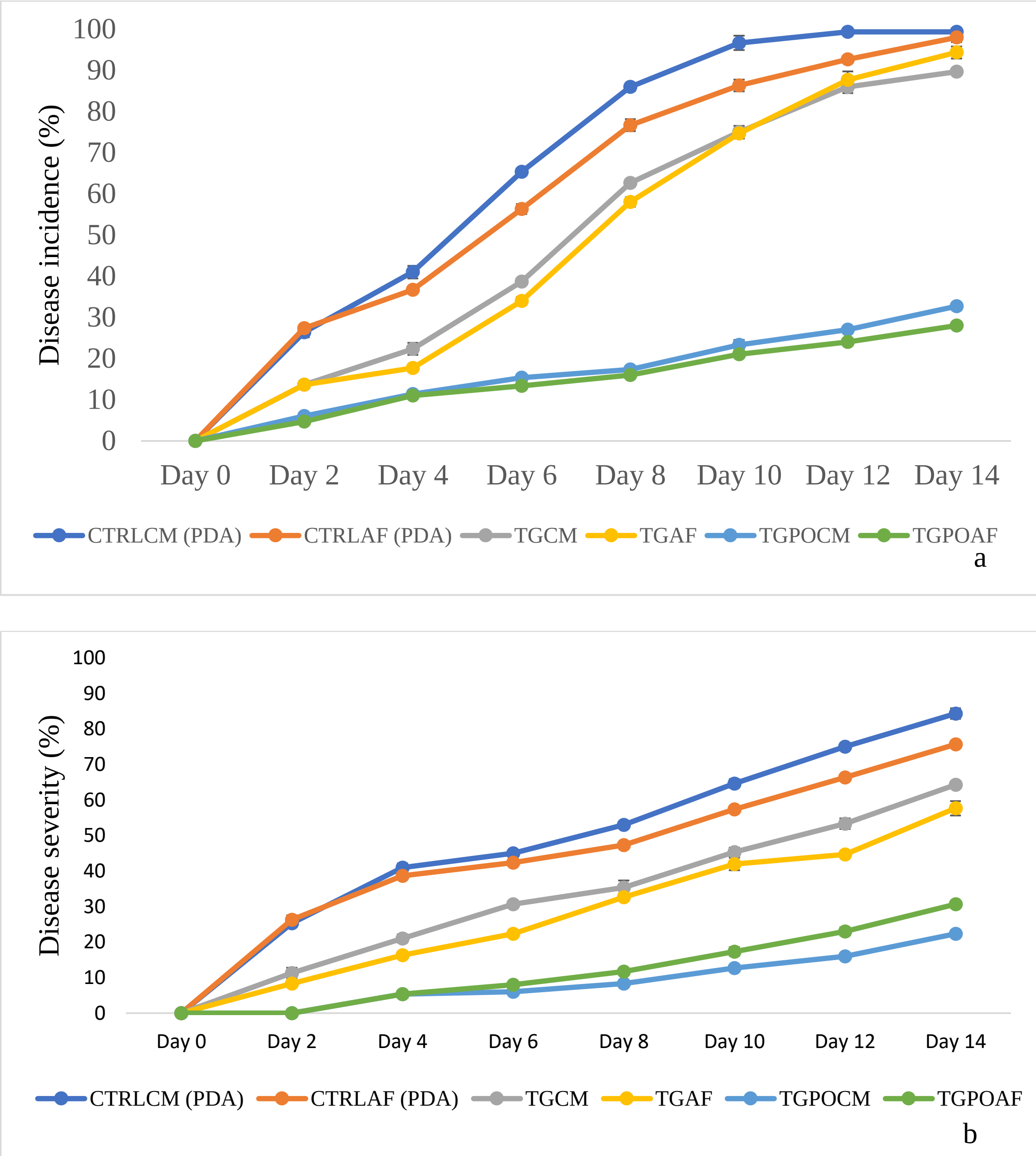


Fig. 3. *In-vivo* Evaluation of Peppermint Oil integrated with Tragacanth gum on *Colletotrichum musae* and *Aspergillus fumigatus*.
TGCM: Tragacanth Gum on *Colletotrichum musae*
TGPOCM: Peppermint oil integrated with Tragacanth Gum on *Colletotrichum musae*
CTRLCM: Control solution on *Colletotrichum musae*
TGAF: Tragacanth Gum on *Aspergillus fumigatus*
TGPOAF: Peppermint oil integrated with Tragacanth Gum on on *Aspergillus fumigatus*
CTRLAF: Control solution on *Aspergillus fumigatus*

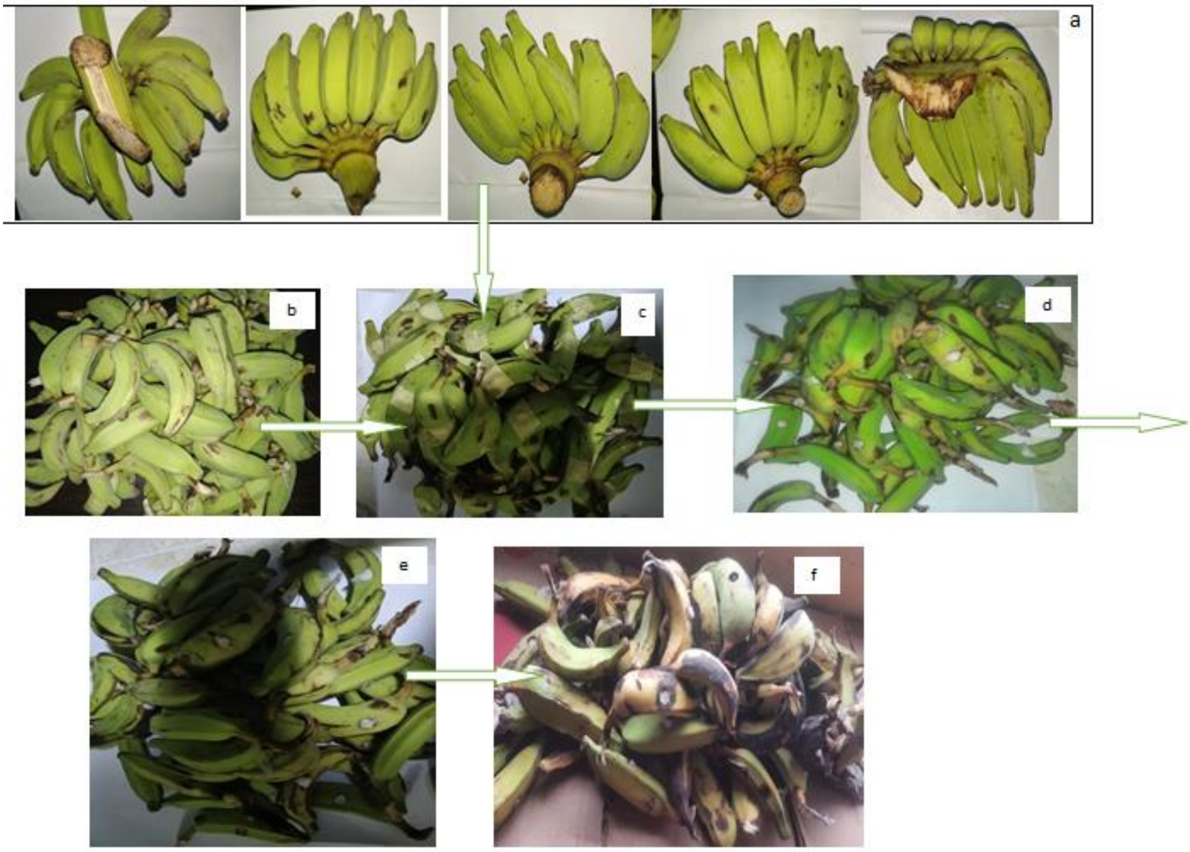


Fig. 4. Assessed fruits from disease incidence and severity evaluation.

a: Bunches of banana fruits to be processed for *in-vivo* assessment
b: Banana fruits after coating for *in-vivo* assessment
c: Banana fruits after inoculation with *Colletotrichum musae* and *Aspergillus fumigatus* at Day 0
d: Banana fruits after inoculation with *Colletotrichum musae* and *Aspergillus fumigatus* at Day 2
e: Banana fruits after inoculation with *Colletotrichum musae* and *Aspergillus fumigatus* at Day 4
f: Banana fruits after inoculation with *Colletotrichum musae* and *Aspergillus fumigatus* at Day 14

Antioxidant Enzymatic Activities

Figure 5a depicts catalase (CAT) activity, showing a marked increase from day 0 to day 4, followed by a decline from day 4 to day 8 across all test agents: peppermint oil integrated with tragacanth gum (TGPOCM and TGPOAF), tragacanth gum alone (TGCM and TGAF), and the untreated control (CTRLCM and CTRLAF) for banana fruits infected with *Colletotrichum musae* and *Aspergillus fumigatus*. Banana fruits treated with TGPOCM and infected with *C. musae* demonstrated a CAT activity of 19.33 ± 0.57 units g^{-1} on Day 2, which slightly declined to 19.00 ± 1.0 units g^{-1} by Day 8. In the case of *A. fumigatus* inoculation, the CAT activity decreased from 22.33 ± 0.57 units g^{-1} on day 2 to 21.33 ± 1.16 units g^{-1} by day 8, demonstrating a more gradual decline in enzyme activity relative to other treatments. Conversely, TGCM-treated banana fruits inoculated with *C. musae* demonstrated a relative decline from 15.00 ±1 units g^{-1} on day 2 to 10.67 ± 1.53 units g^{-1} by day 8, whereas those inoculated with *A. fumigatus* experienced a decrease from 17.67 ± 0.56 units g^{-1} on day 2 to 13.67 ± 1.16 units g^{-1} by day 8. The untreated control samples exhibited the most significant reduction, with *C. musae*-inoculated banana fruits decreasing from 19.33 ± 0.57 units g^{-1} on day 2 to 19.00 ± 1 units g^{-1} by day 8, and *A. fumigatus*-inoculated fruits declining from 22.33 ± 0.57 units g^{-1} to 21.33 ± 1.16 units g^{-1} during the same timeframe. Notably, from days 2 to 4, all test agents demonstrated a transient elevation in CAT activity. In Peppermint Oil integrated with Tragacanth gum, with inoculated *C. musae* (TGPOCM), there was an increase from 19.33 ± 0.57 units g^{-1} to 27 ± 1.0 units g^{-1}, but with inoculated *A. fumigatus* (TGPOAF) increased from 22.33 ± 0.57 units g^{-1} to 29.00 ± 1 units g^{-1}. In Tragacanath gum-treated fruits, inoculation with *C. musae* (TGCM) resulted in an increase from 15.00±1.00 units g^{-1} to 19.67 ± 1.16 units g^{-1}, whereas for *A. fumigatus* (TGAF), the activity rose from 17.67 ± 0.56 units g^{-1} to 24.00 ± 1.0 units g^{-1}. In the control group, *C. musae*-inoculated banana fruits (CTRLCM) exhibited a rise from 10.00 ± 1.00 units g^{-1} to 12.00 ± 1.0 units g^{-1}, whereas *A. fumigatus* (CTRLAF) samples rose from 13.00 ± 1.00 units g^{-1} at day 2 to 15.00 ± 1.00 units g^{-1} by day 4.

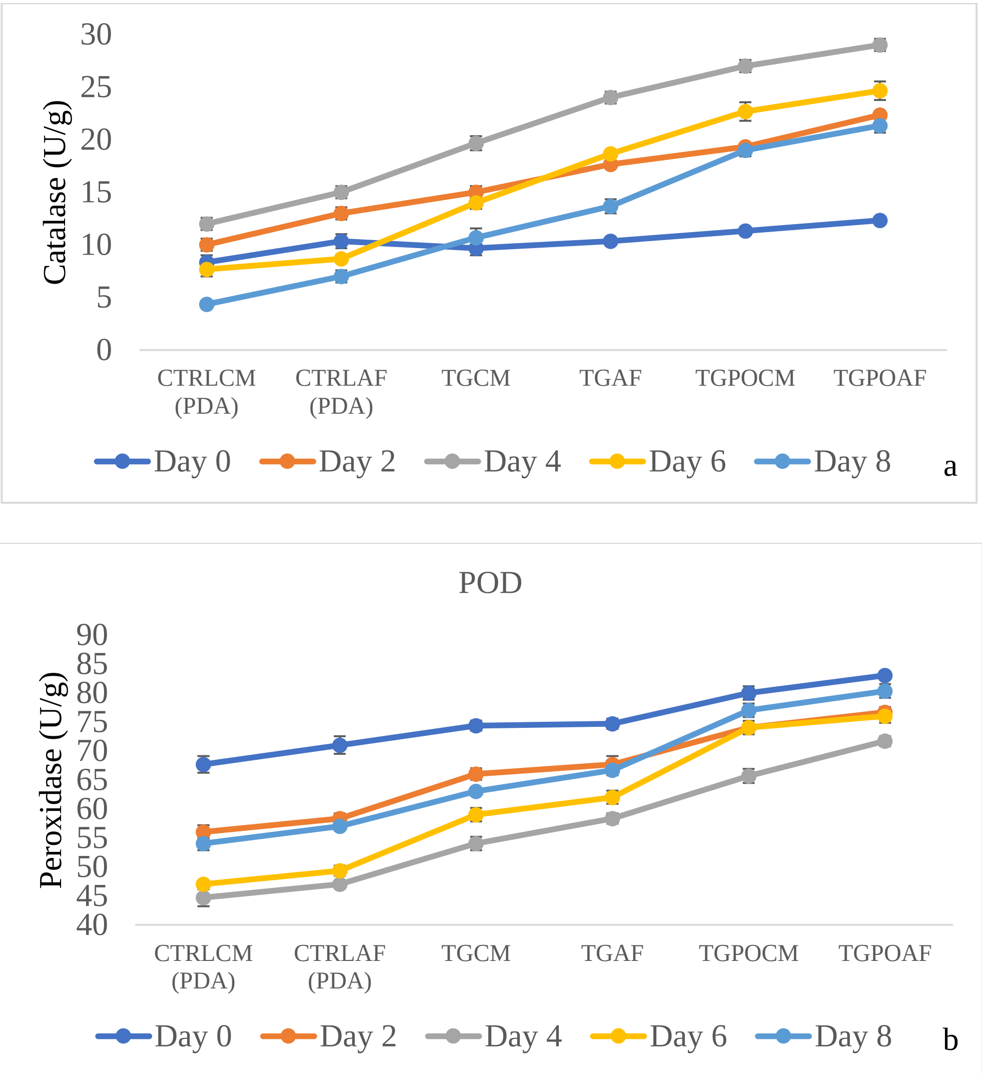


Fig. 5. Impact of the coating treatments on (a) Catalase (CAT) and (b) Peroxidase (POD) activity in banana fruits throughout an 8-day storage period.
POD: Peroxidase, CAT: Catalase
TGCM: Tragacanth Gum on *Colletotrichum musae*
TGPOCM: Peppermint oil integrated with Tragacanth Gum on *Colletotrichum musae*
CTRLCM: Control solution on *Colletotrichum musae*
TGAF: Tragacanth Gum on *Aspergillus fumigatus*
TGPOAF: Peppermint oil integrated with Tragacanth Gum on on *Aspergillus fumigatus*
CTRLAF: Control solution on *Aspergillus fumigatus*

Figure 5b illustrates the peroxidase (POD) activity, indicating a progressive decline from day 0 to day 6, followed by an increase from day 6 to day 8 across all groups, with Peppermint Oil integrated with Tragacanth gum-treated banana fruits exhibiting superior enzyme activity relative to the other treatments. In *C. musae*, the POD activity in TGPOCM-treated banana fruits diminished from 80.00 ± 2.00 unit's g^{-1} on day 0 to 77.00 ± 2.00 units g^{-1} by day 8, whereas *A. fumigatus* inoculation (TGPOAF) exhibited a decline from 83.00 ± 1.00 units g^{-1} to 80.30 ± 2.08 units g^{-1}. The Tragacanth gum alone-treated (TGCM) fruits inoculated with *C. musae* showed a reduction in POD activity from 74.33 ± 1.53 units g^{-1} on day 0 to 63.00 ± 1.00 units g^{-1} by day 5, while in *A. fumigatus* (TGAF), POD activity decreased from 74.67 ± 1.53 units g^{-1} to 66.67 ± 1.53 units g^{-1}. Control fruits had the lowest peroxidase activity, with *C. musae*-inoculated samples (CTRLCM) decreasing from 67.67 ± 2.52 units g^{-1} to 54.00 ± 2.00 units g^{-1}, and *A. fumigatus*-inoculated fruits declining from 71.00 ± 2.65 units g^{-1} to 57.00 ± 1.00 units g^{-1} over the same interval.

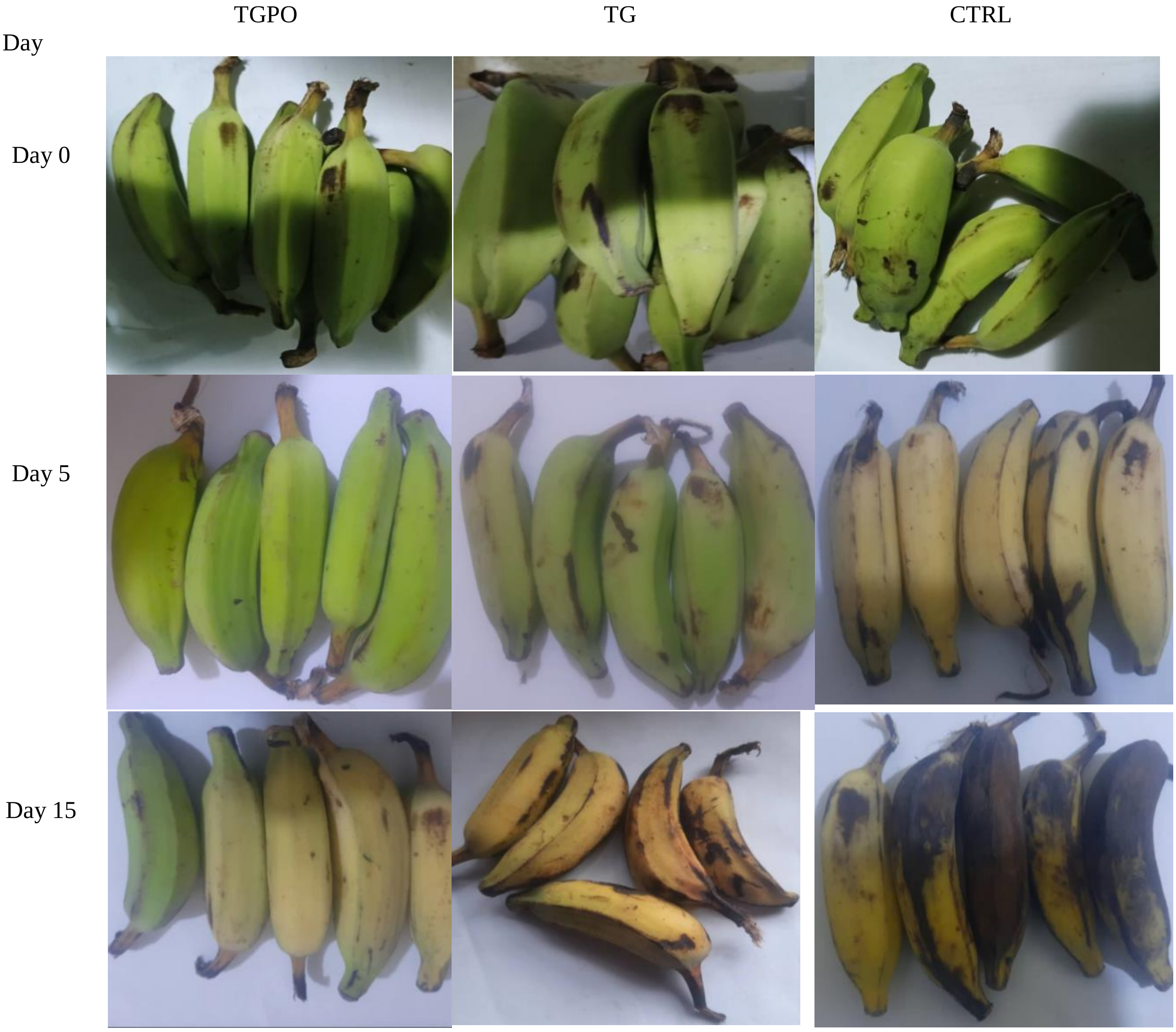


Fig. 6. Visual assessment of coated banana fruits stored for 15 days.
TG: Tragacanth Gum
TGPO: Peppermint oil integrated with Tragacanth Gum
CTRL: Control solution

Quality assessment

The control treatment (CTRL) had more noticeable effects on fruit appearance on day 15 of storage, as depicted in Figure 6. This figure shows the visual appearance of the fruits at day 0, day 5 and day 15.

It was observed based on Figure 7 and Figure 8 that there were significant differences in both pH and total soluble solids (TSS) across different treatment groups of banana fruits during storage. The pH measurements demonstrated that banana fruits treated with Tragacanth gum incorporated with peppermint oil (TGPO) maintained higher pH levels compared to both Tragacanth gum alone (TG) and fruits samples without any treatment (CTRL). Starting from similar baseline pH values (TG: 4.72 ± 0.049, TGPO: 4.44 ± 0.02, CTRL: 4.41 ± 0.04) on Day 5, the treatments showed divergent patterns over the 15-day storage period. By Day 15, TGPO-treated banana fruits maintained a pH of 5.29 ± 0.02, while TG-treated fruits increased to 6.44 ± 0.01, and untreated controls showed the most substantial increased to 6.94 ± 0.03.

Regarding TSS content (Fig. 8), all groups exhibited progressive increases throughout the storage period, but at different rates. TGPO-treated bananas showed the most moderate increase, from 12.33 ± 0.33 °Brix initially to 16.00 ± 0.58 °Brix by day 15. TG-treated fruits displayed an intermediate response, rising from 12.33±0.33 °Brix to 21.67±0.33 °Brix. The untreated control group demonstrated the most pronounced increase in TSS, climbing from 12.00 ± 0.00 °Brix to 25.00 ± 0.58 °Brix over the same period.

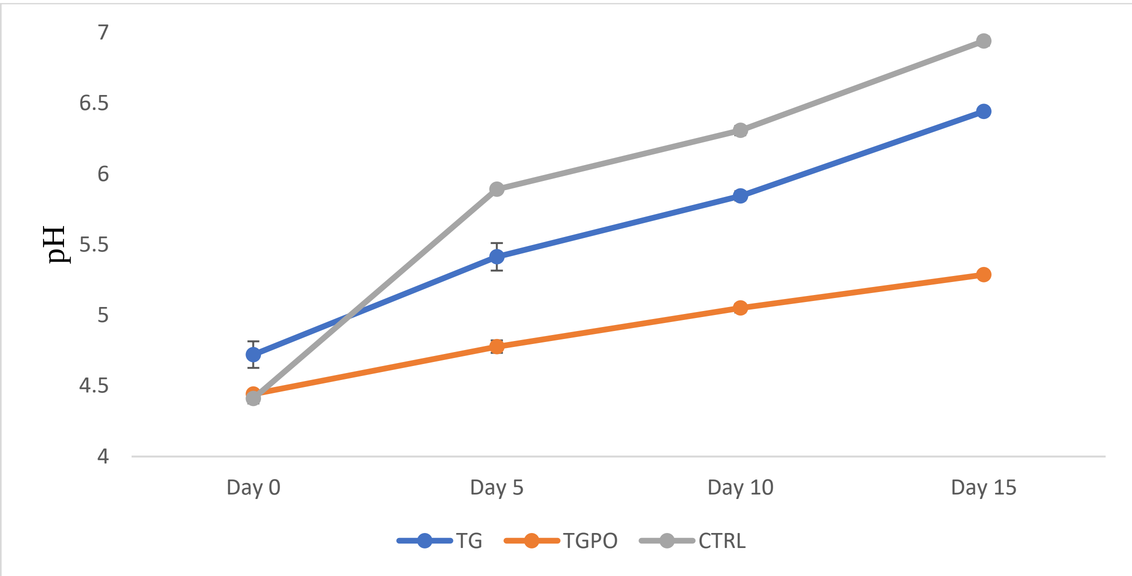


Fig. 7. The effect of Tragacanth gum incorporated with peppermint oil and Tragacanth gum alone on the pH of banana fruits during 15 days of storage.

TGPO: Peppermint oil integrated with Tragacanth gum, TG: Tragacanth gum, Untreated banana fruits (CTRL)

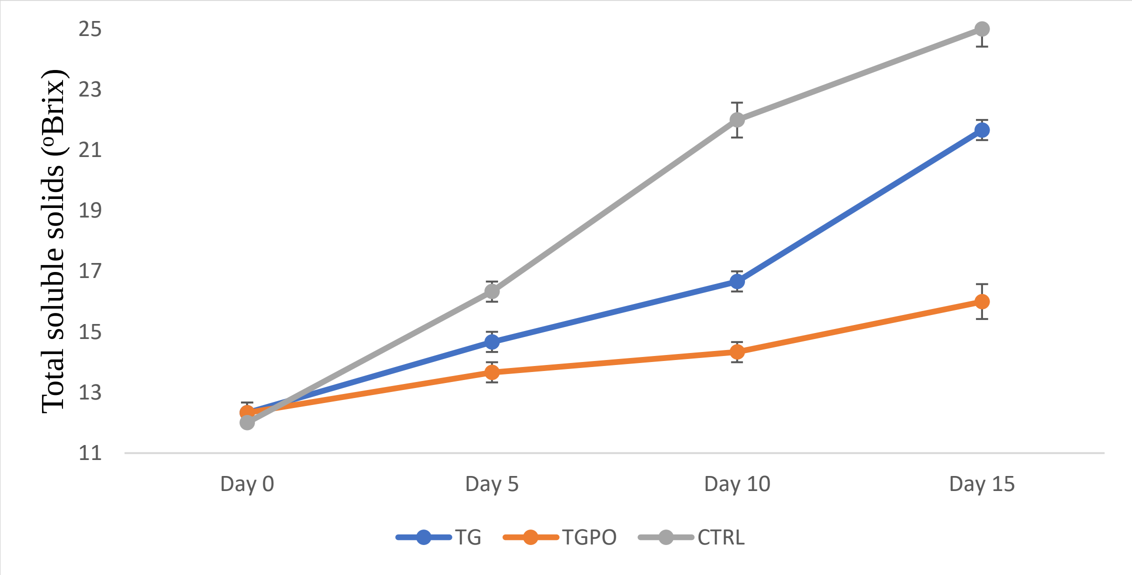


Fig. 8. The effect of Tragacanth gum incorporated with peppermint oil and Tragacanth gum alone on the total soluble solids of banana fruits during 15 days of storage.

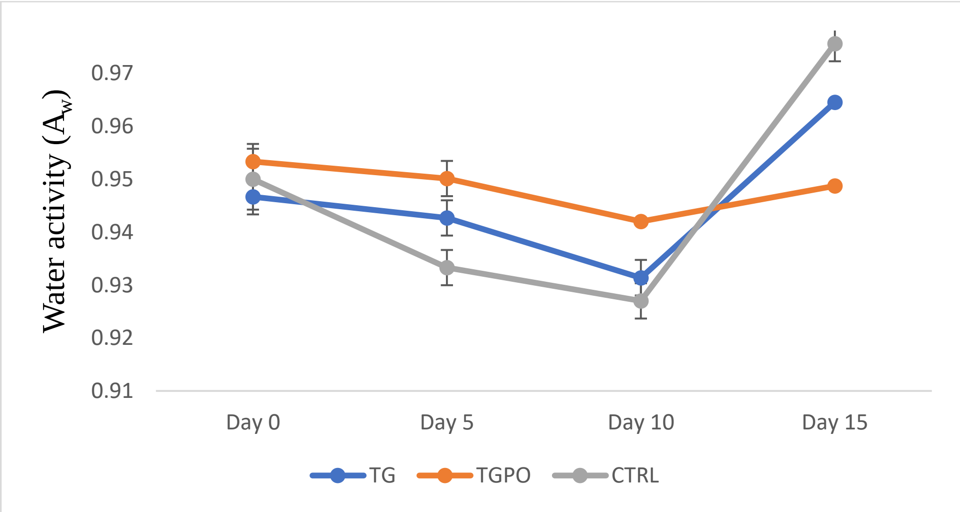


Fig. 9. The effect of Tragacanth gum incorporated with peppermint oil and Tragacanth gum alone on the water activity of banana fruits during 15 days of storage.

Figure 9 illustrates a trend in water activity characterized by a gradual decline from day 0 to day 10, followed by a subsequent rise from day 10 to day 15, observed across various treatments during a 15-day storage period. Banana fruits coated with a combination of Peppermint oil and Tragacanth gum (TGPO) exhibited a minimal reduction in water activity, starting at 0.9567 ± 0.06 on day 0, decreasing to 0.96 ± 0.06 on day 5, and further declining to 0.942 ± 0.04 on day 10. However, an increase in water activity was recorded on day 15, reaching 0.9467 ± 0.043. Similarly, fruits treated exclusively with Tragacanth gum (TG) displayed a comparable pattern, with water activity values decreasing from 0.95 ± 0.008 on Day 0 to 0.93 ± 0.03 on day 5 and 0.927 ± 0.05 on day 10, followed by an increase to 0.9756 ± 0.05 on day 15. Furthermore, untreated control fruits (CTRL) demonstrated the highest water activity levels, starting at 0.9467 ± 0.06 on Day 0, decreasing to 0.9427 ± 0.04 on day 5, and further dropping to 0.9314 ± 0.06 on day 10. A notable increase was observed by the end of the storage period, with water activity rising to 0.9645 ± 0.054 on day 15.

Figure 10 illustrates the decline in fruit firmness across banana fruits treated with TGPO, TG, and CTRL during the 15-day storage period. Firmness consistently decreased in all test samples, with TGPO-treated fruits retaining higher firmness values (day 0: 33.67 ± 0.6N; day 15: 24.52±0.58 N) compared to those treated with TG (day 0: 33.30 ± 0.08N; day 15: 17.38 ± 0.57N). Untreated fruits exhibited the steepest reduction in firmness, with values dropping from 34.20 ± 1.15N on day 0 to 11.33 ± 1.45 N by day 15.

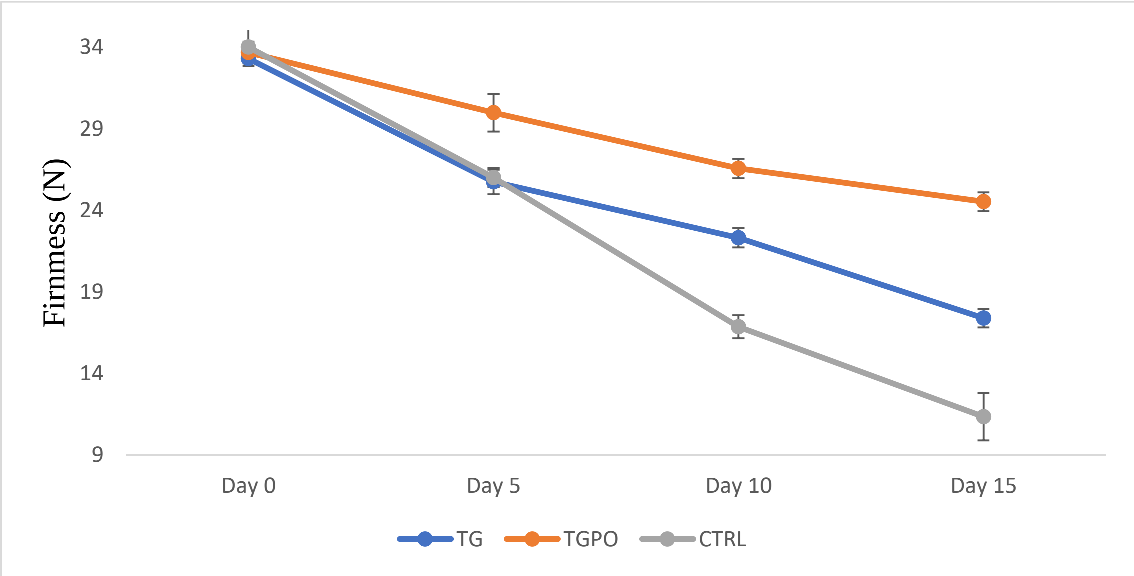


Fig. 10. The effect of Tragacanth gum incorporated with peppermint oil and Tragacanth gum alone on the firmness of banana fruits during 15 days of storage.

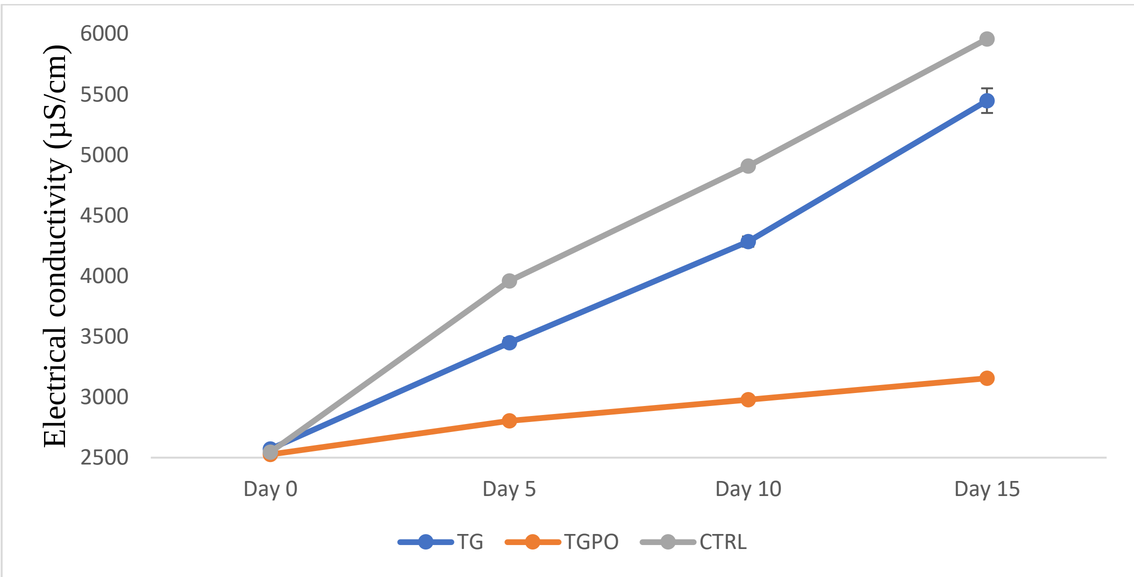


Fig. 11. The effect of Tragacanth gum incorporated with peppermint oil and Tragacanth gum alone on the electrical conductivity (EC) of banana fruits during 15 days of storage.

Figure 11 depicts variations in the electrical conductivity of banana fruits over a 15-day storage period. Banana fruits treated with TGPO displayed a gradual rise in electrical conductivity, starting at 2527.33 ± 59.61µS/cm on day 0 and reaching 3156.33 ± 23.60µS/cm by day 15. Similarly, fruits coated exclusively with TG showed an increase from 2572.33 ± 21.06 µS/cm on day 0 to 5447.67 ± 101.84 µS/cm on day 15. In comparison, the untreated fruits (CTRL) exhibited the most significant rise, from 2543.67 ± 1.33 µS/cm on Day 0 to 5956.67 ± 33.65 µS/cm by Day 15.

DISCUSSION

There is wide evidence about the contribution of fungal diseases to the inedibility of banana fruits over time due to the invasion of these organisms, and several methods to address these issues have been well established (Mairami, 2024; Kuyu & Tola, 2018). The use of synthetic preservatives and natural antimicrobial agents is well documented (Pandey & Negi, 2018). To the best of our knowledge, no research has been conducted on the use of antimicrobial agents like tragacanth gum incorporated with peppermint essential oil on fruits such as bananas (Surekha & Reddy, 2014; Teshome et al., 2022). Consequently, this study aims to determine the impact of tragacanth gum incorporated with peppermint oil on the post-harvest quality of banana fruit stored at room temperature.

This study confirmed that *Colletotrichum musae* and *Aspergillus fumigatus* are the key fungal pathogens responsible for banana fruit rot, as demonstrated through pathogenicity tests. Both fungi have also been identified by Ali et al. (2021) and Kuyu and Tola (2018) as significant contributors to banana fruit deterioration. *Colletotrichum musae*, closely related to *C. gloeosporioides*, is widely recognized as a major cause of anthracnose disease, resulting in severe postharvest decay. Likewise, *Aspergillus fumigatus*, similar to *A. niger*, is a common postharvest pathogen known for causing fruit rot and leading to significant economic losses (Matrose et al., 2021). It has been established that these fungi can penetrate fruit tissues, causing rot and affecting the quality and shelf life of fruits such as bananas (Murmu & Mishra, 2018). The presence of these pathogens has also been observed in fruits like mangoes, avocados, and strawberries (Eckert & Ogawa, 1985). Their virulent nature, particularly under warm and humid conditions, makes them highly problematic for fruit storage and transportation (Zakaria, 2021).

The results on the *in-vitro* effects demonstrated that the integration of Tragacanth gum with Peppermint oil was more effective at inhibiting *Colletotrichum musae* and *Aspergillus fumigatus* compared to using Tragacanth gum alone or leaving the fruits untreated. The study also showed that Peppermint oil played a key role in enhancing the antifungal activity of Tragacanth gum (TGPOCM and TGPOAF) against *C. musae* and *A. fumigatus*. These findings are supported by previous research, including studies by Pawar et al. (2024) and Vilaplana et al. (2018), which highlighted Peppermint oil's effectiveness in reducing postharvest fungal diseases in fruits like bananas.

Several studies have highlighted the effectiveness of edible coatings, such as tragacanth gum, in preserving the volatility of essential oils like peppermint oil, which plays a key role in controlling fungal pathogens in fruits and vegetables (Galus et al., 2020; Perumal et al., 2022). Ghayempour and Montazer (2019) and Godarzi et al. (2021) further reported that the incorporation of essential oils into tragacanth gum enhances the stability and delivery of their bioactive components, ensuring prolonged efficacy. Additionally, Abdi et al. (2024) and Felicia et al. (2024) confirmed that tragacanth gum significantly boosts the antifungal properties of essential oils like peppermint oil. These coatings adhere to fungal cell surfaces, creating a protective barrier that enables the controlled release of active compounds. This close interaction with fungal membranes intensifies the oils' ability to disrupt cell walls and inhibit fungal growth.

The biofungicidal activity of peppermint oil in this study might be attributed to its phytoconstituents, as established in the literature. Bansod and Rai (2008) and Chaemsanit et al. (2018) indicated that peppermint oil, with main phytoconstituents such as menthol and menthone, is responsible for inhibiting *C. musae* and *A. fumigatus*, which cause diseases in crops like banana fruits. The integration of Tragacanth gum with Peppermint oil demonstrated a significant reduction in disease severity and incidence caused by *C. musae* and *A. fumigatus* when compared to the application of Tragacanth gum alone and the control treatments. This enhanced antifungal efficacy has been thoroughly supported by the works of de Oliveira et al. (2017), de Oliveira et al. (2023), and Gonçalves et al. (2021), which confirmed the active role of peppermint oil in mitigating the impact of these fungal pathogens, particularly in controlling both the severity and spread of infections associated with *C. musae* and *A. fumigatus*.

The controlled reduction of catalase (CAT) in banana fruits inoculated with *Colletotrichum musae* (TGPOCM) and *Aspergillus fumigatus* (TGPOAF), and treated with Tragacanth gum incorporated with Peppermint oil, as well as those treated only with Tragacanth gum (TGCM and TGAF), can be attributed to the modulating properties of Peppermint oil. These properties help protect the fruits from oxidative stress. Additionally, Tragacanth gum, integrated with Peppermint oil, forms a physical barrier against oxygen and moisture, which reduces the rate of enzymatic degradation and microbial contamination on the fruit surface (Pillai et al., 2024; Qu et al., 2020; Radev & Pashova, 2020; Saxena et al., 2020). Similar bioactivity of peppermint has been well documented in dragon fruit, strawberries, bananas, and mangoes (Chaemsanit et al., 2018; dos Passos Braga et al., 2019; Felicia et al., 2022).

The pH of TGPO, TG, and CTRL showed upward trends throughout the storage period, with the highest increase observed in CTRL, followed by TG, and the least in TGPO. Several studies have attributed this pH increase to the accumulation of solid matter and molecular breakdown resulting from cellular membrane deterioration. These changes contribute to the alteration of the fruits' mechanical, metabolic, and molecular characteristics over time. The enhanced cellular membrane stability in TGPO-treated banana fruits can be attributed to the integrated activities of peppermint oil and Tragacanth gum, which act as metabolic process regulators due to their antimicrobial properties and ability to form a protective barrier (Adekunle et al., 2021; Afedzi et al., 2022; Iacovino et al., 2024; Nasiri et al., 2018).

Analysis of sugar content in banana fruits after 15 days of storage revealed significant differences in total soluble solids (TSS) among the treatments. Both the TG (Tragacanth gum) and CTRL (control) groups exhibited increased TSS levels, suggesting that these treatments may have facilitated the retention or accumulation of soluble solids during ripening. The increase in TSS is primarily due to the natural ripening process in bananas, which involves the conversion of starch into sugar, leading to higher TSS. This process is driven by ethylene production, a key factor in the ripening of climacteric fruits like bananas, where the release of ethylene accelerates the conversion of starch into sugars, thus increasing TSS (Akkurt et al., 2024).

The lower TSS values in bananas treated with TGPO (Tragacanth gum incorporated with peppermint oil) suggest that TGPO influences the fruit's metabolic processes, particularly those related to sugar and carbohydrate metabolism (Felicia et al., 2022). The reduced TSS in TGPO-treated fruits could be attributed to the structural characteristics of the TGPO coating, which may alter gas exchange or moisture retention. The interaction between Tragacanth gum and peppermint oil may have slowed down the ripening process, possibly by modifying the fruit's respiration rate or limiting ethylene access to the fruit, thereby reducing the rate of starch conversion to sugar (Almeida et al., 2024; Shakil et al., 2023).

Zore et al. (2021) established that factors such as ethylene production significantly affect TSS increase during storage of fruits such as banana. Ethylene production, for instance, is a key trigger for the ripening process, and as bananas ripen, the release of ethylene further accelerates starch conversion into sugar. Additionally, Pamungkas et al. (2023) posited that coatings, such as Tragacanth gum combined with essential oils like peppermint oil, create a barrier that reduces moisture loss, inhibits fungal growth, and delays ripening, thus helping to limit TSS increases.

The effect of the coating treatments on banana fruits was evaluated during a 15-day storage period. The results indicate that control samples (CTRL) without treatment and those treated with Tragacanth gum alone (TG) exhibited a significantly greater increase in water activity compared to bananas treated with Tragacanth gum incorporated with peppermint oil (TGPO). This difference can be attributed to the interaction between bound and unbound water molecules, which plays a pivotal role in maintaining the microbial, structural, and chemical stability of the fruits (Rockland & Stewart, 1981). This study further established an initial decline in water activity (a_w), likely due to moisture loss. This reduction may be partly influenced by Tragacanth gum incorporated with Peppermint oil (TGPO) and Tragacanth alone (TG), both contributing to the decline (Barak et al., 2020). According to the literature, Tragacanth gum acts as a barrier to moisture evaporation. TGPO, which contains peppermint oil, shows slight variations in moisture retention compared to TG (Pamungkas et al., 2023; Zare-Bavani et al., 2024). After Day 10, a rise in a_w was observed, likely due to continued metabolic activity, carbohydrate breakdown releasing bound water, or coating degradation affecting moisture regulation (Almeida et al., 2024). The control group showed the highest aw on Day 15, suggesting the poorest moisture regulation. A study by Pamungkas et al. (2023) on carrageenan-based edible coatings incorporating peppermint essential oil on banana fruits supports our observation on the influence of edible coatings with essential oils. Other studies by Karnwal et al. (2025); Moreira et al. (2022); Soppelsa et al. (2023) also demonstrated the influence of edible coatings, such as tragacanth gum and essential oils like peppermint oil, in positively modulating water activity in fruits like apples, guavas, and bananas.

The impact of TG, TGPO, and CTRL on the tissue rigidity of banana fruits, as observed in this study, indicated that structural degradation was most pronounced in CTRL-treated fruits, followed by those treated with tragacanth gum alone (TG). In contrast, fruits treated with tragacanth gum incorporated with peppermint oil (TGPO) retained optimal firmness. The delayed softening observed in TGPO-treated fruits may be attributed to the antimicrobial properties of peppermint oil, which inhibit rapid cellular breakdown (Yousuf et al., 2021). Furthermore, the significant reduction in firmness in CTRL-treated fruits is likely due to increased activity of pectin-degrading enzymes, which are closely associated with fruit softening and accelerated metabolic processes (Saleh et al., 2019). However, the application of edible coatings such as tragacanth gum, especially when combined with essential oils like peppermint oil, appeared to inhibit water loss by moderating metabolic activity and reducing the production of pectin-degrading enzymes, thereby maintaining fruit firmness (Saleh et al., 2019). These findings align with the results reported by Saleh et al. (2019) in their study on pear fruits. It was also established in this study that the low electrical leakage in TGPO-treated banana fruit suggests enhanced cellular membrane preservation, as posited by Banti (2020). This potentially extends the preservation period by minimizing oxidative degradation.

CONCLUSION

This study established that integrating Tragacanth gum with Peppermint Oil (TGPO) offers a sustainable and eco-friendly solution for extending banana shelf life and maintaining quality during storage. TGPO-treated bananas showed enhanced firmness, better moisture retention, and reduced oxidative stress by preserving antioxidant enzyme activity. The coating also effectively controlled postharvest pathogens (*Colletotrichum musae* and *Aspergillus fumigatus*), highlighting its antimicrobial properties. By combining antioxidant defense with pathogen control, TGPO presents a natural, sustainable alternative to synthetic preservatives, addressing both quality preservation and environmental concerns.

Conflict of interest
The authors declare that there is no conflict of interest.

Acknowledgments
The authors appreciate the Department of Botany, University of Lagos for the provision of some reagents that were used for this study.

REFERENCES

Abdi, G., Jain, M., Patil, N., Tariq, M., Choudhary, S., Kumar, P., Raj, N. S., Mohsen Ali, S. S., & Uthappa, U. T. (2024). (2024). Tragacanth gum-based hydrogels for drug delivery and tissue engineering applications. *Frontiers in Materials*, *11*, 1296399. https://doi.org/10.3389/fmats.2024.1296399

Adekunle, A., Adeogun, O., & Olorunsuyi, Y. J. (2021). Effect of leaf extract of Lantana camara with Maize-based coating on the quality of fresh-cut fruits of *Ananas comosus* and *Musa acuminata*. *Cogent Food and Agriculture*, *7*(1). https://doi.org/10.1080/23311932.2021.1917834

Adeogun, O. O., Ebabhi, A. M., & Adongbede, E. M. (2023). Influence of leaf extract of *Lantana camara* Integrated with Maize-based coating on the quality of fresh *Talinum triangulare* and *Telfairia occidentalis* leaves. *Journal of Sustainable Agriculture*, *38*(1), 85–98. http://dx.doi.org/10.20961/carakatani.v38i1.57446

Afedzi, A. E. K., Ahadjie, V., & Quansah, L. (2022). Gum arabic and beeswax as edible coatings for extending the postharvest shelf life of tomato (*Lycopersicon esculentum*. L) fruit. *Ghana Journal of Science*, *63*(2), 31–42. https://doi.org/10.4314/gjs.v63i2.4

Akkurt, F., Yılmaz, E., & Şen, F. (2024). Determination of the effects of some post-harvest treatments on the quality of banana fruits during storage and after ripening. *Meyve Bilimi*, *11*(1), 43–51. https://doi.org/10.51532/meyve.1463473

Alhassan, N. & Ndomakaah, A. (2024). *Aloe vera* gel coating maintains physicochemical parameters, extends the storage life, and preserves the qualities of Lantundan and Cavendish bananas. *Journal of Horticulture and Postharvest Research*, *7*(3), 287-300. https://doi.org/10.22077/jhpr.2024.7190.1357

Ali, F., Akhtar, N., Shafique, S., & Shafique, S. (2021). Isolation and identification of Aspergilli causing banana fruit rot. *Open Journal of Chemistry*, *4*(1), 8–18. https://doi.org/10.30538/psrp-ojc2021.0019

Almeida, M. M. M. de, Pizato, S., Basaglia, R. R., Pacco-Huamani, M. C., Pinedo, R. A., & Cortez-Vega, W. R. (2024). Effect of tragacanth gum (*Astragalus gummifer*) and melaleuca essential oil to extend the shelf life of minimally processed pineapples. *Acta Scientiarum. Technology*, *46*(1), e65407. https://doi.org/10.4025/actascitechnol.v46i1.65407

Anis, A., Pal, K., & Al-Zahrani, S. M. (2021). Essential oil-containing polysaccharide-based edible films and coatings for food security applications. *Polymers*, *13*(4), 1–32. https://doi.org/10.3390/polym13040575

Azarikia, F., & Abbasi, S. (2016). Mechanism of soluble complex formation of milk proteins with native gums (Tragacanth and Persian gum). *Food Hydrocolloids*, *59*, 35–44. https://doi.org/10.1016/j.foodhyd.2015.10.018

Bansod, S., & Rai, M. (2008). Antifungal activity of essential oils from indian medicinal plants against human pathogenic *Aspergillus fumigatus* and *A. niger*. *World Journal of Medical Sciences*, *3*(2), 81–88.

Banti, M. (2020). Review on electrical conductivity in food, the case in fruits and vegetables. *World Journal of Food Science and Technology*, *4*(4), 80. https://doi.org/10.11648/j.wjfst.20200404.11

Beers, R. F., Jr., & Sizer, I. W. (1952). A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *Journal of Biological Chemistry*, *195*(1), 133–140. https://pubmed.ncbi.nlm.nih.gov/14938361

Chaemsanit, S., Matan, N., & Matan, N. (2018). Effect of peppermint oil on the shelf-life of dragon fruit during storage. *Food Control*, *90*, 172–179. https://doi.org/10.1016/j.foodcont.2018.03.001

Chen, H., Cao, S., Fang, X., Mu, H., Yang, H., Wang, X., Xu, Q., & Gao, H. (2015). Changes in fruit firmness, cell wall composition and cell wall degrading enzymes in postharvest blueberries during storage. *Scientia Horticulturae*, *188*, 44–48. https://doi.org/10.1016/j.scienta.2015.03.018

de Oliveira, K. Á. R., Berger, L. R. R., de Araújo, S. A., Câmara, M. P. S., & de Souza, E. L. (2017). Synergistic mixtures of chitosan and *Mentha piperita* L. essential oil to inhibit Colletotrichum species and anthracnose development in mango cultivar Tommy Atkins. *Food Microbiology*, *66*, 96–103. https://doi.org/10.1016/j.fm.2017.04.012

de Oliveira, T. S., Costa, A. M. M., Cabral, L. M. C., Freitas-Silva, O., Rosenthal, A., & Tonon, R. V. (2023). Anthracnose controlled by essential oils: are nanoemulsion-based films and coatings a viable and efficient technology for tropical fruit preservation?. *Foods*, *12*(2), 279. https://doi.org/10.3390/foods

de Souza, E. L., Lundgren, G. A., de Oliveira, K. Á., Berger, L. R., & Magnani, M. (2019). An analysis of the published literature on the effects of edible coatings formed by polysaccharides and essential oils on postharvest microbial control and overall quality of fruit. *Comprehensive Reviews in Food Science and Food Safety*, *18*(6), 1947-1967.https://doi.org/10.1111/1541-4337.12498

dos Passos Braga, S., Lundgren, G. A., Macedo, S. A., Tavares, J. F., dos Santos Vieira, W. A., Câmara, M. P. S., & de Souza, E. L. (2019). Application of coatings formed by chitosan and Mentha essential oils to control anthracnose caused by Colletotrichum gloesporioides and C. brevisporum in papaya (*Carica papaya* L.) fruit. *International Journal of Biological Macromolecules*, *139*, 631–639. https://doi.org/10.1016/j.ijbiomac.2019.08.010

Eckert, J. W., & Ogawa, J. M. (1985). The chemical control of postharvest diseases: subtropical and tropical fruits. *Annual Review of Phytopathology*, *23*(1), 421-454. https://doi.org/10.1146/annurev.py.23.090185.002225

Mairami, F. M. (2024). Assessment of Fungal Species Associated With Banana and Orange Spoilage in Kwali Market Abuja, Nigeria. *Biological and Environmental Sciences Journal for the Tropics*, *21*(1), 81–88. https://doi.org/10.4314/bestj.v21i1.7

Felicia, W. X. L., Rovina, K., Mamat, H., Aziz, A. H. A., Lim, L. S., Jaziri, A. A., & Nurdiani, R. (2024). Advancements in fruit preservation technologies: Harnessing chitosan, aloe vera gel, and plant-based essential oils for coating applications. *Applied Food Research*, *4*(2), 100439. https://doi.org/10.1016/j.afres.2024.100439

Galus, S., Arik Kibar, E. A., Gniewosz, M., & Kraśniewska, K. (2020). Novel materials in the preparation of edible films and coatings—A review. *Coatings*, *10*(7), 674. https://doi.org/10.3390/coatings10070674

Gavlighi, H. A., Meyer, A. S., Zaidel, D. N. A., Mohammadifar, M. A., & Mikkelsen, J. D. (2013). Stabilization of emulsions by gum tragacanth (Astragalus spp.) correlates to the galacturonic acid content and methoxylation degree of the gum. *Food Hydrocolloids*, *31*(1), 5–14. https://doi.org/10.1016/j.foodhyd.2012.09.004

Ghayempour, S., & Montazer, M. (2019). A novel controlled release system based on Tragacanth nanofibers loaded Peppermint oil. *Carbohydrate Polymers*, *205*, 589–595. https://doi.org/10.1016/j.carbpol.2018.10.078

Godarzi, H., Mohammadifar, M. A., Rad, A. H., Pirouzian, H. R., Ansari, F., & Pourjafar, H. (2021). Physicochemical properties of oil in water emulsions prepared with irradiated gum tragacanth in acidic conditions. *Journal of Food Measurement and Characterization*, *15*(5), 4735–4746. https://doi.org/10.1007/s11694-021-01052-z

Gonçalves, D., Ribeiro, W. R., Gonçalves, D. C., Menini, L., & Costa, H. (2021). Recent advances and future perspective of essential oils in control Colletotrichum spp.: A sustainable alternative in postharvest treatment of fruits. *Food Research International*, *150*, 110758.

Hojnik, N., Modic, M., Ni, Y., Filipič, G., Cvelbar, U., & Walsh, J. L. (2019). Effective Fungal Spore Inactivation with an Environmentally Friendly Approach Based on Atmospheric Pressure Air Plasma. *Environmental Science and Technology*, *53*(4), 1893–1904. https://doi.org/10.1021/acs.est.8b05386

Hossain, M. S., & Iqbal, A. (2016). Effect of shrimp chitosan coating on postharvest quality of banana *(Musa sapientum L.)* fruits. *International Food Research Journal*, *23(1)*, 277-283.

Iacovino, S., Cofelice, M., Sorrentino, E., Cuomo, F., Messia, M. C., & Lopez, F. (2024). Alginate-based emulsions and hydrogels for extending the shelf life of banana fruit. *Gels*, *10*(4). https://doi.org/10.3390/gels10040245

Jiao, W., Liu, X., Li, Y., Li, B., Du, Y., Zhang, Z., ... & Fu, M. (2022). Organic acid, a virulence factor for pathogenic fungi, causing postharvest decay in fruits. *Molecular Plant Pathology*, *23*(2), 304-312. https://doi.org/10.1111/mpp.13159

Karnwal, A., Kumar, G., Singh, R., Selvaraj, M., Malik, T., & Al Tawaha, A. R. M. (2025). Natural biopolymers in edible coatings: Applications in food preservation. *Food Chemistry: X*, 102171. https://doi.org/10.1016/j.fochx.2025.102171

Kumar, D., Arya, S. K., Shamim, M., Srivastava, D., Tyagi, M., & Singh, K. N. (2021). Impact of food selection and usage pattern on consumers' attitude towards food label information. *Journal of Postharvest Technology*, *9*(1), 53–63. www.jpht.in

Kuyu, C. G., & Tola, Y. B. (2018). Assessment of banana fruit handling practices and associated fungal pathogens in Jimma town market, southwest Ethiopia. *Food Science and Nutrition*, *6*(3), 609–616. https://doi.org/10.1002/fsn3.591

López-Mata, M. A., Ruiz-Cruz, S., Ornelas-Paz, J. D. J., Cira-Chávez, L. A., & Silva-Beltrán, N. P. (2015). Antibacterial and antioxidant properties of edible chitosan coatings incorporated with essential oils. *International Journal of Pharma and Bio Sciences*, *6*(4), P251-P264.www.ijpbs.net

Matche, R. S., & Adeogun, O. O. (2022). Physicochemical characterisations of Nanoencapsulated Eucalyptus globulus oil with gum Arabic and gum Arabic nanocapsule and their biocontrol effect on anthracnose disease of Syzygium malaccense Fruits. *Scientific African*, e01421. https://doi.org/10.1016/j.sciaf.2022.e01421

Matrose, N. A., Obikeze, K., Belay, Z. A., & Caleb, O. J. (2021). Plant extracts and other natural compounds as alternatives for post-harvest management of fruit fungal pathogens: A review. *Food Bioscience*, *41*, 100840. https://doi.org/10.1016/j.fbio.2020.100840

Mendy, T. K., Misran, A., Mahmud, T. M. M., & Ismail, S. I. (2019). Application of Aloe vera coating delays ripening and extend the shelf life of papaya fruit. *Scientia Horticulturae*, *246*, 769–776. https://doi.org/10.1016/j.scienta.2018.11.054

Mesías, F. J., Martín, A., & Hernández, A. (2021). Mesías, F. J., Martín, A., & Hernández, A. (2021). Consumers' growing appetite for natural foods: Perceptions towards the use of natural preservatives in fresh fruit. *Food Research International*, *150*, 110749. https://doi.org/10.1016/j.foodres.2021.110749

Moalemiyan, M., Ramaswamy, H. S., & Maftoonazad, N. (2012). Pectin-based edible coating for shelf-life extension of Ataulfo mango. *Journal of Food Process Engineering*, *35*(4), 572–600. https://doi.org/10.1111/j.1745-4530.2010.00609.x

Mohammadi, M., Azizi, M. H., & Zoghi, A. (2020). Antimicrobial activity of carboxymethyl cellulose–gelatin film containing Dianthus barbatus essential oil against aflatoxin-producing molds. *Food Science and Nutrition*, *8*(2), 1244–1253. https://doi.org/10.1002/fsn3.1413

Mohammed Idris, F., Mohammed Ibrahim, A., & Fikreyesus Forsido, S. (2015). Essential oils to control Colletotrichum musae in vitro and in vivo on banana fruits. *American-Eurasian Journal of Agricultural and Environmental Science*, *15*(3), 291-302.

https://doi.org/10.5829/idosi.aejaes.2015.15.3.12551

Moreira, E. D. S., SILVA, N. M. C. D., Brandão, M. R. S., Santos, H. C., & Ferreira, T. A. P. D. C. (2021). Effect of modified starch and gelatin by-product based edible coating on the postharvest quality and shelf life of guava fruits. *Food Science and Technology*, *42*, e26221. https://doi.org/10.1590/fst.26221

Murmu, S. B., & Mishra, H. N. (2018). Post-harvest shelf-life of banana and guava: Mechanisms of common degradation problems and emerging counteracting strategies. *Innovative Food Science & Emerging Technologies*, *49*, 20-30. https://doi.org/10.1016/j.ifset.2018.07.011

Nasiri, M., Barzegar, M., Sahari, M. A., & Niakousari, M. (2018). Application of Tragacanth gum impregnated with Satureja khuzistanica essential oil as a natural coating for enhancement of postharvest quality and shelf life of button mushroom (Agaricus bisporus). *International Journal of Biological Macromolecules*, *106*, 218–226. https://doi.org/10.1016/j.ijbiomac.2017.08.003

Pamungkas, A., Siregar, Z. A., Sedayu, B. B., Fauzi, A., & Novianto, T. D. (2023). A carrageenan-based edible coating incorporating with peppermint essential oils to increase shelf life of bananas (Musa acuminata cavendish). *Jurnal Ilmiah Rekayasa Pertanian Dan Biosistem*, *11*(2), 232–245. https://doi.org/10.29303/jrpb.v11i2.543

Pandey, A., & Negi, P. S. (2018). Pandey, A., & Negi, P. S. (2018). Use of natural preservatives for shelf life extension of fruit juices. In *Fruit juices* (pp. 571-605). Academic Press. https://doi.org/10.1016/B978-0-12-802230-6.00029-1

Pawar, S., Rathod, G., & Nanjunadappa, M. (2024). Characterization and Management of Post-harvest Fungal Diseases in Banana (*Musa paradisiaca*). *Journal of Advances in Biology & Biotechnology*, *27*(8), 1093–1103. https://doi.org/10.9734/jabb/2024/v27i81230

Perez-Vazquez, A., Barciela, P., Carpena, M., & Prieto, M. A. (2023). Edible coatings as a natural packaging system to improve fruit and vegetable shelf life and quality. *Foods*, *12*(19), 3570. https://doi.org/10.3390/foods12193570

Perumal, A. B., Huang, L., Nambiar, R. B., He, Y., Li, X., & Sellamuthu, P. S. (2022). Application of essential oils in packaging films for the preservation of fruits and vegetables: A review. *Food Chemistry*, *375*, 131810. https://doi.org/10.1016/j.foodchem.2021.131810

Pillai, A. R., Eapen, A. S., Zhang, W., & Roy, S. (2024). Polysaccharide-based edible biopolymer-based coatings for fruit preservation: A review. *Foods*, *13*(10), 1529. https://doi.org/10.3390/foods13101529

Purewal, S. S., Kaur, A., Bangar, S. P., Singh, P., & Singh, H. (2023). Protein-based films and coatings: an innovative approach. *Coatings*, *14*(1), 32. https://doi.org/10.3390/coatings14010032

Qu, T., Li, B., Huang, X., Li, X., Ding, Y., Chen, J., & Tang, X. (2020). Effect of peppermint oil on the storage quality of white button mushrooms (*Agaricus bisporus*). *Food and Bioprocess Technology*, *13*(3), 404–418. https://doi.org/10.1007/s11947-019-02385-w

Radev, R., & Pashova, S. (2020). Application of edible films and coatings for fresh fruit and vegetables. *Qual. Access Success*, *21*(177), 108-112. https://www.researchgate.net/publication/343850924

Raghav, P. K., Agarwal, N., & Saini, M. (2016). Edible coating of fruits and vegetables: A review. *Education*, *1*(2), 188-204. www.rdmodernresearch.com

Rajapaksha, L., Gunathilake, D. C., Pathirana, S., & Fernando, T. (2021). Reducing post-harvest losses in fruits and vegetables for ensuring food security – Case of Sri Lanka. *MOJ Food Processing & Technology*, *9*(1), 7–16. https://doi.org/10.15406/mojfpt.2021.09.00255

Rockland, L. B. ., & Stewart, G. F. (1981). *Water activity : influences on food quality : a treatise on the influence of bound and free water on the quality and stability of foods and other natural products*. Academic Press.

Saleh, M. A., Zaied, N. S., Maksoud, M. A., & Hafez, O. M. (2019). Application of arabic gum and essential oils as the postharvest treatments of le conte pear fruits during cold storage. *Asian Journal of Agricultural and Horticultural Research*, *3*(3), 1–11. https://doi.org/10.9734/ajahr/2019/v3i329999

Samtiya, M., Aluko, R. E., Dhewa, T., & Moreno-Rojas, J. M. (2021). Potential health benefits of plant food-derived bioactive components: An overview. *Foods*, *10*(4), 839. https://doi.org/10.3390/foods10040839

Sanchís, E., Ghidelli, C., Sheth, C. C., Mateos, M., Palou, L., & Pérez-Gago, M. B. (2017). Integration of antimicrobial pectin-based edible coating and active modified atmosphere packaging to preserve the quality and microbial safety of fresh-cut persimmon (*Diospyros kaki* Thunb. cv. Rojo Brillante). *Journal of the Science of Food and Agriculture*, *97*(1), 252-260. https://doi.org/10.1002/jsfa.7722

Saxena, A., Sharma, L., & Maity, T. (2020). Enrichment of edible coatings and films with plant extracts or essential oils for the preservation of fruits and vegetables. In *Biopolymer-Based Formulations: Biomedical and Food Applications* (pp. 859–880). Elsevier. https://doi.org/10.1016/B978-0-12-816897-4.00034-5

Sellamuthu, P. S., Mafune, M., Sivakumar, D., & Soundy, P. (2013). Thyme oil vapour and modified atmosphere packaging reduce anthracnose incidence and maintain fruit quality in avocado. *Journal of the Science of Food and Agriculture*, *93*(12), 3024–3031. https://doi.org/10.1002/jsfa.6135

Shakil, M., Islam, S., Yasmin, S., Hossain Sarker, M. S., & Noor, F. (2023). Effectiveness of aloe vera gel coating and paraffin wax-coated paperboard packaging on post-harvest quality of hog plum (Spondius mangifera L.). *Heliyon*, *9*(7). https://doi.org/10.1016/j.heliyon.2023.e17738

Singh, R., Shushni, M. A. M., & Belkheir, A. (2015). Antibacterial and antioxidant activities of Mentha piperita L. *Arabian Journal of Chemistry*, *8*(3), 322–328. https://doi.org/10.1016/j.arabjc.2011.01.019

Slavin, J. L., & Lloyd, B. (2012). Health benefits of fruits and vegetables. *Advances in Nutrition*, *3*(4), 506-516. https://doi.org/10.3945/an.112.002154

Soppelsa, S., Van Hemelrijck, W., Bylemans, D., & Andreotti, C. (2023). Essential oils and chitosan applications to protect apples against postharvest diseases and to extend shelf life. *Agronomy*, *13*(3). https://doi.org/10.3390/agronomy13030822

Surekha, M., & Reddy, S. M. (2014). Preservatives: Classification and Properties. In *Encyclopedia of Food Microbiology: Second Edition* (pp. 69–75). Elsevier Inc. https://doi.org/10.1016/B978-0-12-384730-0.00257-3

Tapia, M. S., Alzamora, S. M., & Chirife, J. (2008). Effects of water activity (aw) on microbial stability: as a hurdle in food preservation. In *Water Activity in Foods: Fundamentals and Applications* (pp. 239–271). Blackwell Publishing Ltd. https://doi.org/10.1002/9780470376454.ch10

Tesfay, S. Z., Magwaza, L. S., Mbili, N., & Mditshwa, A. (2017). Carboxyl methylcellulose (CMC) containing moringa plant extracts as new postharvest organic edible coating for Avocado (Persea americana Mill.) fruit. *Scientia Horticulturae*, *226*, 201–207. https://doi.org/10.1016/j.scienta.2017.08.047

Teshome, E., Forsido, S. F., Rupasinghe, H. V., & Olika Keyata, E. (2022). Potentials of natural preservatives to enhance food safety and shelf life: A review. *The Scientific World Journal*, *2022*(1), 9901018. https://doi.org/10.1155/2022/9901018

Thakur, R., Pristijono, P., Golding, J. B., Stathopoulos, C. E., Scarlett, C. J., Bowyer, M., Singh, S. P., & Vuong, Q. V. (2018). Development and application of rice starch based edible coating to improve the postharvest storage potential and quality of plum fruit (Prunus salicina). *Scientia Horticulturae*, *237*, 59–66. https://doi.org/10.1016/j.scienta.2018.04.005

Török, Á., Yeh, C. H., Menozzi, D., Balogh, P., & Czine, P. (2023). European consumers' preferences for fresh fruit and vegetables – A cross-country analysis. *Journal of Agriculture and Food Research*, *14*, 100883. https://doi.org/10.1016/j.jafr.2023.100883

Tugce Senturk Parreidt, K. M. and M. S. (2018). *Alginate-Based Edible Films and Coatings for Food Packaging Applications*. 1–38. https://doi.org/10.3390/foods7100170

Venkatarayappa, T., Fletcher, R. A., & Thompson, J. E. (1984). Retardation and reversal of senescence in bean leaves by benzyladenine and decapitation. *Plant and Cell Physiology*, *25*(3), 407-418. https://academic.oup.com/pcp/article-abstract/25/3/407/1867469

Vilaplana, R., Pazmiño, L., & Valencia-Chamorro, S. (2018). Control of anthracnose, caused by Colletotrichum musae, on postharvest organic banana by thyme oil. *Postharvest Biology and Technology*, *138*, 56–63. https://doi.org/10.1016/j.postharvbio.2017.12.008

Wang, Z., Sui, Y., Li, J., Tian, X., & Wang, Q. (2022). Biological control of postharvest fungal decays in citrus: a review. In *Critical Reviews in Food Science and Nutrition*, *62*(4), 861–870. Taylor and Francis Ltd. https://doi.org/10.1080/10408398.2020.1829542

Yousuf, B., Wu, S., & Siddiqui, M. W. (2021). Incorporating essential oils or compounds derived thereof into edible coatings: Effect on quality and shelf life of fresh/fresh-cut produce. In *Trends in Food Science and Technology*, *108*, 245–257. Elsevier Ltd. https://doi.org/10.1016/j.tifs.2021.01.016

Zakaria, L. (2021). Diversity of colletotrichum species associated with anthracnose disease in tropical fruit crops - A review. *Agriculture (Switzerland)*, *11*(4), 297. https://doi.org/10.3390/agriculture11040297

Zare-Bavani, M. R., Rahmati-Joneidabad, M., & Jooyandeh, H. (2024). Gum tragacanth, a novel edible coating, maintains biochemical quality, antioxidant capacity, and storage life in bell pepper fruits. *Food Science and Nutrition*, *12*(6), 3935–3948. https://doi.org/10.1002/fsn3.4052

Zore, K., Rajenimbalkar, V., & Shaikh, N. (2021). Ripening behaviour of banana treated with different ethylene sources. *International Journal of Farm Sciences*, *11*(1and2), 51–56. https://doi.org/10.5958/2250-0499.2021.00009.4

JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH
2025, VOL. 8(4), 451-464

Journal of Horticulture and Postharvest Research

Journal homepage: www.jhpr.birjand.ac.ir

University of Birjand

Water footprint for citrus production in Egypt: a case study of Navel orange

Waleed Fouad Abobatta[1,*] and Ahmed A. Farag[2]

1, Horticulture Research Institute, Agricultural Research Center, Giza, Egypt
2, Central Laboratory for Agricultural Climate, Agricultural Research Center, Giza, Egypt

ARTICLE INFO

Original Article

Article history:
Received 22 November 2024
Revised 2 April 2025
Accepted 13 April 2025

Keywords:
Citrus
Fruit quality
Navel orange
Water use efficiency
Yield

DOI: 10.22077/jhpr.2025.8436.1450
P-ISSN: 2588-4883
E-ISSN: 2588-6169

***Corresponding author:**
Horticulture Research Institute, Agricultural Research Center, Giza, Egypt.

Email: wabobatta@arc.sci.eg

ABSTRACT

Purpose: Citrus is one of the most significant fruit crops in the world, and there are huge amounts of citrus in Egypt, especially orange. Shortage of water resources is the main challenge for citrus production, therefore, proper management of water resources for orange orchards is essential in Egypt. **Research Method:** The current study's objective was to calculate the water footprint components of orange production in four governorates (Beheira, Gharbia, Menoufia, and Sharqiya) during 2020-2023. **Findings:** Data indicated that different irrigation rates affected tree growth, tree yield, total yield, yield efficiency, and fruit quality. Results showed that trees grown in the Salhyia area recorded the highest values of canopy ratio increment (42.21%), N leaf content (2.46%), yield efficiency (5.92 kg/m3), tree yield (132.00 kg/tree), total yield (52.80 tons/ha), TSS/TA ratio value (11.75), and the lowest acidity value (0.99 %). The highest values of leaf K content (1.76%), and vitamin C (42.83) were recorded in Al Mahalla El Kubra region, while the highest P leaf content (0.314%) was observed in Ashmoun district. Data showed that water use efficiency was lower in surface irrigation with a value of 3.71 kg orange/m^3 water and higher in drip irrigation with a value of 3.81 kg orange/m^3 water. **Research limitations:** There was no limitation. **Originality/Value:** Regarding water footprint components, data revealed that the drip irrigation system had lower green, grey and total water footprint values than surface irrigation. In contrast, the blue water footprint was the height under the drip irrigation system rather than the surface irrigation system.

INTRODUCTION

In Egypt, the Nile Delta, Nubaria region in Beheira Governorate, and Salhyia region in Sharqiya Governorate are among the most important citrus-growing areas. Egypt produces a wide variety of citrus fruits, Oranges, Mandarins, lemons, and grapefruits are among the most popular and mainly consumed as fresh fruit, juice, or exporting (Abobatta, 2019). Oranges have a significant position in Egyptian citrus production and represent the major citrus crop in Egypt. Navel orange (*Citrus sinensis* (L.) Osbeck), is one of the significant varieties and occupies the first place in citrus production in Egypt, known for its unique characteristics and exceptional taste, so, it has become a favorite among local and international consumers. The cultivated areas of Navel oranges have steadily increased to reach 67850 ha, representing 31% of the total citrus cultivated (Annual Report, 2023). Under irrigated cultivation systems, enhancing plant growth is greatly depending mineral fertilizing. Citrus orchards require reasonable amounts of irrigation water about 10,000 m^3 /ha/ yearly and 300 nitrogen units/ha / yearly for growth and fruiting in Egypt (Arafat & Helal, 2021).

The water footprint (WF) concept has emerged as a new attitude for assessing the sustainability of water use, particularly in the agricultural sector. The water footprint provides a comprehensive measure of water use by considering not only direct water consumption (irrigation water) but also the indirect water use embedded in the production of agricultural products (Lovarelli et al., 2016). Water footprint is a great indicator of the efficiency of water used in different processes, and it includes direct and indirect use of water to produce goods or services during a certain period measured in liters used per unit produced of product or service (Hoekstra et al., 2009). Analyzing WF helps researchers to define the water use situation, and then estimate the imported/exported amounts of water (Tuninetti et al., 2015). The water footprint assessment provides a quantitative framework to analyze the amount of water set in agricultural goods and the efficiency of water use when the metric is computed per unit weight of the products (hereafter referred to as the unit water footprint (Galán-Martín et al., 2017). The water footprint has three components, including green, blue and grey. The footprint of green water mentions to the consumption of rainwater stored in the soil as the soil moisture (Yi et al., 2024). Bluewater is defined as the surface water and groundwater consumed in the chain of production (Galán-Martín et al., 2017). Grey water refers to the volume of freshwater required to dilute the chemical fertilizers and pesticides (Hoekstra et al., 2011). Due to population increases, food security and sustainable management of water resources are essential; hence, WF is a dynamic concept for management of water resources in any sector, especially in agriculture. These concepts are considered by many researchers in various fields; for instance, the study of water footprints on citrus fruits in South Africa (Munro et al., 2016). The water footprint is affected by agricultural management more willingly than the regional climate and could be controlled by better management of all agricultural inputs and improving water use efficiency in agriculture (Lu et al., 2016).

The WF takes into account the impacts that arise from the cumulative effect of all activities, with the understanding that the agricultural water footprint (WF) is the sum of the WF of each crop grown in an area to identify the periods in which extractions are unsustainable (Lovarelli et al., 2016).

This study evaluates the water footprint of navel oranges under flood and drip irrigation systems in citrus orchards by studying five main regions of citrus production in Egypt and estimating the green, blue, and grey water footprint to improve water management in Egypt. Knowledge of water footprint in citriculture is an important issue in planning efficient water use and improving productivity, sustainability and competitiveness of irrigated crops (Imbernón-Mulero et al., 2024).

The main goals of this work are the estimated water footprint of navel oranges, increasing water use efficiency, improving tree growth, and sustaining citrus crop production at different locations in Egypt.

MATERIALS AND METHODS

Study areas

In Egypt, Navel orange is mainly grown in Delta, Nubaria and Salhyia regions. The study and water management measurements were conducted on mature orange (*Citrus sinensis* L.) trees (15 years old) in commercial orchards in four governorates (Beheira, Gharbia, Menoufia, and Sharqiya) as shown in (Table 1).

Data collection

Certain criteria were used to determine the WFP of Orange trees in response to various irrigation water quantities and N fertilization rates. Data obtained were vegetative growth, leaf mineral contents, yield ($ton.ha^{-1}$), fruit quality, and water use efficiency during the study period from 2020 to 2023.

The vegetative growth of the tree is expressed as the canopy volume increment percentage as follows (1):

$$\text{The yearly increment percentage} = [(TCV2 - TCV1)/TCV1] \times 100 \qquad (1)$$

Where TCV1: is the tree canopy volume at the beginning of the growth season and TCV2 is the tree canopy volume at the end (m^3) according to equation (2) of Zekri (2000).

$$\text{Canopy volume} = 0.52 \times \text{tree height} \times (\text{diameter}^2) \qquad (2)$$

Various agricultural practices were conducted, including pruning, organic fertilization, pest control, etc., according to the Egyptian Ministry of Agriculture and Land Reclamation recommendations.

Table 1. Four years (2020–2023) of water supply (m^3/ha), Rainfall (mm), and nitrogen fertilizer (kg/ ha) applications for Navel Orange orchards in the four governorates.

Site	Al-Shorouk	Nubaria	El- Mahalla El-Kubra	Ashmoun	Salhyia
	Irrigation water supply (m^3/ha)				
First season	10560	9600	9423	9120	10800
Second season	10800	10320	8880	9360	10560
Third Season	11400	10800	9360	9840	11100
Fourth Season	12720	12000	10320	10320	11880
Average	11370	10680	9496	9660	11085
	Rainfall (mm)				
First season	90.8	90.8	72.9	68.6	15.3
Second season	20.8	20.8	44.9	33.1	12.2
Third Season	33.4	33.4	27.6	25.2	15.0
Fourth Season	19.9	19.9	21.4	17.9	15.2
	N supply (kg/ ha)				
First season	432	350	550	389	411
Second season	428	353	414	375	425
Third Season	400	386	361	428	439
Fourth Season	452	400	425	400	425
Average	428.00	372.25	437.50	398.00	425.00

Determination of leaf mineral contents

Every season and from each site, 20 mature leaves were washed with distilled water and dried at 70°C, and plant samples were wet digested using H_2O_2 and H_2SO_4 according to the procedure described by Bankaji et al. (2023) to determine the macro elements.

Yield and its components

Twenty fruits were picked up at harvesting time in the second half of December each season (2020, 2021, 2022, and 2023) from each experimental site to determine fruit quality parameters. The number and weight of fruit per tree were counted in the orchards to get the tree yield (kg), yield (ton) /ha, and yield efficiency (kg/m^3), according (Biratu et al., 2023). TSS % was determined using hand refractometer. Total titratable acidity percentage in fruit juice was estimated as anhydrous citric acid and Vitamin C (as mg/ 100 g pulp) was determined according (A.O.A.C., 2000), then T.S.S/acid ratio was calculated.

Effective Rainfall

An empirical formula developed by FAO/AGLW based on analysis for different arid and sub-humid climates. These formula are as follows (3), (4):

$$\text{Effective Rainfall} = 0.6 \times \text{Total Rainfall} - 10 \text{ For (Total Rainfall} < 70 \text{ mm)} \quad (3)$$

$$\text{Effective Rainfall} = 0.8 \times \text{Total Rainfall} - 24 \text{ For (Total Rainfall} > 70 \text{ mm)} \quad (4)$$

Water Use Efficiency (WUE)

WUE is the ratio of plant production (carbon assimilation) per unit of water use and is commonly used to indicate vegetation performance and yield. WUE is calculated by dividing total yield (kg ha^{-1}) by water requirement (m^3) (5).

$$WUE = Y / WR \quad (5)$$

Y = yield (Kg) and WR = water requirement (m^3)

Water footprint calculation

Water footprint concept was studied (blue, gray and green) of orange orchards over the crop growing period for the last 4 years from 2020 to 2023 in five different locations in Egypt. The FAO Penman-Monteith equation method was used to calculate the water requirement of orange productions. For the water footprint concept estimation of the sum of water footprint Green, water footprint Blue and water footprint Gray of orange production, we follow the method of Hoekstra et al. (2011). The water footprint Green, water footprint Blue and water footprint Gray are estimated as follows (6), (7), and (8):

$$WF\ (Green) = \frac{(Pe)\times 10}{Y} \quad (6)$$

$$WF\ (Blue) = \frac{(Etc-Pe)\times 10}{Y} \quad (7)$$

$$WF\ (Gray) = \frac{a\times NAR}{(Cmax-Cnet)\times Y} \quad (8)$$

$$WFT = WF\ Green + WF\ Blue + WF\ Grey$$

WF Green, WF Blue, and WF Gray are the green, blue and gray water footprint measured by unit (m^3 kg^{-1}).

The Pe is the total effective rainfall for orange growth (mm) calculated with the USDA method, Y is the orange yield (kg/ha), α is the leaching runoff fraction, AR is the rate of nitrogen fertilizer use to the field per hectare (kg/ha), CMax is defined as the maximum acceptable concentration (kg/m^3), and CNat is defined as the natural concentration for the pollutants (kg/m^3). The α value is identified as the average of 10% of consumed nitrogen fertilizer under irrigated conditions similar to those applied by Hoekstra et al. (2011). The CMax is 50 mg/L NO_3 or 10 mg/L NO_3- N recommended by the WHO and USEPA, respectively. In this work, the USEPA standard was suggested by Chapagain, et al., (2006) was applied for equation, and the CNat here was supposed to be zero (Chapagain & Hoekstra, 2011).

Statistical analysis

Data obtained from trees at the research sites were analyzed by MSTAT-C package (Freed, 1985) with a probability of 0.05, and differences between means were compared using Duncan's multiple-range test according to (Wallar & Duncan, 1969).

RESULTS

Citrus are Egypt's most significant fruit crop and the most exported fruit in terms of quantity and significance; therefore, they were chosen for this study. The effect of different fertilizing rates and quantities of irrigation water on tree growth was monitored by determining tree canopy on March 1st and October 1st every season at experimental sites.

Table 2. Effect of tested irrigation rates on tree canopy increment and leaf mineral contents of Navel orange trees during the experimental seasons.

Site	Tree volume increments (%)	N%	P%	K%
First season				
1	34.07A	2.46 A	0.211 B	1.70 A
2	22.24D	2.12 B	0.300 AB	1.4٥ D
3	31.24B	2.25 AB	0.217 B	1.60 B
4	27.00C	2.18 B	0.314 A	1.43 D
5	31.35B	2.32 AB	0.253 B	1.5٤ C
Second season				
1	33.43A	2.41 A	0.241 B	1.64 A
2	14.04D	2.05 B	0.313 A	1.49 B
3	27.26C	2.24 AB	0.260 AB	1.51 B
4	14.85D	2.16 B	0.296 AB	1.47 B
5	31.44B	2.30 AB	0.258 AB	1.54 B
Third Season				
1	24.79C	2.25 AB	0.277 AB	1.48 B
2	22.55D	2.22 AB	0.287 AB	1.40 B
3	15.41E	2.13 B	0.306 A	1.41 B
4	32.33B	2.40 A	0.239 B	1.61 A
5	35.42A	2.45 A	0.229 B	1.66 A
Fourth season				
1	38.08 B	2.36 AB	0.241 B	1.63 A
2	29.03 E	2.13 B	0.306 A	1.42 B
3	33.15 C	2.45 A	0.263 AB	1.76 A
4	30.46 D	2.40 A	0.311 A	1.43 B
5	42.21 A	2.46 A	0.270 AB	1.71 A

*Values in the same column followed by the same letter(s) do not significantly differ from each other according to Duncan's multiple range test at 5% level. *Site1: Al-Shorouk; Site2: Nubaria; Site3: El-Mahalla El-Kubra; Site4: Ashmoun; Site5: Salhyia.

Data in Table 2 shows that canopy increase varied between different sites. The highest canopy ratio increment (42.21%) was recorded in Site 5 (Salhyia) region in the last season, followed by Site 1 (38.08 %) Al-Shorouk region, in (2023), while, trees in Site 2 Nubaria region had the lowest increment ration (14.04%) in (2021).

Estimating mineral elements in the leaves, show a large variation in available nutrients in leaves through the investigation, i.e. N % (2.46 to 2.05 %), P % (0.314 to 0.211%), and K % (1.76 to 1.40 %). These variations were statistically highly significant compared to the tree responses to different N fertilizing averages and irrigation water quantities.

Data presented in Table 2 indicated that the tree that was grown in Nubaria (site 2) fertilized with 353 N units/ha and received 10,320 m^3/ha water in the second season had the lowest N leaf content (2.05 %) during the experiment. On the contrary, trees grown in Salhyia site (5) received 425 N units/ha with (11,880 m^3/ha) of irrigation water, recording the highest N leaf content (2.46 %) during the experiment.

Data in hand Table 2 showed that the highest leaf K content (1.76%) was observed in the last season of the tree grown in the Al Mahalla El Kubra region, fertilized with 425 N units/h and irrigated with 10,320 m^3/ha, followed by trees grown in Salhyia region and received the same N quantity (425 unit/ha) but watering with 11,880 m^3 water/ha. Trees grown in El-Nubaria district that received 386 N units/ha and irrigated with 10,800 m3 water/ha, recorded the lowest K values (1.40 %) in the third season.

Concerning the effect of various irrigation rates and applied N units on P leaf content data in Table 2 cleared that the highest P leaf content (0.314 %) was recorded in trees growing in Ashmoun district and received 9,120 m^3 water and fertilized with 389 N units/hectare in the first season. The minimum P leaf content (0.211 %) was recorded in Al-Shorouk region whereas trees were fertilized with 432 N units and watered by 10,560 m^3/ha in the same season.

There are variations in tree yield according to the quantity of irrigation water and nitrogen fertilizer rates at different experiment sites, ranging from 132.00 to 78.00 kg/tree, as shown in Table 3. The maximum tree yield (132.00 kg/ tree) was recorded in the last season from trees grown in Salhyia region, which received 425 N units/ha and irrigated with 11,100 m^3/ha. The lowest tree yield (78.00 kg/tree) was recorded in site 2 in the second season and site 3 during the third season.

Total yield (Ton/ha) had the same trend, whereas using 425 N units with 11,100 m^3/ha irrigation water in site 5 produced the maximum yield (52.80 tons/ha). On the contrary, Trees grown in sit 2 have a minimum total yield (31.20 tons/ha) was recorded in site 2 in the second season and site 3 in the third one.

The study demonstrated that irrigation water quantities and nitrogen rates affected productivity, and the following ranking was observed for total yield: site 5 > site 1 > site 4 > site 2 > site 3.

Outcome data from Table 3 revealed that the quantity of irrigation water and fertilizing rates in different experimental sites affected fruit quality parameters. TSS/acidity is one of the main maturity indexes for citrus fruits reducing acidity and the accumulation of soluble solid determining fruit maturity according to variety.

The quantity of irrigation water and N rate caused a variation in TSS. The highest TSS rate (11.93) was recorded in site 3, whereas the trees were fertilized by 361 N units and irrigated with 9,360 m^3/ha in the third season. Trees grown in site 2 that were watered by 9600 m^3/ha and received 350 N units/ha recorded the lowest TSS value (11.23).

Table 3. Effect of the tested irrigation rates on tree yield (kg/tree), yield (Ton/ha), and internal fruit quality parameters of Navel orange trees during experimental seasons.

Site	Tree yield (kg/tree)	Yield (Ton/ha)	TSS	Acidity %	TSS/Acid ratio	vitamin C (mg/100g)
First season						
1	96.00 B	38.٤0 B	11.60 A	1.087 A	10.68 A	42.33 A
2	84.00 D	33.60 B	11.23 C	1.060 A	10.60 A	42.07 A
3	84.00 D	33.60 B	11.33 BC	1.053 A	10.77 A	42.83 A
4	89.25 C	35.70 B	11.58 AB	1.107 A	10.55 A	42.60 A
5	108.00 A	43.20 A	11.43 ABC	1.043 A	11.05 A	42.43 A
Second season						
1	102.00 B	40.٨٠ B	11.67 A	1.023 A	11.48 A	42.70 A
2	78.00 C	31.20 C	11.47 BC	1.030 A	11.14 A	42.40 A
3	81.00 C	32.40 C	11.58 ABC	1.053 A	11.04 A	40.33 B
4	84.00 C	33.60 C	11.27 C	1.000 A	11.43 A	42.33 A
5	120.00 A	48.00 A	11.63 AB	0.990 A	11.75 A	41.50 AB
Third season						
1	102.00 B	40.٨٠ B	11.57 B	1.037 BC	11.16 B	41.87 A
2	90.00 BC	36.00 C	11.68 AB	1.070 BC	10.94 B	42.03 A
3	78.00 C	31.20 C	11.93 A	1.133 A	10.54 C	41.00 A
4	90.00 BC	36.00 C	11.27 C	1.087AB	10.38 C	41.00 A
5	126.00 A	50.40 A	11.82 AB	1.020 C	11.59 A	40.97 A
Fourth season						
1	120.00 A	48.00 AB	11.75 A	1.023 A	11.48 A	40.47 A
2	102.00 B	40.80 C	11.59 AB	1.053 A	11.00 BC	40.69 A
3	96.00 B	38.40 C	11.51 B	1.073 A	10.72 C	42.07 A
4	1087.00B	43.20 BC	11.55 AB	1.070A	10.82 C	40.70A
5	132.00 A	52.80 A	11.60 AB	1.030 A	11.27 AB	40.53 A

Note: Values in the same column followed by the same letter(s) do not significantly differ from each other according to Duncan's multiple range test at 5% level. *Site1: Al-Shorouk; Site2: Nubaria; Site3: El-Mahalla El-Kubra; Site4: Ashmoun; Site5: Salhyia.

A higher acid content frequently results in lower-quality orange fruit, whereas a moderate acid amount improves flavor. The difference in acid content was observed among the five sites, results indicated that the lowest acidity value (0.990 %) was recorded in the second season from trees growing in Salhyia. In contrast, the highest acidity ratio (1.133%) was recorded in El-Nubaria district in the third season. Our study showed that N fertilization benefits fruit sugar accumulation and positively affects TSS and VC concentrations, but significantly negatively affects acid content. The effect of various fertilizing rates and quantity of irrigation water in different experimental sites, affect TSS/Acid ratio. The highest TSS/Acid ratio value (11.75) was recorded from trees growing in Salhyia in the second season. The trees growing in Ashmoun district had the lowest TSS/Acid ratio (10.38) in the second season. Vitamin C (ascorbic acid) is one of the most vital water-soluble vitamins and is naturally produced in various fruits and vegetables. Results in hand implied that a high N dose might stimulate VC accumulation in fruit. Regarding VC content in fruit juice, data in Table 3 cleared that, the highest value (42.83 mg/100g) was recorded in site 3 in the third season, and trees growing in site 2 had the lowest VC content (40.12 mg/100g) in the third season.

Regarding the effect of various irrigation rates and applied N units on yield efficiency (Kg/m^3) Figure 1 showed that, the highest yield efficiency (5.92) was recorded in the second season from trees grown in Salhyia district that received 425 N units and watering with 11,10 m^3 water/ ha. On the contrary, the lowest yield efficiency (4.01) was in site 3 in the third season, when trees received 361 N units and 9,360 m^3 water/ ha.

The Water footprint and water use efficiency at Al-Shorouk, north of Tahrir –Beheira presented in Table 4 data shows that water use efficiency ranged from 3.58 to 3.78 kg orange /m^3 water with an average for four years of 3.70 kg orange /m^3 water. The green water footprint ranged from 5 to 122 m^3 water /ton orange depending on rainfall in different years. The blue water footprint was lower in 2020 with a value of 122 m^3 water /ton orange and higher in 2023 with a value of 261 m^3 water /ton orange with an average for four years of 232 m^3 water /ton orange. The gray water footprint was higher in 2020 with a value of 117m^3 water /ton orange and lower in 2023 with a value of 94 m^3 water /ton. The grey water footprint of orange average for four years of 105 m^3 water /ton orange. The average water footprint for Al-Shorouk was 370 m^3 water /ton orange.

The Water footprint and water use efficiency at Nubaria-Beheira presented in Table 4 data shows that water use efficiency was lower in 2021 with a value of 3.02 kg orange/ m^3 water and was the height in 2020 with a value of 3.50 Kg orange / m^3 water. The average of water use efficiency for Nubaria was 3.31m^3 kg orange /m^3 water. The green water footprint ranged from 8 to 127 m^3 water /ton orange depending on rainfall in different years. The blue water footprint was lower in 2020 with a value of 159 m^3 water /ton orange and higher in 2021 with a value of 323 m^3 water /ton orange with an average of four years of 261 m^3 water /ton orange. The gray water footprint ranged from 88 to 118 m^3 water /ton orange. The average gray water footprint was 106m^3 water /ton orange. The water footprint was lower in 2022 with a value of 382m^3 water /ton orange and higher in 2021 with a value of 448m^3 water /ton orange with an average of four-year 402 m^3 water /ton orange. Date in Table 4 presented the Water footprint and water use efficiency at Mahalla al-Kubra- Gharbia data showed that water use efficiency was lower in 2022 with a value of 3.33 kg orange /m^3 water and was height in 2023 with a value of 3.72 kg orange /m^3 water. The average of water use efficiency was 3.57 m^3 kg orange /m^3 water. The green water footprint ranged from 8 to 138 m^3 water /ton orange depending on rainfall in different years with an average of 54 m^3 water /ton orange. The blue water footprint was lower in 2020 with a value of 143 m^3 water /ton orange and higher in 2022 with a value of 280 m^3 water /ton orange. The average blue water footprint was 226 m^3 water /ton orange. The lowest gray water footprint was 118 m^3 water /ton orange and the highest was 170 m^3 water /ton orange. The average gray water footprint was 146 m^3 water /ton orange. The average for Mahalla al-Kubra was 429 m^3 water /ton orange. The water footprint was lower in 2022 with a value of 398 m^3 water /ton orange and higher in 2020 with a value of 478 m^3 water /ton orange.

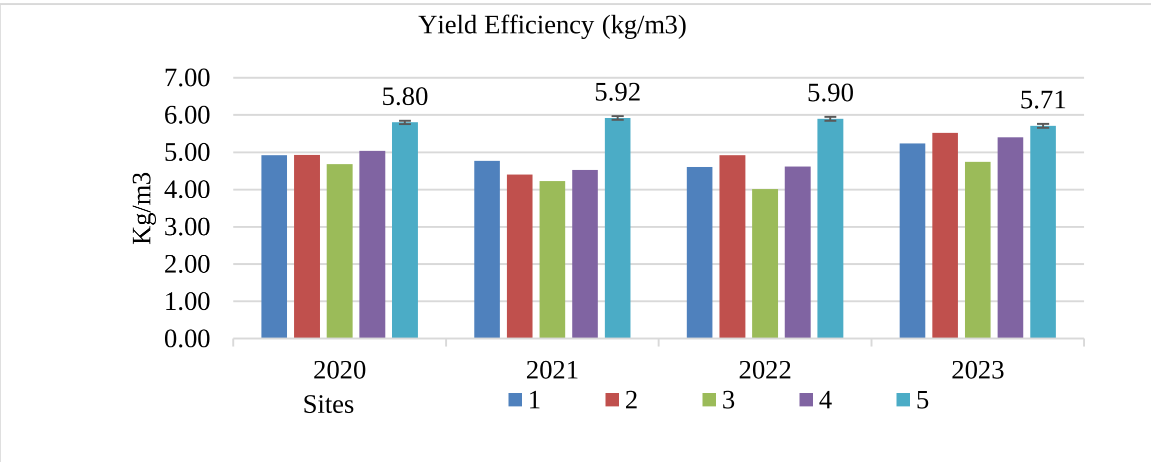


Fig. 1. Yield efficiency (Kg/m3), as affected by the tested irrigation rates on Navel orange trees in different experimental sites. *Site1: Al-Shorouk; Site2: Nubaria; Site3: El-Mahalla El-Kubra; Site4: Ashmoun; Site5: Salhyia.

Table 4. Navel orange water use, water use efficiency, green water, blue water, gray water, and total water footprint for various sites during the experimental seasons.

Year	Yield (ton/ ha)	WU (m^3/ha)	WUE (Kg/m^3)	WF_{green} (m^3/ton)	WF_{blue} (m^3/ton)	WF_{grey} (m^3/ton)	WF (m^3/ton)
Al-Shorouk,							
2020	38.4	10560	3.64	122	153	117	392
2021	40.8	10800	3.78	6	259	108	373
2022	40.8	11400	3.58	24	256	100	356
2023	48.0	12720	3.78	5	261	94	359
mean	42	11370	3.70	39	232	105	370
Nubaria							
2020	33.6	9600	3.50	127	159	107	393
2021	31.2	10320	3.02	8	323	118	448
2022	36.0	10800	3.33	31	273	109	382
2023	40.8	12000	3.40	6	290	88	384
mean	35.4	10680	3.31	43	261	106	402
Mahalla							
2020	33.6	9423	3.56	138	143	170	478
2021	32.4	8880	3.65	50	224	147	421
2022	31.2	9360	3.33	19	280	118	398
2023	38.4	10320	3.72	8	262	149	419
mean	33.6	9496	3.57	54	227	146	429
Ashmoun							
2020	36	9120	3.95	83	170	111	365
2021	33.6	9360	3.59	28	250	114	393
2022	36	9840	3.66	15	260	123	382
2023	43.20	10320	4.18	2	237	93	332
mean	37.2	9660	3.85	32	229	110	368
Salhyia							
2020	43.2	10800	4.29	0	233	97	330
2021	48	10560	4.45	0	220	90	310
2022	50.4	11100	4.54	0	220	92	312
2023	52.80	11880	4.44	0	225	79	304
mean	48.6	11085	4.43	0	225	90	314

The Water footprint and water use efficiency at Ashmoun –Menoufia obtainable in Table 4 data revealed that water use efficiency was ranged from 3.59 to 4.18 kg orange /m^3 water with average for four years 3.85 kg orange /m^3 water. The green water footprint was ranged from 2 to 83 m^3 water /ton orange depend on rainfall amount. The blue water footprint was lower at 2020 with value 170 m^3 water /ton orange and higher at 2022 with value 260 m^3 water /ton orange with average for four years 229 m^3 water /ton orange. The gray water footprint was higher in 2022 with value 123 m^3 water /ton orange and lower in 2023 with value 93 m^3 water /ton orange with average for four year 110 m^3 water /ton orange. The average of water footprint for Ashmoun was 368 m^3 water /ton orange.

The water footprint and water use efficiency at Al-Shorouk, located north of Tahrir-Beheira, as shown in Table 4 data indicates that water use efficiency varied from 3.58 to 3. 78 kg orange/m3 water, with an average over four years of 3.70 kg orange/m3 water. The green water footprint varied from 5 to 122 m^3 water/ton orange, depending on the rainfall in various years. The blue water footprint was lower in 2021 and 2022 with a value of 220 m^3 water /ton orange and higher in 2020 with a value of 233 m^3 water /ton orange with an average for four year 225 m^3 water /ton orange. The gray water footprint ranged from 79 to 97 m^3 water /ton orange. The average gray water footprint was 90 m^3 water /ton orange. The water footprint was lower in 2023 with a value of 304 m^3 water /ton orange and higher in 2020 with a value of 330 m^3 water /ton orange with an average of four years 314 m^3 water /ton orange. Table 5 presented the Water footprint and water use efficiency under surface and drip irrigation data showed that water use efficiency was lower in surface irrigation with a value of 3.71 kg orange /m^3 water and was in height drip irrigation with a value of 3.81 kg orange /m^3 water.

The green water footprint under drip irrigation was 27.3 m^3 water /ton orange and was higher under surface irrigation at 43m^3 water /ton with an incensed percentage of about 63%. The blue water footprint under surface irrigation was 228m^3 water /ton orange and under drip irrigation were 239.3 m^3 water /ton orange. The water footprint was lower under the drip irrigation system with a value of 362 m^3/ton than the surface irrigation system with a value of 398.5 m^3/ton.

Furthermore, Figure 2 shows the comparison between surface and drip irrigation systems for water footprint component data revealed that the drip irrigation system is lower in green, gray and total water footprint than surface irrigation. The blue water footprint was the height under drip irrigation system than surface irrigation system.

Table 5. Navel orange yield, water use, water use efficiency, green water, blue water, gray water, and total water footprint under drip and surface irrigation.

Site	Drip irrigation				Surface irrigation		
	1	2	5	Mean	3	4	Mean
Yield (ton/ha)	42	35.4	48.6	42	33.9	37.2	35.5
WU (m^3/ha)	11370	10680	11085	11045	9496	9660	9578
WUE (Kg/m^3)	3.7	3.31	4.43	3.81	3.57	3.85	3.71
WF_{green} (m^3/ton)	39	43	0	27.3	54	32	43
WF_{blue} (m^3/ton)	232	261	225	239.3	227	229	228
WF_{grey} (m^3/ton)	105	106	90	100.3	146	110	128
WF (m^3/ton)	370	402	314	362	429	368	398.5

*Site1: Al-Shorouk; Site2: Nubaria; Site3: El-Mahalla El-Kubra; Site4: Ashmoun; Site5: Salhyia.

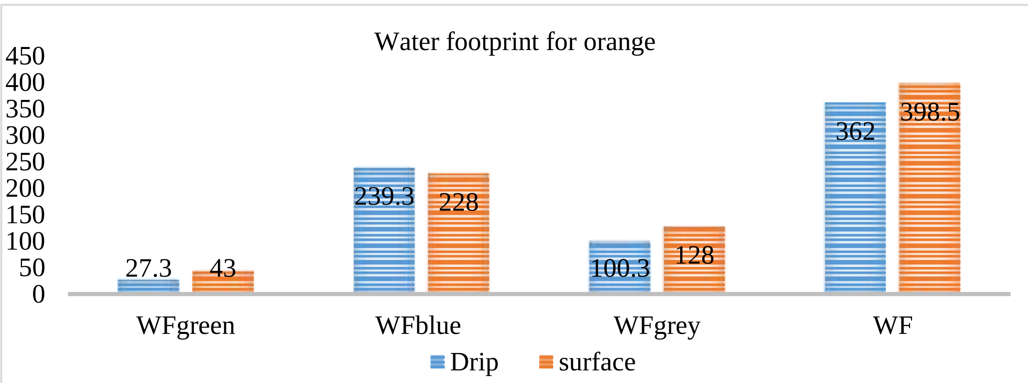


Fig. 2. The mean of green water footprint, blue water footprint, gray water footprint, and total water footprint under drip and surface irrigation for Navel orange.

DISCUSSION

This study investigates the Water Footprint components and water use efficiency of Navel Orange trees at five different locations in Egypt during 2020–2023. It is well documented that water is one of the limiting factors in the fruiting and productivity of fruit trees and affects plant metabolism. Various irrigation practices are used in citrus orchards in Egypt, including reduced water and N manual application), including reduced water and N fertilizer input, increased water productivity (WP) and water use efficiency (Li et al., 2021).

Our results indicate that the productivity of citrus orchards using drip irrigation may be significantly higher than that under surface irrigation and manual N fertilization. There are different responses to water quantity under experimental sites conditions on tree yield and fruit quality parameters, in addition, nitrogen levels significantly affect total fruit yield and fruit characters. There is a positive correlation between the availability of water and nitrogen in the soil during the growth cycle and productivity (Fikry et al., 2022; Panigrahi & Srivastava, 2016).

Increasing water availability improves the growth and productivity of fruit trees. The highest tree volume increment was in site (5) in the fourth season when trees were irrigated with 11,880 m^3 and fertilized with 425 N units.

Our findings in agree with (Rakha et al., 2024; Liao et al., 2019) who reported that high N fertilizers increase the total acidity of citrus fruit juice and reduce TSS/Acidity and vitamin content.

Our study indicated that moderate irrigation water with moderate N fertilizers in clay soil increases TSS in fruit juice. While in sandy soil with high irrigation and a high N rate, the TSS/Acidity ratio increased. Furthermore, acidity increased with increasing nitrogen rate, this could be due to the limited differences in nitrogen levels between the study sites. These results indicated that nitrogen fertilization treatments provided adequate nutrition and increased acidity (Liao et al., 2019).

Mekonnen and Hoekstra's model serves as an effective way to compare water footprints for crops grown worldwide. When comparing crop water footprint numbers, it is important to note the impact of crop yields on water footprint. Due to the role of yields in determining water footprints, these values are influenced by the different factors that impact yields apart from water availability, including nutrient source, crop varieties, farmer access to agricultural inputs, the severity of rainfall, soil type and condition and pest and disease incidence. Water appropriation can differ depending on the region, climate conditions (rainfall and evapotranspiration), the irrigation methods used (surface, or drip), and the fertilizers amount and pesticides allowed. The water consumption depends on crop characteristics, especially the tree age and fruit yield (Mekonnen & Hoekstra, 2014). Especially in arid and semi-arid regions, the amount of irrigation water plays numerous roles in the productive yield of oranges by affecting the yield and water use efficiency. Therefore, good irrigation management is required for maximum quality yield in citrus (Zekri, 2000).

The water use efficiency increased with micro-sprinkler irrigation systems under 80% ETc, which resulted in 2.57 and 2.67 kg of fruit per cubic meter of irrigation water needed in the first and second seasons, respectively (Youssef et al., 2023). In addition, water productivity or water use efficiency for oranges ranged from 3.6 to 3.0 kg m-3 and decreased with increasing irrigation water applied (Hammami & Mellouli, 2011).

Green water talks about to the precipitation on soil that does not run off or recharge the groundwater but is stored in the soil or temporarily stops on top of the soil or vegetation. The green water can also leave the soil through evaporation or subsurface runoff, but it is considered productive only when used for plant transpiration. The lower green water footprint is expected in our study due to the semi-arid climate of Egypt and depends on rainfall amounts for different locations in this study. The share of green water footprint is low in the total OF, which concludes that the share of effective rainfall is very low. The green water footprint is related to effective precipitation. Since the effective rainfall Peff is low in the extra-arid regions, hence the share of green water footprint is lower than the other water footprint components (Sun et al., 2012).

The blue water footprint is a role of the consumptive irrigated water amount and the produced yield (Mekonnen & Hoekstra, 2020). Blue water footprint is related to the use of irrigation water (net water requirement). Irrigation water is a vital element of improving plant productivity and minimizing the yield gap, mainly in arid regions with severe water scarcity. The water consumed by different irrigation techniques (surface and drip) and irrigation strategies were not the same? The blue water footprint indicates the amount of potable water contributed to a process and/or product, it will be high in agriculture. This result matches Bazrafshan et al. (2018) who reported that the high water requirement and low yield per area have led to a high blue water footprint.

The greywater footprint is defined to the use of freshwater to dilute the pollutants (nitrogen fertilizers) when the nitrogen fertilizer amount was incensed the graywater footprint increased.

Increased use of nitrogen fertilizer has both positive and negative effects. The rise in crop yields production over the past decades is partly due to the increased use of fertilizer in agriculture. However, a large fraction of nitrogen used to croplands in the form of fertilizer and manure ends up entering the freshwater system causing degradation of the water quality. The Grey Water Footprint focuses on the release of nitrogen to freshwater systems and translates the nitrogen load into a volume of water to assimilate. These results agree with Tozzini et al. (2021) who show that the quantity of nitrogen fertilizer was the main factor that affected greywater footprint and that increasing nitrogen fertilizer dosage can increase greywater footprint values.

The mean source of freshwater contamination affected by fertilizers and surface run-off of nitrogen Pollution levels are determined by a variety of factors, including soil texture, terrain, and, most importantly, crop and farm management (Delin & Stenberg, 2014). The water footprint under study condition was 362 m^3/ton (0.362 m^3/kg) under the drip irrigation system and the surface irrigation system with a value of 398.5 m^3/ton (0.398 m^3/kg), this result agreed with (Arabi-Yazdi et al., 2009) who reported that the weighted average of citrus WFT is 0.36 m^3/ kg in Iran. In addition, previous work reported that the average world water footprint for oranges is about 510 m^3/ton (0.510 m^3/kg), according to (Mekonnen & Hoekstra, 2014).

CONCLUSION

Results showed that using 11085 m3/ha in Salhyia district produced maximum yield and improved most fruit quality parameters. Moreover, water use efficiency in drip irrigation (3.81 kg orange/m3 water) was better than surface irrigation (3.71 kg orange/m3 water). Regarding water footprint components, data revealed that the drip irrigation system is lower in green, gray and total water footprint than surface irrigation. The blue water footprint was the height under the drip irrigation system rather than the surface irrigation system. This work provides practical approaches for researchers and policymakers in the agriculture area to manage the water footprint and optimize the water consumption and productivity of Navel oranges in Egypt.

Conflict of interest
The authors declare that there is no conflict of interest**.**

REFERENCES

Abobatta, W. F. (2019). Citrus varieties in Egypt: An impression. *International Research Journal of Applied Sciences*, *1*, 63-66.

Abobatta, W. F. (2023). Citrus production in climate change era. In *Cultivation for Climate Change Resilience*, Volume 2 (pp. 68-93). CRC Press. https://doi.org/10.1201/9781003351153-5

Annual Reports of the Statistical Institute and Agricultural Economic Research in Egypt, (2023).

Arabi, Y. A., Alizadeh A., & Mohammadian F. (2010). Study on ecological water footprint in agricultural section of Iran. *Water and Soil*, *23*, 1–5.

Arafat, I. E., & Helal, M. E. M. (2021). Impacts of climate change on irrigation requirements and water productivity of citrus and olive crops in Egypt. *Fundamental and Applied Agriculture*, *6*(2), 144-154.

Bankaji, I., Kouki, R., Dridi, N., Ferreira, R., Hidouri, S., Duarte, B., ... & Caçador, I. (2023). Comparison of digestion methods using atomic absorption spectrometry for the determination of metal levels in plants. *Separations*, *10*(1), 40. https://doi.org/10.3390/separations10010040

Bazrafshan, O., Dehghanpir, S., & Holisaz, A. (2018). Estimation of virtual water trade in the hormozgan province over the past decade. *Desert Management*, *5*(10), 116-129.

Biratu, W., Abebe, H., Berhe, M., Tesfay, K., Gebremeskel, H., Tuemay, M., ... & Purba, J. H. (2023). Growth, henological, yield and yield components evaluation of sweet orange (*Citrus sinensis* L.) cultivars in Raya Azebo Woreda of Southern Tigra, Ethiopia *AGROLAND The Agricultural Sciences Journal (e-Journal)*, *10*(1). https://doi.org/10.22487/agroland.v0i...

Chapagain, A. K., & Hoekstra, A. Y. (2011). The blue, green and grey water footprint of rice from production and consumption perspectives. *Ecological economics*, *70*(4), 749-758.

Chapagain, A. K., Hoekstra, A. Y., & Savenije, H. H. (2006). Water saving through international trade of agricultural products. *Hydrology and Earth System Sciences*, *10*(3), 455-468. https://doi.org/10.5194/hess-10-455-2006

Delin, S., & Stenberg, M. (2014). Effect of nitrogen fertilization on nitrate leaching in relation to grain yield response on loamy sand in Sweden. *European Journal of Agronomy*, *52*, 291-296. https://doi.org/10.1016/j.eja.2013.08.007

Fikry, A. M., Radhi, K. S., Abourehab, M. A., Abou Sayed-Ahmed, T. A., Ibrahim, M. M., Mohsen, F. S., ... & El-Saadony, M. T. (2022). Effect of inorganic and organic nitrogen sources and biofertilizer on murcott mandarin fruit quality. *Life*, *12*(12), 2120. https://doi.org/10.3390/life12122120

Freed, R. D. (1985). MSTAT-C Statistical Package, Version 2.0. 0. *Crop and Soil Science Department, Michigan State University, East Lansing*.

Galán-Martín, Á., Vaskan, P., Antón, A., Esteller, L. J., & Guillén-Gosálbez, G. (2017). Multi-objective optimization of rainfed and irrigated agricultural areas considering production and environmental criteria: a case study of wheat production in Spain. *Journal of Cleaner Production*, *140*, 816-830. https://doi.org/10.1016/j.jclepro.2016.06.099.

Hammami, A., & Mellouli, J. (2011, October). Drip irrigation scheduling of citrus orchard in Tunisia. In *Proceedings of the 21st ICID International Congress of Irrigation and Drainage, Teheran, Iran* (pp. 15-23).

Hoekstra, A., Chapagain, A. K., Aldaya, M. M., & Mekonnen, M. M. (2012). *The water footprint assessment manual: Setting the global standard*. Routledge.

Hoekstra, A. Y., Chapagain, A., Martinez-Aldaya, M., & Mekonnen, M. (2009). Water footprint manual: State of the art 2009. http://waterfootprint.org/media/downloads/WaterFootprintManual2009.pdf

Imbernón-Mulero, A., Martínez-Alvarez, V., Ben Abdallah, S., Gallego-Elvira, B., & Maestre-Valero, J. F. (2024). A comparative water footprint analysis of conventional versus organic citrus production: a case study in Spain. *Agriculture*, *14*(7), 1029. https://doi.org/10.3390/agriculture14071029

Li, H., Mei, X., Wang, J., Huang, F., Hao, W., & Li, B. (2021). Drip fertigation significantly increased crop yield, water productivity and nitrogen use efficiency with respect to traditional irrigation and fertilization practices: A meta-analysis in China. *Agricultural Water Management*, *244*, 106534. https://doi.org/10.1016/j.agwat.2020.106534

Liao, L., Dong, T., Qiu, X., Rong, Y., Wang, Z., & Zhu, J. (2019). Nitrogen nutrition is a key modulator of the sugar and organic acid content in citrus fruit. *PLoS One*, *14*(10), e0223356. https://doi.org/10.1371/journal.pone.0223356

Lu, Y., Zhang, X., Chen, S., Shao, L., & Sun, H. (2016). Changes in water use efficiency and water footprint in grain production over the past 35 years: a case study in the North China Plain. *Journal of cleaner production*, *116*, 71-79.

Lovarelli, D., Bacenetti, J., & Fiala, M. (2016). Water Footprint of crop productions: A review. *Science of the Total Environment*, *548*, 236-251.

Mekonnen, M. M., & Hoekstra, A. Y. (2020). Sustainability of the blue water footprint of crops. *Advances in Water Resources*, *143*, 103679.

Mekonnen, M. M., & Hoekstra, A. Y. (2014). Water footprint benchmarks for crop production: A first global assessment. *Ecological indicators*, *46*, 214-223. https://doi.org/10.1016/j.ecolind.2014.06.013.

Munro, S. A., Fraser, G. C., Snowball, J. D., & Pahlow, M. (2016). Water footprint assessment of citrus production in South Africa: A case study of the Lower Sundays River Valley. *Journal of Cleaner Production*, *135*, 668-678.

Panigrahi, P., & Srivastava, A. K. (2016). Effective management of irrigation water in citrus orchards under a water scarce hot sub-humid region. *Scientia Horticulturae*, *210*, 6-13. https://doi.org/10.1016/j.scienta.2016.07.008

Rakha, A. M., Eisa, R. A., Abourayya, M. S., Kaseem, N. E., & Mahmoud, T. S. M. (2024). Effects of different sources of nitrogen fertilizer on the yield and fruit quality of persian lime under nubaria conditions. *Applied Fruit Science*, *66*(5), 1929-1935. https://doi.org/10.1007/s10341-024-01158-w

Sun, S. K., Wu, P. T., Wang, Y. B., & Zhao, X. N. (2012). Impacts of climate change on water footprint of spring wheat production: the case of an irrigation district in China. *Spanish Journal of Agricultural Research*, *10*(4), 1176-1187.

Tozzini, L., Pannunzio, A., & Soria, P. T. (2021). Water footprint of soybean, maize and wheat in Pergamino, Argentina. *Agricultural Sciences*, *12*(3), 305-323. https://doi.org/10.4236/as.2021.123020

Tuninetti, M., Tamea, S., D'Odorico, P., Laio, F., & Ridolfi, L. (2015). Global sensitivity of high-resolution estimates of crop water footprint. *Water Resources Research*, *51*(10), 8257-8272. https://doi.org/10.1002/2015WR017148

Wallar, A., & Duncan, D. B., (1969). Multiple ranges and multiple tests. *Biometrics* 11, 1-24.

Yi, J., Gerbens-Leenes, P. W., & Aldaya, M. M. (2024). Crop grey water footprints in China: The impact of pesticides on water pollution. *Science of the Total Environment*, *935*, 173464. https://doi.org/10.1016/j.scitotenv.2024.173464

Youssef, E. A., Mahmoud, T. A., & Abo-Eid, M. A. (2023). Effect of some irrigation systems on water stress levels of Washington navel orange trees. *Bulletin of the National Research Centre*, *47*(1), 163. https://doi.org/10.1186/s42269-023-01140-8

Zekri, M., (2000). Citrus rootstocks affect scion nutrition, fruit quality, growth, yield and economical return. *Fruits*, *55*(4), 231-239.

JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH
2025, VOL. 8(4), 465-476

Journal of Horticulture and Postharvest Research

Journal homepage: www.jhpr.birjand.ac.ir

University of Birjand

The impact of heat units on the physical and chemical characteristics of two grape varieties in Egypt

Ahmed S. Abd El-Rahman[1,*], Fawzia I. Moursy[1], Amira Sh. Soliman[1], Mohamed A. Abd El-Wahab[2] and Alaa A. Khalil[3]

1, Department of Natural Resources, Faculty of African Postgraduate Studies, Cairo University, 12613 Giza, Egypt
2, Viticulture Research Department, Horticulture Research Institute, Agricultural Research Center, Giza, Egypt
3, Agricultural Meteorological Applications Research Department, Central Laboratory for Agricultural Climate, Agricultural Research Center, Giza, Egypt

ARTICLE INFO

Original Article

Article history:
Received 28 November 2024
Revised 13 March 2025
Accepted 2 April 2025

Keywords:
Climate
Grape
Phenology
Veraison
Yield

DOI: 10.22077/jhpr.2025.8464.1453
P-ISSN: 2588-4883
E-ISSN: 2588-6169

***Corresponding author:**
Department of Natural Resources, Faculty of African Postgraduate Studies, Cairo University, 12613 Giza, Egypt.

Email: Ahmedsamy.2025@hotmail.com

ABSTRACT

Purpose: Climatic circumstances are significant determinants in the formation and growth of the vine. Due to variations in climatic parameters, high-temperature affects phenology, the ripening period, and physicochemical characteristics are detrimental to the quality of the grapes produced and gradually decrease the yield. **Research method:** This investigation studies the effect of heat units on the yield and fruit quality of some grape cultivars in different regions of Egypt. This trial evaluates two grape cultivars (Flame Seedless and Crimson Seedless) grown in three distinct locations (El-Behira, El-Menoufia, and El-Minia governorates) during seasons (2021 and 2022). **Findings:** Heat units negatively affect the phenological dates of the grape growth cycle. However, the warmer regions (El-Minia governorate) accelerated various phases or stages in the phenological development of grapevines, including bud burst, full flowering, fruit set, veraison, and grape maturity as compared to the moderate regions (El-Behira and El-Menoufia governorates). Regarding yield and its attributes, the moderate regions (El-Behira and El-Menoufia governorates) had the highest yield. They improved the bunch physiochemical attributes of Flame Seedless and Crimson Seedless grapes compared to the warmer region (El-Minia governorate). **Research limitations:** There were no limits. **Originality/Value:** Heat units negatively affect the phenological stages of grape growth (bud break, full flowering, fruit set, veraison, and grape maturity) and physicochemical characteristics.

INTRODUCTION

Among all cultivated plants, grapevines are considered one of the most responsive to their environment (Jackson, 2001). Grapes (*Vitis vinifera* L.) are among the most important horticultural products and have high nutritional value. The most significant grape species used specially to make grapevines is *Vitis vinifera*, which is also commercially very valuable. Due to their extreme environmental sensitivity, these species are found in a rather small climatic niche, which is typically found between latitudes 30–50°N and 30–40°S (Wang et al., 2020). Difference in climate parameters is considered a main factor in inter-annual variability in plant development. All crops are affected by different heat stresses during the growing season and differential response to temperature changes across crops has been observed in various production environments (Kalra, 2008). Simple to complex temperature indices are the most commonly used measurements to assess which types of grapes can be grown in which weather (Jones et al., 2010). Any change in the ideal temperature during its differentiation negatively affects the initiation and duration of different phenological events and yields (Singh et al., 2007). Meteorological conditions exert a considerable influence on the phenology of fruit trees (Gupta et al., 2020). During the veraison-maturity stage, increased temperature can significantly affect the accumulation of sugar (Greer & Weedon, 2013). A decrease in the amount of anthocyanin production may also result from this occurrence (Conde et al., 2016). Grapes can also have low acidity and high sugar levels during this time. According to recent studies, there are negative correlations between anthocyanin temperature and berry weight at technical maturity (Gouot et al., 2019). Vines are a perennial crop that requires both adequate warm and cold conditions to complete their biological processes (high temperatures for berry ripening and low temperatures for fruiting and hardening). As a result, temperature can be thought of as one of the primary determinants of the vegetative cycle's evolution, the berries' ultimate maturity, and their composition. Conversely, the high-temperature regime during the ripening phase might be advantageous to the vine. On the other hand, high temperatures can stress plants and decrease their ability to photosynthesize. Thus, this investigation aimed to study the effect of heat units on the physiochemical of some grape cultivars in different regions of Egypt.

MATERIALS AND METHODS

This survey was conducted for two consecutive seasons (2021 and 2022) in three different regions located in the Arab Republic of Egypt, namely the El-Nubaria region of El-Behira governorate, El-Sadat city of the governorate of El-Menoufia and the district of Matai of the governorate of El-Minia Governorate, where the most grape-productive areas in Egypt are located, to study the impact of thermal units on the physical chemistry of Flame Seedless and Crimson Seedless grape cultivars. The Egyptian Meteorological Authority (Table 1) was recorded.

Table 1. List of study area locations for the Egyptian Meteorological Authority.

Region	Governorate	Latitude (N)	Longitude (E)	Elevation (m)
El-Nubaria	El-Behira	30.53	30.83	9.60
El-Sadat	El-Menoufia	31.03	30.53	17.90
Matai	El-Minia	28.41	30.77	34.20

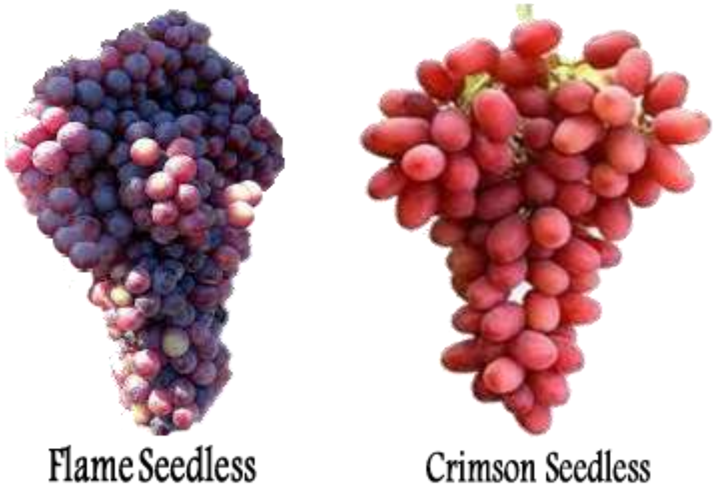


Fig. 1. Appearance of Flame Seedless and Crimson Seedless grape clusters.

Table 2. Climate data for the governorates of El-Behira, El-Menoufia, and El-Minia during the 2021 and 2022 seasons.

Governorate	El-Behira				El-Menoufia				El-Minia			
Month	Max Temp. (°C)	Min Temp. (°C)	Ppt (mm)	R.H. (%)	Max Temp. (°C)	Min Temp. (°C)	Ppt (mm)	R.H. (%)	Max Temp. (°C)	Min Temp. (°C)	Ppt (mm)	R.H. (%)
	Season 2021											
January	27.1	6.7	19.0	68.1	27.1	5.2	2.5	63.9	28.4	3.0	0.0	52.4
February	28.2	7.4	55.1	68.4	28.0	6.1	12.4	65.1	28.9	3.4	0.7	49.4
March	32.0	7.3	2.5	67.1	33.0	5.9	0.4	63.2	38.0	4.5	0.0	43.7
April	40.5	7.7	0.5	60.3	40.9	7.4	0.2	53.9	41.9	6.0	0.0	31.8
May	42.6	15.6	0.1	49.6	42.7	15.1	0.0	44.1	43.9	15.5	0.0	24.4
June	41.0	17.2	0.0	51.6	41.5	16.3	0.0	47.6	42.3	16.5	0.0	30.1
July	43.4	21.3	0.0	52.9	44.1	20.3	0.0	48.2	44.4	21.7	0.0	29.9
August	43.8	22.2	0.0	55.1	44.0	20.9	0.0	50.5	43.8	21.3	0.0	31.0
September	41.1	19.6	0.0	56.0	41.1	18.9	0.0	53.6	41.0	17.9	0.0	40.4
October	36.0	17.6	4.0	58.6	35.5	16.2	1.0	56.9	36.2	14.8	0.0	44.8
November	33.5	13.2	11.1	66.5	33.2	13.0	9.1	63.9	33.9	10.7	0.0	49.6
December	24.6	7.4	45.4	73.0	24.2	6.8	32.8	69.3	23.8	2.6	0.6	60.2
	Season 2022											
January	21.4	3.2	29.5	72.7	21.6	2.4	5.7	68.4	22.6	2.1	0.4	62.5
February	24.4	4.8	13.3	72.3	24.7	5.1	4.5	67.3	25.7	3.4	6.0	56.6
March	28.5	5.0	8.1	67.8	30.0	4.0	2.5	62.4	31.8	3.2	0.0	46.4
April	39.9	9.9	0.0	59.8	40.8	9.0	0.0	51.4	41.9	9.1	0.0	31.1
May	42.7	13.8	0.2	53.8	42.3	12.7	0.1	47.8	41.7	12.6	0.0	28.4
June	44.6	19.1	0.0	53.0	45.4	18.6	0.1	48.8	44.7	18.9	0.1	30.8
July	40.6	20.8	0.0	53.5	41.3	19.9	0.0	49.6	40.4	19.4	0.0	31.1
August	41.9	21.8	0.1	55.5	41.9	21.3	0.0	51.2	42.5	21.7	0.1	33.6
September	41.4	20.1	0.0	55.4	41.3	19.7	0.1	52.1	42.2	19.3	0.0	36.6
October	40.5	17.1	7.5	60.2	40.4	16.1	6.3	58.9	41.6	14.8	0.1	47.4
November	29.3	11.5	6.7	61.1	29.4	10.3	2.3	60.3	28.7	10.1	0.0	50.4
December	28.1	9.5	19.4	68.1	28.2	8.9	13.5	65.9	27.4	6.7	0.3	56.8

The grape is divided into early, medium, and late ripening. Climate plays an important role in phenological dates, yield, and characteristics of vine bunches (Spayd et al., 2002). Flame Seedless grapes were chosen as representative of early varieties and Crimson Seedless grapes as representative of late varieties to determine their degree of response to climatic elements (Fig. 1).

The governorates of Lower Egypt differ from the governorates of Upper Egypt in terms of climate. Behira and Menoufia governorates were chosen as representatives of Lower Egypt governorates, and Minia governorate was chosen as representative of Upper Egypt governorates in terms of climate in their influence on the studied vines (Table 2).

The selected vines were seven years old, planted in rows two by three meters apart, in sandy soil (Table 3), and received drip watering. The Spanish baron system was followed for pruning and trellising the vines. For both study seasons, the vines were trimmed in the second week of January to maintain a load of 84 buds/vine (7 canes × 12 buds/vine). Every cultivar was tested in three repetitions, with five vines in each replicate. The experimental vines were given the same horticultural treatments and had comparable growth vigor and health.

Heat units

Daily maximum temperature (Tmax), minimum temperature (Tmin), precipitation (Ppt), and relative humidity (RH) were recorded at the agro-meteorological observatory installed at the Central Agricultural Climate Laboratory of the Egyptian Research Center Agricultural (Table 2).

Growing degree days (GDD) for different grape phenologies were calculated using a base temperature of 10 °C (Tb) (Winkler et al., 1974) according to the following formulas (1) and accumulated from the date (i.e. January 1) until the date of appearance.

$$GDD=\sum \left(\frac{Tmax+Tmin}{2}\right) -T_b \qquad (1)$$

Grape phenology

The factors taken into account were: the first, known as the budburst date, which occurs when 50% of the buds open; the second, known as the full flowering date, which occurs when the calyptra falls on 70–80% of the flowers; the third, known as the fruit set date, which occurs when the fertilized flower starts to turn into a berry; the fourth date of veraison, which is when the berries attain the color stage or starts to soften as sugars build up and acids start to decrease; The berries achieve their full-color stage and the TSS reaches about 16–17% according to Hamie et al. (2023). Every calendar year, the number of days for phenological dates (Julian Day) was computed.

Yield

Yield/vine (kg) was determined as the number of bunches/vine × bunch weight (g).

Physical characteristics of the bunch

Average weight of bunch (g), average weight of the berries (g), and average volume of the berries (cm^3) were measured.

Chemical characteristics of the bunch

Total sugars were determined in (%) in the juice by Miller (1959) as described in A.O.A.C. (2005). Total acidity was determined in (%) according to the A.O.A.C. (2005). Total anthocyanin was determined in (mg/100g fresh berry skin) according to Hsia et al. (2006).

Table 3. Physical and chemical analysis of the soil of the vineyards of El-Behira, El-Menoufia, and El-Minia Governorates.

Characters	El-Behira Governorate	El-Menoufia Governorate	El-Minia Governorate
Sand (%)	74.3	71.6	78.2
Silt (%)	14.2	16.5	11.5
Clay (%)	11.5	11.9	10.3
Texture	Sandy	Sandy	Sandy
Organic carbon (%)	0.84	0.87	0.79
pH (1:25)	7.52	7.41	7.64
EC (Mmhos/cm)	0.96	0.91	0.93
Ca Co_3 (%)	1.33	1.37	1.31
Total N (%)	0.18	0.21	0.17
P (%)	0.12	0.14	0.11
K (%)	0.35	0.36	0.34

The following parameters were adopted for Flame Seedless and Crimson Seedless grape cultivars in three different regions.

Experimental design and statistical analysis

A completely randomized design was adopted for this experiment. The statistical analysis of the present data was carried out according to Snedecor and Cochran (1980). Averages were compared using the L.S.D. values at the 5% level.

RESULTS AND DISCUSSION

Heat units

Data in (Table 4) mentioned that Flame Seedless grapevines recorded the lowest heat requirements which led to faster ripening as compared to Crimson Seedless grapevines, which recorded heat requirements in both seasons. However, Flame Seedless grapevines required heat requirements (45422 and 46202 GDD) for the El-Behira governorate, (46827 and 47631 GDD) for the El-Menoufia governorate, and (42753 and 43487 GDD) for the El-Minia governorate in both seasons, respectively. In contrast, Crimson Seedless grapevines required heat requirements (66504 and 67646 GDD) for El-Behira governorate, (68561 and 69738 GDD) for El-Menoufia governorate, and (62596 and 63671 GDD) for El-Minia governorate in both seasons, respectively. These findings align with those of Karvonen (2020) who demonstrated that the apparent yearly temperature increase greatly sped up the Rondo vines' growth cycle (from budburst to harvest) by 11 days Regarding this, Ferretti et al. (2021) discovered that a consistent and regular rise in temperature, up to 4 °C, during the Gewürztraminer grape variety's growth season led a faster-growing cycle and ripening, resulting in a harvest that occurred three to five weeks early. The cumulative growth degree day range for ripening types was also mentioned by Gupta et al. (2020): 1303-1530 °C for early varieties, 1617-1712 °C for medium variations, and 1912-1959 °C for late kinds.

Grape phenology

Table 5 displays the data on the number of Julian days (days in a calendar year) for phenological dates. The data exhibited a significant degree of variation among the sites under investigation. When compared to other types, the Minia region had the fewest days for every phenological date in this regard. On the other hand, during both seasons, the El-Behira and El-Menoufia regions recorded the most days for every phenological date. In terms of the cultivar effect, in both seasons the Flame Seedless grape had fewer days than the Crimson Seedless grape for every phenological factor. There are various phases or stages in the phenological development of grapevines, including bud burst, full flowering, fruit set, veraison, and grape maturity. Climate plays a major role in determining these phases of the grapevine's vegetative and reproductive cycles (Fraga et al., 2012). Temperature series spanning several millennia have been reconstructed using harvest dates (Chuine et al., 2014). Varieties differ significantly in phenology (Parker et al., 2013). Local grape varietals may be subjected to high temperatures during grape ripening if they migrate beyond their optimal ripening window due to the presence of thermal units (Lereboullet et al., 2014). The vine's phenology advances with higher temperatures (Parker et al., 2013). As a result, grapes ripen in warmer climates earlier in the growing season (Molitor & Junk, 2019). In this sense, the outcomes align with the findings of (Molitor & Junk, 2019) conducted research in the Sopron and Zala districts (Western Carpathian Basin, northwest Hungary) on the phenological reaction of grapevines to temperature units. They demonstrated an 11-day early harvest and an approximate 7–8 days advance in bud break, flowering, and veraison, respectively, pointing to a substantial alteration in the vine's yearly vegetative cycle. The phenological intervals have also shortened, with 4.5 days now separating bud break from flowering. However, Biasi et al. (2019) found that there are differences between native and foreign grape types in the ripening date response, which is heavily influenced by genotype. Droulia and Charalampoulos (2022) reported that the Rondo variety's growth cycle (from bud break to harvest) was accelerated by an average of 11 days due to the apparent increase in yearly air temperature. Harvest frequency was earlier, with an average harvest start date six days earlier.

Table 4. Heat unit requirements (10 °C) for ‘Flame Seedless’ and ‘Crimson Seedless’ grape cultivars under three different regions during the 2021 and 2022 seasons.

Governorate	Stage	Flame Seedless		Crimson Seedless	
		Season 2021	Season 2022	Season 2021	Season 2022
El-Behira	Bud burst	5965	6372	6856	7324
	Full bloom	11108	11356	12768	13053
	Ripening	45422	46202	66504	67646
El-Menoufia	Bud burst	6149	6569	7068	7551
	Full bloom	11452	11708	13163	13457
	Ripening	46827	47631	68561	69738
El-Minia	Budburst	5614	5998	6453	6894
	Full bloom	10456	10689	12018	12286
	Ripening	42753	43487	62596	63671

Table 5. Impact of heat units on the number of days per calendar year (Julian day) for the phenological dates of the ‘Flame Seedless’ and ‘Crimson Seedless’ grape varieties during the 2021 and 2022 seasons.

Governorate	Budburst date		Full bloom date		Fruit set date		Veraison date		Ripening date	
	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022
	Flame Seedless									
El-Behira	70	73	122	124	126	129	154	156	171	174
El-Menoufia	73	77	127	128	129	133	159	160	174	178
El-Minia	66	68	116	119	122	124	148	151	167	169
	Crimson Seedless									
El-Behira	86	88	126	129	134	135	169	170	246	248
El-Menoufia	92	93	130	134	140	139	174	174	252	253
El-Minia	79	82	121	123	127	130	163	165	239	242

Yield

Climate significantly affected Flame Seedless and Crimson Seedless grape productivity in both seasons, according to data in (Table 6). The hot region (El-Minia governorate) and the temperate regions (El-Behira and El-Menoufia governorates) differed significantly in productivity. El-Behira Governorate had the highest yield, followed by El-Menoufia Governorate. While El-Minia Governorate had the significantly lowest values in both seasons, there was no significant difference in effect between El-Behira and El-Menoufia governorates.

The effects of temperature on grapevine productivity were not uniform. Elevations beyond 35 °C interfere with the physiological functions of plants, like photosynthesis in grapevines, hence limiting overall yield (Drappier et al., 2019). Because of bud divergence, the period from April 15 to May 15 is critical for vineyard yield the following year. Over a few vital weeks, maximum temperatures have risen dramatically and now reach critical levels (>30°C). The outcomes are in line with those of Koch and Oehl (2018). Who found that a 2.1 °C temperature increase in Seinfeld, southwest Germany, caused a 15 tons/ha reduction in Pinot Gris output. Pinot Noir grapes yield 17 to 30 tons/ha, Riesling grapes yield 22 to 25 tons/ha, Sylvaner grapes yield 25 to 41 tons/ha, and Müller-Thorgau grapes yield 35 to 50 tons/ha. In a recent study, Gentilucci et al. (2020) revealed a rise in maximum values of 1.2 °C (showing an increase in extreme temperatures) and an increase in annual average temperatures from the past to the present, with a larger increase of more than 0.5 °C. With grape output declining with rising temperatures in every standard climatological mean period, a strong inverse relationship between temperature and productivity was shown. The average tons/ha of grape output during the years 1971-2000, 1981-2010, and 1991-2020 were 10.23, 9.84, and 9.21, respectively. A strong correlation was observed between the temperature increase from one period to the next and the decrease in grape yield, underscoring the challenges linked to rising temperatures.

Physical characteristics of the bunch

The data in Table 7 clearly shows that heat units remarkably affect the physical characteristics of the Flame Seedless and Crimson Seedless grape cultivars during the 2021 and 2022 seasons. Regarding average bunch weight, it is obvious that thermal units had a significant effect on the mean weight of Flame Seedless and Crimson Seedless grape bunches in both seasons. The highest mean weight of bunches was obtained in the El-Behira governorate, followed by the El-Menoufia governorate. There was no significant effect between the El-Behira and El-Menoufia governorates, while the El-Minia governorate significantly gave the lowest values of mean weight of bunches in both seasons. Concerning average berry weight, thermal units significantly affected the average weight of Flame Seedless and Crimson Seedless berries in both seasons. In general, grape berries from the El-Behira governorate significantly reached the highest values of average berry weight, followed by the El-Menoufia governorate. There was no significant effect between the El-Behira and El-Menoufia governorates, while the El-Minia governorate significantly reached the lowest values in both seasons. Concerning average berry volume, the data indicate that thermal units had a significant effect on the mean berry size of Flame Seedless and Crimson Seedless grapes in both seasons. The highest significant mean berry volume was obtained in the El-Behira governorate, followed by the El-Menoufia governorate. There was no significant effect between the El-Behira and El-Menoufia governorates, while the El-Minia governorate significantly gave the lowest values of mean berry volume in both seasons.

Table 6. Impact of heat units on the yield/vine (kg) of the 'Flame Seedless' and 'Crimson Seedless' grape cultivars during the 2021 and 2022 seasons.

Governorate	Flame Seedless		Crimson Seedless	
	Season 2021 yield/vine (kg)	Season 2022 yield/vine (kg)	Season 2021 yield/vine (kg)	Season 2022 yield/vine (kg)
El-Behira	16.49	17.25	15.08	15.57
El-Menoufia	14.57	15.23	13.32	13.75
El-Minia	12.28	12.84	11.23	11.59
LSD at 0.05	1.96	2.03	1.77	1.84

Table 7. Impact of heat units on physical characteristics of the 'Flame Seedless' and 'Crimson Seedless' bunches cultivars during the 2021 and 2022 seasons.

Governorate	Average bunch weight (g)		Average berry weight (g)		Average berry volume (cm3)	
	2021	2022	2021	2022	2021	2022
	Flame Seedless					
El-Behira	634.9	662.7	3.49	3.59	3.32	3.43
El-Menoufia	560.5	585.1	3.23	3.31	3.07	3.16
El-Minia	472.7	493.4	3.08	3.12	2.93	2.98
LSD at 0.05	74.9	78.3	0.27	0.29	0.26	0.28
	Crimson Seedless					
El-Behira	574.2	599.4	4.15	4.24	4.12	4.19
El-Menoufia	506.9	529.2	4.02	4.09	3.90	3.96
El-Minia	427.5	446.3	3.91	3.97	3.81	3.85
LSD at 0.05	71.2	71.5	0.15	0.18	0.23	0.26

Table 8. Impact of heat units on chemical characteristics of the 'Flame Seedless' and 'Crimson Seedless grape cultivars during the 2021 and 2022 seasons.

Governorate	Total sugars (%)		Total acidity (%)		Total anthocyanin (mg/g F.W.)	
	2021	2022	2021	2022	2021	2022
	Flame Seedless					
El-Behira	13.48	13.96	0.64	0.66	37.42	38.36
El-Menoufia	13.59	14.11	0.61	0.64	36.34	37.23
El-Minia	13.74	14.23	0.59	0.63	35.83	36.75
LSD at 0.05	0.13	0.16	0.04	0.03	1.17	1.23
	Crimson Seedless					
El-Behira	13.08	13.37	0.58	0.56	32.59	33.47
El-Menoufia	13.21	13.48	0.55	0.52	31.42	32.24
El-Minia	13.37	13.61	0.53	0.51	30.86	31.53
LSD at 0.05	0.15	0.17	0.04	0.05	1.31	1.39

Chemical characteristics of the bunch

The data in Table 8 clearly shows that heat units remarkably affect the chemical characteristics of the Flame Seedless and Crimson Seedless grape cultivars during the 2021 and 2022 seasons.

Regarding total sugars, it is evident that thermal units significantly affected the total sugar content of Flame Seedless and Crimson Seedless berries in both seasons. In general, grape berries from the El-Behira governorate had significantly lower levels of total sugars, followed by the El-Menoufia governorate. There was no significant effect between the El-Behira and El-Menoufia governorates, while the El-Minia governorate significantly reached the highest percentage of total sugars in both seasons. Concerning total acidity, the data clearly show that thermal units had a significant effect on the total acidity of Flame Seedless and Crimson Seedless berry juice in both seasons. The most significant berry juice in terms of total acidity was obtained in the El-Behira governorate, followed by the El-Menoufia governorate. There was no significant effect between the El-Behira and El-Menoufia governorates, while the El-Minia governorate significantly gave the lowest values of total acidity in both seasons. Concerning total anthocyanin, it is clear that thermal units significantly affected the skin of Flame Seedless and Crimson Seedless berries in terms of total anthocyanin in both seasons. In general, grape berries from the El-Behira governorate had significantly higher levels of total anthocyanin, followed by those from the El-Menoufia governorate. There was no significant effect between the El-Behira and El-Menoufia governorates, while the El-Minia governorate significantly reached the lowest values of total anthocyanin in both seasons.

Soil type, management practices, genotype, and climate all affect berry quality (Jackson & Lombard, 1993). Temperature is one of the environmental variables that have been found to have a significant impact on grapevine development and berry composition (Soar et al.., 2008). High temperature can have a considerable impact on sugar buildup (Greer & Weedon, 2013) and reduce the content of anthocyanin biosynthesis (Conde et al., 2016) during the veraison-ripening period. Recent research has demonstrated negative connections between temperature and anthocyanin and berry weight at technical maturity (Costa et al., 2020).

CONCLUSION

The previous results allow us to conclude that the beginning and end of the main phenological stages of the grape throughout the growing period and the chemical properties of the berries are negatively affected by the different thermal units since temperature is the atmospheric element that has the greatest influence on grape phenology. In general, temperature contributes to the harvest time and product quality for the Flame Seedless and Crimson Seedless grape varieties. It was also observed that high temperature had a positive effect on sugar accumulation and reduced the content of anthocyanin biosynthesis. The results of the current study may be useful for developing models to predict grape variety productivity based on temperature indicators, for expanding the cultivation of new varieties, and for establishing a map of grape varieties in regions suitable for their productivity.

Conflict of interest

The study presented in this publication, according to the authors, was not affected by any of their known financial conflicts or personal relationships. The authors declare that this article does not involve any conflict of interest.

Funding

The authors declare that no funding of any kind has been obtained for this work.

Data Availability

The authors acknowledge that the meteorological data are sourced from the Egyptian Agricultural Research Center and the Central Agricultural Climatic Laboratory, which approved the use of the data in this research paper.

Acknowledgments
The authors would like to express their sincere gratitude to the Egyptian Agricultural Research Center for providing the essential data necessary for my research project. Furthermore, sincere thanks are due to Cairo University for the invaluable scientific support they have offered us throughout our research.

REFERENCES

Association of Official Agricultural Chemists, A.O.A.C. (2005). *Official methods of analysis* (18th ed.). A.O.A.C., Benjamin Franklin Station.

Biasi, R., Brunori, E., Ferrara, C., & Salvati, L. (2019). Assessing impacts of climate change on phenology and quality traits of *Vitis vinifera* L.: The contribution of local knowledge. *Plants*, *8*, 121. https://doi.org/10.3390/plants8050121

Chuine, I., Yiou, P., Viovy, N., Seguin, B., Daux, V., & Leroy-Ladurie, E. L. R. (2014). Historical phenology: Grape ripening as a past climate indicator. *Nature*, *432*, 289–290. https://doi.org/10.1038/432289a

Conde, A., Pimentel, D., Neves, A., Dinis, L. T., Bernardo, S., Correia, C. M., Geros, H., & Moutinho-Pereira, J. (2016). Kaolin foliar application has a stimulatory effect on phenylpropanoid and flavonoid pathways in grape berries. *Frontiers in Plant Science*, *7*, 1150. https://doi.org/10.3389/fpls.2016.01150

Costa, C., Graça, A., Fontes, N., Teixeira, M., Gerós, H., & Santos, J. A. (2020). The interplay between atmospheric conditions and grape berry quality parameters in Portugal. *Applied Sciences*, *10*, 4943. https://doi.org/10.3390/app10144943

Drappier, J., Thibon, C., Rabot, A., & Geny-Denis, L. (2019). Relationship between grape composition and temperature: Impact on Bordeaux grape typicity in the context of global warming—Review. *Critical Reviews in Food Science and Nutrition*, *59*, 14–30. https://doi.org/10.1080/10408398.2017.1355776

Droulia, F., & Charalampopoulos, I. (2022). A review of the observed climate change in Europe and its impacts on viticulture. *Atmosphere*, *13*(5), 837. https://doi.org/10.3390/atmos13050837

Ferretti, C. (2021). Topo-climate and grape quality: Results of research on the Gewürztraminer grape variety in South Tyrol, Northern Italy. *OENO One*, *55*, 313–335. https://doi.org/10.20870/oeno-one.2021.55.1.4531

Fraga, H., Malheiro, A. C., Moutinho-Pereira, J., & Santos, J. A. (2012). An overview of climate change impacts on European viticulture. *Food and Energy Security*, *1*, 94–110. https://doi.org/10.1002/fes3.14

Gentilucci, M., Materazzi, M., Pambianchi, G., Burt, P., & Guerriero, G. (2020). Temperature variations in Central Italy (Marche Region) and effects on grape production. *Theoretical and Applied Climatology*, *140*, 303–312. https://doi.org/10.1007/s00704-020-03089-4

Gouot, J. C., Smith, J. P., Holzapfel, B. P., & Barril, C. (2019). Impact of short temperature exposure of *Vitis vinifera* L. Cv. Shiraz grapevine bunches on berry development, primary metabolism and tannin accumulation. *Environmental and Experimental Botany*, *168*, 103866. https://doi.org/10.1016/j.envexpbot.2019.103866

Greer, D. H., & Weedon, M. M. (2013). The impact of high temperatures on *Vitis vinifera* cv. Semi-lion grapevine performance, and berry ripening. *Frontiers in Plant Science*, *4*, 491. https://doi.org/10.3389/fpls.2013.00491

Gupta, N., Pal, R., Kour, A., & Mishra, S. K. (2020). Thermal unit requirement of grape (*Vitis vinifera* L.) varieties under southwestern Punjab conditions. *Journal of Agrometeorology*, *22*(4), 469–476. https://doi.org/10.54386/jam.v22i4.456

Hamie, N., Nacouzi, D., Choker, M., Salameh, M., Darwiche, L., & El Kayal, W. (2023). Maturity assessment of different table grape cultivars grown at six different altitudes in Lebanon. *Plants*, *12*(18), 3237. https://doi.org/10.3390/plants12183237

Hsia, C. L., Luh, B. S., & Chichester, C. D. (1965). Anthocyanin in freestone peach. *Journal of Food Science*, *30*, 5–12. https://doi.org/10.1111/j.1365-2621.1965.tb00253.x

Jackson, D., & Lombard, P. (1993). Environmental and management practices affecting grape composition and grape quality. A review. *American Journal of Enology and Viticulture*, *44*, 409–430. https://doi.org/10.5344/ajev.1993.44.4.409

Jackson, D. I., & Schuster, D. (2001). *The production of grapes and wine in cool climates*. Gypsum Press and Daphne Brasell Associates Ltd.

Jones, G. V., Duff, A. A., Hall, A., & Myers, J. W. (2010). Spatial analysis of climate in wine grape growing regions in the western United States. *American Journal of Enology and Viticulture*, *61*(3), 313–326. https://doi.org/10.1177/000298761006100305

Kalra, N., Chakraborty, D., Sharma, A., Rai, H. K., Jolly, M., Chander, S., & Sehgal, M. (2008). Effect of increasing temperature on yield of some winter crops in northwest India. *Current Science*, *94*(1), 82–88.

Karvonen, J. (2020). Changes in the grapevine's growth cycle in Southern Finland in the 2000s—Comparison between two first decades. *Climate Change*, *6*, 94–99.

Koch, B., & Oehl, F. (2018). Climate change favors grapevine production in temperate zones. *Agricultural Sciences*, *9*, 247–263. https://doi.org/10.4236/as.2018.93019

Lereboullet, A. L., Beltrando, G., Bardsley, D. K., & Rouvellac, E. (2014). The viticultural system and climate change: Coping with long-term trends in temperature and rainfall in Roussillon, France. *Regional Environmental Change*, *14*, 1955–1966. https://doi.org/10.1007/s10113-013-0446-2

Miller, G. J. (1959). Use of dinitrosalicylic acid reagent for the determination of reducing sugars. *Analytical Chemistry*, *31*, 426–428. https://doi.org/10.1021/ac60147a030

Molitor, D., & Junk, J. (2019). Climate change is implicating a two-fold impact on air temperature increase in the ripening period under the conditions of the Luxembourgish grape growing region. *OENO One*, *53*(3), 2329. https://doi.org/10.20870/oeno-one.2019.53.3.2329

Parker, A., Garcia de Cortázar, I., Chuine, I., Barbeau, G., Bois, B., Boursiquot, J. M., Cahurel, J. Y., Claverie, M., Dufourcq, T., & Gény, L. (2013). Classification of varieties for their timing of flowering and veraison using a study modeling approach. A case for the grapevine species *Vitis vinifera* L. *Agricultural and Forest Meteorology*, *180*, 249–264. https://doi.org/10.1016/j.agrformet.2013.06.005

Singh, I. A., Rao, U. V. M., Singh, D., & Singh, R. (2007). Study on agrometeorological indices for soybean crop under different growing environments. *Journal of Agrometeorology*, *9*, 81–85. https://doi.org/10.5958/0976-058X.2015.00046.3

Snedecor, G. W., & Cochran, W. G. (1980). *Statistical methods* (7th ed.). The Iowa State University Press. https://doi.org/10.1201/9780203738580

Soar, C., Sadras, V., & Petrie, P. (2008). Climate drivers of red grape quality in four contrasting Australian grape regions. *Australian Journal of Grape and Wine Research*, *14*, 78–90.

Spayd, S. E., Tarara, J. M., Mee, D. L., & Ferguson, J. C. (2002). Separation of sunlight and temperature effects on the composition of *Vitis vinifera* cv. Merlot berries. *American Journal of Enology and Viticulture*, *53*, 171–182. https://doi.org/10.5344/ajev.2002.53.3.171

Wang, X., Wang, H., & Li, H. L. (2020). The influence of recent climate variability on viticultural zoning and variety regionalization of *Vitis vinifera* in China. *OENO One*, *54*(3), 523–541. https://doi.org/10.20870/oeno-one.2020.54.3.2971

Winkler, A. J. (1974). *General viticulture* (4th ed.). University of California Press.

JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH
2025, VOL. 8(4), 477-490

Journal of Horticulture and Postharvest Research

Journal homepage: www.jhpr.birjand.ac.ir

University of Birjand

Postharvest VeSolution treatment mitigates rot in pomegranate (*Punica granatum* L.) fruits

Senthilkumar Shricharan[1,*], Akshay Ramaswamy Deenadayalan[1], Bhavesh Vadher[1], Kumar J Hemanth[1] and Arghyadeep Ashok Bhattacharjee[1]

1, Velabs Research & Development Division, Vegrow, Sector 4, HSR Layout, Bengaluru, Karnataka, India - 560102

ARTICLE INFO

Original Article

Article history:
Received 29 November 2024
Revised 10 March 2025
Accepted 2 April 2025

Keywords:
Fungal rotting
GRAS
Growth inhibition
Pomegranate
Postharvest losses

DOI: 10.22077/jhpr.2025.8500.1455
P-ISSN: 2588-4883
E-ISSN: 2588-6169

***Corresponding author:**
Velabs Research & Development Division, Vegrow, Sector 4, HSR Layout, Bengaluru, Karnataka, India - 560102.

Email: shricharanag@gmail.com

ABSTRACT

Purpose: Pomegranate is of considerable economic significance, with Maharashtra, Karnataka, and Gujarat serving as the primary cultivation regions. Despite the high production levels, postharvest losses are serious, with certain fruits experiencing fruit cracking, fungal infections, and poor handling during transportation, resulting in postharvest losses of up to 35%. For controlling postharvest infections in a variety of fruits and vegetables, synthetic fungicides are incredibly effective. **Research Method:** VeSolution is a GRAS salt-based formulation with antimicrobial properties developed to assess its efficacy in minimizing fruit rotting. Therefore, the present investigation examined the effectiveness of VeSolution in reducing these postharvest losses. The infected fruits were used to isolate and identify fungal pathogens. Subsequently, the antifungal efficacy of the VeSolution formulation was assessed by both *in vitro* and *in vivo* methodologies. The *in vitro* investigations entailed evaluating the formulation's inhibitory effects on mycelial growth of identified fungal pathogens on PDA plates. In the in vivo evaluation, artificially inoculated pomegranate fruits were subjected to VeSolution treatment to test their effectiveness in mitigating rot advancement. Finally, VeSolution-treated pomegranates were exposed to supply chain conditions to assess their practical efficacy. Critical parameters, including rot advancement, in-transit spoiling, and fruit quality were assessed. **Findings:** *Aspergillus* sp., *Alternaria* sp., and *Coinella* sp. were identified as fungi associated with pomegranate fruit rotting. The results indicated that the growth of the fungal colony was substantially inhibited by the 2% and 5% concentrations of VeSolution. The fruit rot development and progression were effectively restricted by the 2% VeSolution, as confirmed by *in vivo* assessments. Subsequent pilot and large-scale trials demonstrated that 1% VeSolution substantially reduced rot during longer (> 24 h) transportation periods. **Research limitations:** There were no limitations. **Originality/Value:** These results emphasise VeSolution as a viable and non-toxic alternative to conventional synthetic fungicides for maintaining the postharvest quality of pomegranates.

INTRODUCTION

Pomegranate (*Punica granatum* L.) is a highly economical fruit due to its wide adaptability to different agroclimatic zones. Indian states including Maharashtra, Karnataka, Gujarat, and Andhra Pradesh are the major contributors to pomegranate production (Jadhav et al., 2023). The varieties like Ganesh, Mrudula, and Bhagwa are commercially cultivated in Solapur, Nashik, Pune, Ahmednagar and Aurangabad districts of Maharashtra. Despite the highest production, India loses 35% of the yield due to cracking, fungal rotting, over-ripening, dehydration, and wound damages during postharvest handling and transportation of fruits (Murthy et al., 2009; Ranjani et al., 2023). Fruit losses are scattered throughout many supply chain stages, including the field, wholesale, and retail locations, which are all connected to transportation (Ambalavanan et al., 2024). In addition to this improper handling of fruits, poor vendor hygiene, unfavourable ambient factors, and sanitary risk may further intensify the market circumstances that encourage postharvest losses. In India, 10% of crop losses occur throughout the cultivation and distribution stages, while an additional 15% occur at the retail stage (Murthy et al., 2009). Fungal rotting including soft rot, anthracnose, black heart rot and gray mold represent about 65% of the total postharvest losses in pomegranate (Mincuzzi et al., 2022).

Minimizing postharvest losses in pomegranate is the prime objective, especially during long-distance transit from source to destination markets. Synthetic fungicides are extremely efficient in managing postharvest infections in a wide range of vegetables and fruits. Waskar et al. (1999) reported that postharvest treatment with fungicides like carbendazim and captan controlled fruit rotting in pomegranate. Similarly, the primary approach to preventing, controlling, or eliminating postharvest pathogens has been the creation of novel synthetic chemicals over the last several decades. Under normal circumstances, fungicides used at postharvest are usually more fungistatic than fungicidal. These fungicides are often applied as fumigants, dips, sprays, treated wraps, and box liners or may be incorporated with waxes and coatings (Ambalavanan et al., 2024).

Although they are effective, these chemicals can disrupt the balance of the ecosystem if used repeatedly (Camele et al., 2010). This may lead to the development of new pathotypes that are resistant to one or more of these chemicals. Additionally, these chemicals may be toxic to organisms that are not the intended target, and they can sometimes accumulate as residues in the food chain, exceeding safe limits. Due to this, alternatives like biocontrol agents and botanicals are gaining commercial importance in the area of crop protection and management (Shricharan et al., 2020). Moreover, owing to health and environmental concerns, the use of postharvest fungicides has been regulated throughout the world.

Basic substances are substances like lecithin, talc, vinegar, chitosan, mustard seed powder, etc. are nontoxic and not predominantly used in plant protection but can be used in plant protection. In recent years, multiple inquiries have been conducted in this domain to explore the antimicrobial characteristics of Generally Recognized as Safe (GRAS) salts (Guimaraes et al., 2019; Martinez-Blay et al., 2020; Allagui et al., 2024). GRAS substances including botanicals, essential oils, inorganic and organic salts including bicarbonates, benzoates, silicates, metabisulphites, etc (Palou et al., 2016) are gaining attention as they can be employed to mitigate postharvest problems due to their exemption from residual limits on all agro-commodities by US FDA (Palou, 2018; Romanazzi et al., 2022). A novel formulation termed VeSolution was developed utilizing GRAS salts (metabisulphite salts) with potent antimicrobial properties. Moreover, studies on controlling fruit rotting particularly for long-distance transportation are lacking in pomegranate.

Therefore the work was carried out with the following objectives (i) to evaluate *in vitro* antifungal activity of different concentrations of VeSolution against the isolated fungi (ii) to assess *in vivo* activity of the most promising concentration(s) of VeSolution to control pathogens associated with fruit rotting, (iii) to study the effectiveness of postharvest dipping of pomegranates in promising concentration(s) of VeSolution to control rotting, (iv) to investigate the impact of VeSolution treatment(s) in reducing rotting under short and long-distance transport conditions.

MATERIALS AND METHODS

VeSolution and fruit sample collection

VeSolution is a formulation (metabisulphite salt + 0.5% Tween 20) (developed by Velabs, Vegrow, Bengaluru, Karnataka) designed to decrease fruit rotting and to maintain the quality of pomegranate fruit. This was developed from an inorganic salt that has been previously recognized as GRAS and reported as an antifungal, antioxidant, and reducing agent in the food industry (Kolaei et al., 2012; Mladenović et al., 2018).

The pomegranate fruit rotting is primarily associated with fungal pathogens. Hence for its isolation, pomegranate fruits (var. Ganesh) that exhibited rotting symptoms as described by Ambalavanan et al. (2024) were collected from fruits received at Vegrow Distribution Centre (DC) (Bengaluru, Karnataka) from Bhuj (Gujarat, India) with a transit length of around 60 to 70 hours. The samples were collected and stored at 4 °C until isolation. The subsequent experimentations and data collection of *in vitro* and *in vivo* were carried out at the in-house R&D facility of Vegrow, Bengaluru, and Karnataka.

Fungal isolation, purification and identification

The symptomatic region from the fruit was cut into small pieces with a sterile blade, surface sterilized in 1% sodium hypochlorite for 1 min, and plated aseptically on PDA pH 5.6±2 supplemented with chloramphenicol 100 µg ml^{-1} to prevent bacterial contamination and incubated at 25±1 °C for 5 days. A pure culture was obtained by single spore method and maintained by sub-culturing the different colonies that developed on PDA plates and incubated at 25±1 °C for 5 days. The colony morphology and slide culture techniques were followed for microscopic examination of fungi. Briefly, a sterilized microscopic slide was placed on a bent glass rod in a sterilized petri plate. About 1×1 cm agar block was cut from a PDA plate and transferred to the glass slide, and the fungi were inoculated using a loop on the top four corners of the agar block. The agar block was covered with a cover slip from the top. The plate was covered and incubated at 25±1 °C for 3-4 days. For microscopic observations, the cover slip from the inoculated agar block was removed and placed inverted on a drop of Lactophenol Cotton Blue (LPCB) stain on a new slide and observed under a microscope for the identification of fungi (Leck, 1999).

In vitro assay of VeSolution

VeSolution was initially tested under *in vitro* conditions against the isolated pathogens. PDA medium was amended with concentrations of VeSolution ranging from 2%, 5%, 10%, and 20% before autoclaving at 121 °C for 20 mins. The PDA without VeSolution served as a negative control (CK-ve) while the PDA with 0.1% Fludioxonil served as a positive control (CK+ve). A mycelial plug (4 mm diameter) was taken using a cork borer from 7 to 10 days old pure culture and placed on the center of the PDA plates with pH 5.6±2. The plates were then incubated at 25±1 °C. The radial mycelial growth was determined in each Petri plate by measuring two perpendicular diameters (in mm) of the fungal colony. The measurements were taken once when the mycelial completely covered the plate in CK-ve. The assay was conducted in triplicates and the results were expressed as percentage inhibition of radial mycelial growth (IRMG) using the formula as described by Bouhlali et al. (2021) (1).

$$\text{IRMG (\%)} = [(dc - dt)/dc] \times 100 \qquad (1)$$

Where, dc: average diameter of fungal colony in control plates (mm); dt: average diameter of fungal colony (mm) in assayed petri plates.

In vivo curative assay of VeSolution in pomegranate fruits

The pomegranate fruits for *in vivo* assay were procured directly from farms around Karnataka. The healthy fruits were selected without wounds, and randomly divided into 4 sets, and their surface was sterilized by dipping them in 1% sodium hypochlorite solution for 1 min, rinsed twice with sterile water, and air dried. For fruit inoculation, the spores were harvested by adding 0.05% Tween 20 to the pure cultures, scraped with a sterile rod and filtered through 3 layers of cheesecloth. The spores were counted with a hemocytometer under a microscope and diluted to the concentration of 1×10^5 spores/ml with 0.05% Tween 20. The surface sterilized pomegranates were wounded aseptically with a 3 mm cork borer and each wounded site of the fruit was inoculated with 20 µl spore suspension (the suspension was mixed by vortexing before inoculation).

The curative assay was carried out by dipping the fruits for 2 mins in a predetermined promising concentration of VeSolution after 24 h of inoculation with spore suspension. The fruits dipped in sterile water were considered as CK-ve and fruits dipped in 0.1% fludioxonil served as CK+ve. The experiment was conducted with three replications with 10 fruits per replication. All the treated fruits were stored in plastic crates at room temperature for 10 days. The rot diameter on each fruit was measured (in mm) perpendicularly on the 10^{th} day and expressed as rot inhibition % using the formula as described by Allagui and Ben Amara (2024) (2).

$$\text{Rot inhibition (\%)} = [(dc - dt)/dc\text{-}] \times 100 \qquad (2)$$

Where, dc- is the rot diameter (mm) of CK-ve and dt is the rot diameter (mm) of the treated fruits.

Pilot scale and implementation trial in transit conditions

Based on the preliminary trial, the pilot scale trial of postharvest treatment of VeSolution was conducted from Lingsugur, Karnataka to different destinations in trucks under ambient conditions. The details of the shipment including the source, destination, and sample quantity are given in Table. 1. Based on the results from the pilot trial, this process was implemented on a large scale from different source locations to the destination markets with transit hours ranging from 15 h to 84 h (Table 2). The rotting percentage was estimated (as described earlier) in both pilot and implementation trials once the shipment reached the destination markets.

Statistical analysis

The experiment was conducted in CRD and the analysis of variance (ANOVA) was carried out in WASP 2.0 (Web Agri Stat Package 2) statistical tool (www.icargoa.res.in/wasp2/index.php). The variables were transformed for normality and the graphs were developed in GraphPad Prism version 9.2.0 for Windows, GraphPad Software, Boston, Massachusetts USA.

Table 1. Details of pilot shipments and the treated quantity in 1% and 2% VeSolution.

Shipment No.	Source	Destination	Total (Kg)	Control (Kg)	Treated (Kg) in 1% VeSolution	Treated (Kg) in 2% VeSolution
1	Lingsugur	Delhi	70	20	0	50
2	Lingsugur	Bengaluru	50	20	30	0
3	Lingsugur	Bengaluru	80	30	50	0
4	Lingsugur	Bengaluru	80	30	50	0
5	Lingsugur	Bengaluru	90	18	36	36
6	Lingsugur	Bengaluru	90	18	36	36

Table 2. The process implementation of postharvest treatment from source to destination and their transit time.

Source	Destination	Transit time (h)
Nashik	Guwahati	84
Nashik	Lucknow	65
Pune	Bhubaneswar	50
Pune	Bengaluru	18
Sangola	Hyderabad	15

RESULTS

Morphological and microscopic characterization of the isolated fungal pathogens

The identification was done after purification of the colonies isolated from the rotten samples of pomegranate. The fungal colonies were identified by observing the colony growth and morphology on potato dextrose agar (PDA) plates and after microscopic examination. The initial isolated fungi colony exhibited white growth with a complete margin, which was subsequently transformed to black pigmentation as a result of spore production. Proper development of septate hyphae was evident in their extensive branching. The isolated fungi was confirmed as *Aspergillus* sp. due to the globose conidia and brown to black conidial head, which were similar to the *Aspergillus* characterised by Romero-Cortes et al. (2019) and Shricharan et al. (2020). The subsequent colony was initially light grey in colour, but it transitioned to a dark brown to black colour with a white margin. Conidiophores were present on the hypahe, which were pale brown in colour. Conidia were dark brown in pigmentation and consisted of 3 to 4 transverse septa. Their morphology was typically ovate to obclavte. The fungi were identified as *Alternaria* sp. based on the characteristics reported by Yu et al. (2016) and Saleem and El-Shahir et al. (2022). The third colony was initially white and was transformed to brown as it grew and developed pycnidia. Hayline, single-celled, elongated, ellipsoid to fusiform conditions were observed. The observations were consistent with those of Uysal et al. (2018) and Mahadevakumar et al. (2019) and were subsequently identified as *Coinella* sp.

VeSolution inhibited the growth of fungal colonies under *in vitro* condition

In general, the radial mycelial growth of all three fungal colonies was inhibited by VeSolution, and the percentage of inhibition increased as the concentration of VeSolution (VS) increased. The control (CK+ve) plates exhibited the highest percentage of inhibition in all three fungal colonies, while the maximal colony diameter was observed in the CK-ve plates. In particular, the colony growth was not significantly affected by 10% VS, 15% VS, or 20% VS, while 2% VS and 5% VS exhibited a significant growth inhibition as compared to CK+ve plates (Fig. 1). Subsequently, the toxicity effect of these concentrations of VeSolution was checked on the pomegranate surface. Pomegranate fruits exhibited toxicity and skin burn symptoms at concentrations exceeding 5% (data not presented). Therefore, considering the fungal growth inhibitory properties of VeSolution and the fruit appearance, 2% VS and 5% VS were chosen as prospective interventions for *in vivo* fruit inoculation studies.

Curative VeSolution treatments reduced the fungal rot progression in pomegranate fruits

Pomegranate fruits that were inoculated with spore suspension exhibited rot progression symptoms 3 days after the curative VeSolution treatment. Initially, the inoculated spores germinated and sporulated, resulting in the visible growth of sporulated fungal growth on CK-ve fruits. The fungal growth had penetrated the fruits and was beginning to infect the arils by six days after treatment (DAT). Notably, the highest diameter (in mm) of rot progression was observed in CK-ve fruits. Whereas, the highest inhibition in rot progression percentage was observed in CK+ve on the 10th day of curative treatment, followed by 2% VS and 5% VS treatments (Fig. 2). Although both the 2% VS and 5% VS treatments significantly reduced rot progression in comparison to CK+ve, there was no significant difference in VeSolution concentrations. The 2% VS treatment was chosen for further investigation due to its apparent ability to impede fungal development (Fig. 3 & Fig. 4).

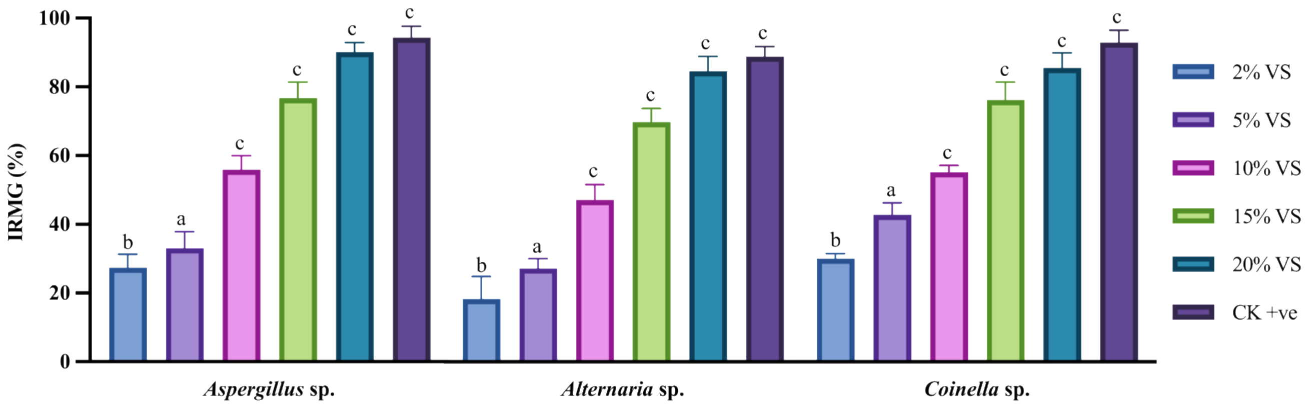


Fig. 1. The inhibition of radial mycelial growth by VeSolution on *Aspergillus* sp., *Alternaria* sp. and *Coinella* sp. under *in vitro* conditions. The bars with different letters are statistically significant ($P \leq 0.05$).

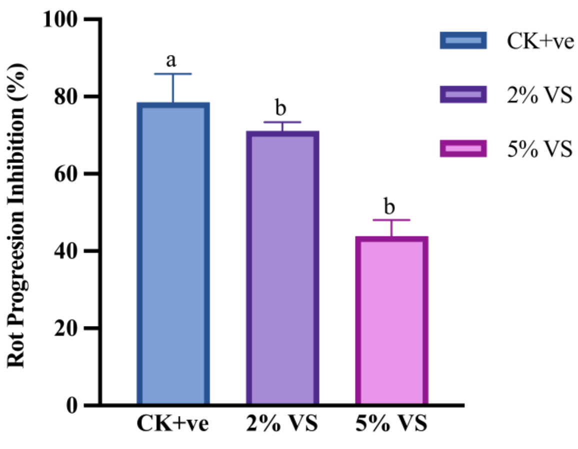


Fig. 2. The Inhibition of rot progression (%) by VeSolution on pomegranate fruits under *in vivo* conditions. The bars with different letters are statistically significant ($P \leq 0.05$).

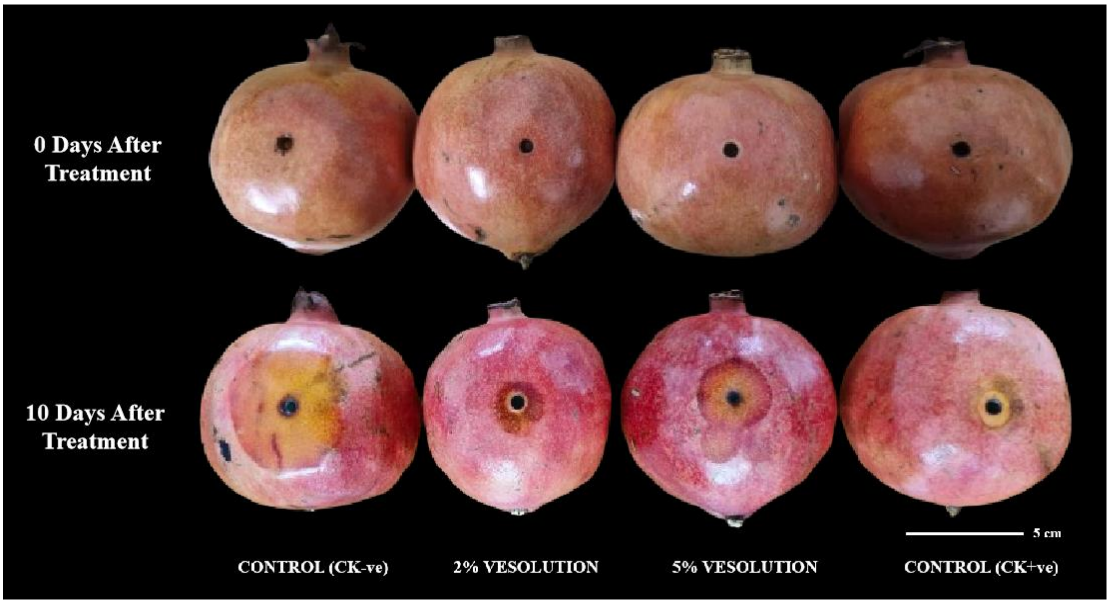


Fig. 3. The progression of fruit rot in pomegranate inoculated with fungal spores and with post curative VeSolution treatment. The treated fruits exhibit variations in rot progression over time demonstrating the treatments efficacy in reducing rot progression compared to control (CK-ve) fruits.

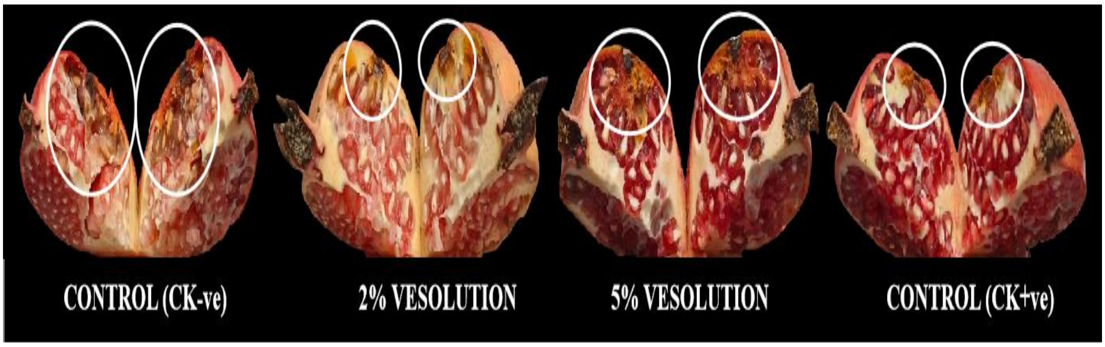


Fig. 4. The severity of rot progression inside pomegranate fruits at 10 DAT. The progression of fungal sporulation and infection within the peel and arils are highlighted within circles.

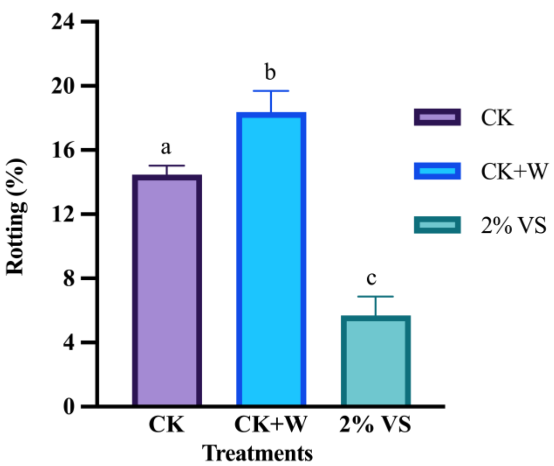


Fig. 5. The effect of postharvest treatment of 2%VS on rotting with controls (dry and wet). The bars with different letters are statistically significant ($P \leq 0.05$).

Significant reduction in rotting percentage achieved with 2% VeSolution treatment

Based on the above investigations, the decaying percentage was significantly reduced by the usage of 2% VeSolution at 6 days after treatment (DAT) in comparison to the control (CK) and water-treated control (CK+W). In CK+W, the highest decaying percentage was observed (Fig. 5), suggesting that moisture is a critical factor in the proliferation of pathogens. This is because the presence of water fosters pathogen growth and infection. In contrast, the dry control (CK) demonstrated a lower decaying percentage, but it was not as effective as the 2% VeSolution treatment. This implies that 2% VeSolution not only effectively regulates fruit spoilage but also preserves the fruit's overall quality and appearance.

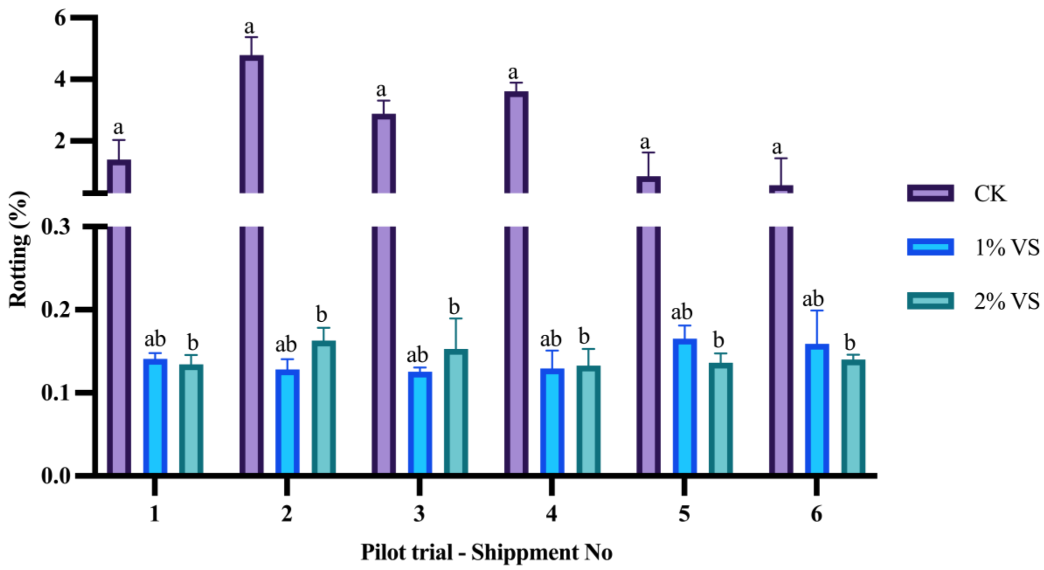


Fig. 6. The effect of postharvest treatment of 1%VS and 2%VS on rotting % during 6 pilot trial shipments. The bars with different letters are statistically significant ($P \leq 0.05$).

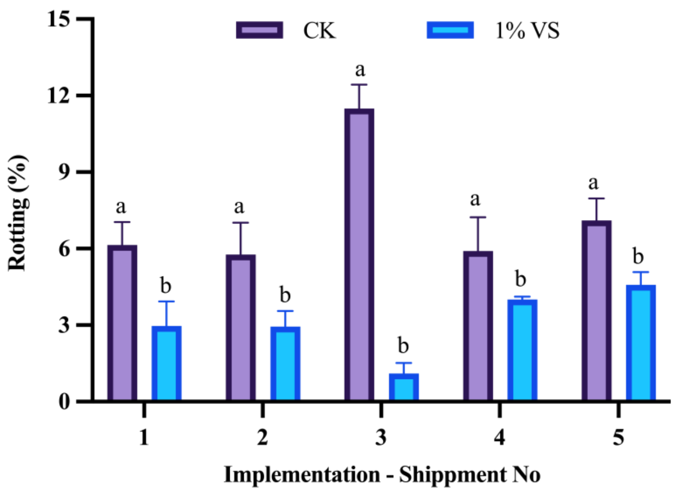


Fig. 7. The effect of postharvest treatment of 1%VS implementation on rotting % during long distance transportation. The bars with different letters are statistically significant ($P \leq 0.05$).

Postharvest VeSolution treatment controlled pomegranate fruit rotting under long distance transit conditions

Eventually, a pilot trial was conducted in which the shipments were dipped in 1% (not optimized earlier) and 2% VeSolution. The results were obvious that CK showed the highest % of rotting while both 1% and 2% VeSolution showed significantly least % of rotting in all shipments to Bengaluru and Delhi from Lingsugur (Fig. 6). In particular, 1% VeSolution showed only the least significant variation as compared to 2% VeSolution. Therefore the process of postharvest treatment with 1% VeSolution was implemented in large shipments with transit time ranging from 15 h to 84 h approximately. The results revealed that CK showed the highest rotting % in all shipments while 1% VeSolution significantly reduced the rotting % as compared to CK (Fig. 7). Particularly, 1% VeSolution was effective in controlling rotting till 84 h with the highest efficacy (91%) in Pune to Bhubaneswar shipment with a transit time of 50 h.

DISCUSSION

The isolated fungal colonies were identified based on its growth, morphology and microscopic characters based on the previous reports on *Aspergillus* (Romero-Cortes et al., 2019; Shricharan et al., 2020), *Alternaria* (Yu et al., 2016; Saleem & El-Shahir, 2022) and *Coinella* (Uysal et al., 2018; Mahadevakumar et al., 2019). Furthermore, the effect of GRAS sodium salt on the spore number and hyphae morphology was reported by Lyousfi et al. (2023). The microscopic observations showed a decrease in spore number and tight aggregations of hyphae with abnormal bulges, disruptures, and swellings when treated with GRAS salts while control samples showed normal morphology. Similarly, morphological damage was detected in *B cinerea* hyphae treated with GRAS salts compared to the control. The GRAS salt-treated hyphae had shriveled and unusual bulges on the surface as compared to the control (Youssef et al., 2019).

Additionally, the evaluation on fungal growth under different concentration of VeSolution showed growth inhibition properties with increase in concentration. Similarly, the antifungal properties of 17 GRAS salts were evaluated against postharvest fungal diseases of citrus fruit. The results revealed that 1% sodium silicate showed 100% inhibitory action against every tested fungus. Conversely, sodium carbonate (1%) had no efficacy against *G. citri-aurantii* or *G. gloeosporioides* but was 100 % effective against *P. digitatum* and *P. italicum* (Zhao et al., 2023). In context to this, Lyousfi et al. (2023) studied the *in vitro* effect of a few organic and inorganic food additive salts against *Monilina fructigena.* The majority of the additives demonstrated a notable reduction in mycelial growth, however, the percentage of inhibition varied depending on the additives and their concentration. Sodium bicarbonate, sodium carbonate, and copper sulphate were the most effective with ammonium carbonate and citric acid showing the least effectiveness. Furthermore, similar results were reported by Allagui and Ben Amara (2024) on *the in vitro* efficacy of sodium metabisulfite (SMB), ammonium bicarbonate, sodium bicarbonate and potassium dihydrogen orthophosphate against *Alternaria alternata*, *Botrytis cinerea*, *Penicillium italicum* and *Penicillium digitatum.* Results revealed that 0.2% SMB showed complete inhibition of all the fungal species. While bicarbonate salts of ammonium and sodium were least effective at 0.2% in inhibiting fungal growth.

In addition, numerous research has been carried on studying the antifungal activity of GRAS salts against postharvest fungal pathogens responsible for fruit rotting in citrus (Guimarães et al., 2019; Soto-Munoz et al., 2020), stone fruit (Martinez-Blay et al., 2021), grapes (Youssef & Roberto, 2014), pomegranate (Palou & Taberner, 2022), kiwi

(Türkkan et al., 2017), banana (Alvindia & Natsuak, 2007), and mango (Kalupahana et al., 2020).

Although, *in vitro* tests may be used to determine the potential of GRAS salts to reduce postharvest rotting pathogens. However, *in vivo* bio assays are still required to validate the *in vitro* findings on the fruit. Our present study results on *in vivo* fruit inoculation showed notable significant decrease in rot progression on the surface and inside the fruit surface treated with 2% and 5% VeSolution as compared to controls. Similar results were also reported by Youssef et al. (2012) that potassium sorbate (GRAS salt) reduced rotting in citrus. Comparably, metabisulphite, sulphite derivates, and citric acid were reported to be effective in controlling molds (Sgroppo et al., 2010).

In a related study, Lyousfi et al. (2023) reported the use of 2% sodium sulfate in controlling brown rot in apples. Chloride and carbonates of calcium treatments minimized stem end rot in mangoes (Montecalvo et al., 2023). Interestingly, Pedrozo et al. (2024) optimized the compatible combinations of biocontrol yeast (*Metschnikowia pulcherrima*) with GRAS salt (sodium bicarbonate) and reported their ability to control blue rot in table grapes.

However, the above reports concentrated on the use of GRAS substance to improve storage while its effect on in-transit fruit rotting has not been studied. Conversely, the results from our present study demonstrated the effectiveness of postharvest dipping of fruit in 1% VeSolution in significantly controlling rotting under long-distance transit conditions. Additionally, the studies related to MAP and CA for managing rotting under prolonged transport and storage were reported earlier in pomegranate (Fuchs et al., 2007), Indian gooseberry (Singh et al., 2023), cherry (Cabañas et al., 2023), guava (Yadav et al., 2022) and the role of 1-MCP in inducing postharvest disease resistance in fruits to improve shelf life was reviewed by Ranjani et al. (2023).

CONCLUSION

The VeSolution presents a promising solution for reducing rotting during transportation in pomegranates. Initially, *Aspergillus* sp., *Alternaria* sp. and *Coinella* sp. were purified after isolation from the rotten pomegranate samples. The primary *in vitro* and *in vivo* studies with different concentrations of VeSolution demonstrated efficiency in minimizing the growth of fungal pathogens associated with pomegranate fruit rotting. However, concentrations above 5% showed toxicity symptoms on the fruit surface. The pilot trials revealed the effectiveness of 1% VS in minimizing pomegranate rotting and the same was implemented in larger shipments which ensured minimal rotting up to 84 h of transit. This implementation offers a practical strategy for long-distance fruit transportation. Overall, this study underscores the potential of using VeSolution as an alternative to traditional fungicides in minimizing postharvest losses and maintaining fruit quality during transportation, thus enhancing the economic viability of pomegranate production.

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgment

The authors duly acknowledge the financial support received from Vegrow (Chifu Agritech Pvt Ltd), Bengaluru, Karnataka, India.

Conceptualization of research work and designing of experiments (SS, ARD); Execution of lab and shipment trial experiments and data collection (SS, ARD); Analysis of data and interpretation (SS, BV); Preparation of initial draft of the manuscript (SS); Contributed to writing through review and editing (KJH, AAB). All authors read and approved the final version of the manuscript.

REFERENCES

Allagui, M. B., & Ben Amara, M. (2024). Effectiveness of several GRAS salts against fungal rot of fruit after harvest and assessment of the phytotoxicity of sodium metabisulfite in treated fruit. *Journal of Fungi*, *10*(5), 359. https://doi.org/10.3390/jof10050359

Alvindia, D. G., & Natsuaki, K. T. (2007). Control of crown rot-causing fungal pathogens of banana by inorganic salts and a surfactant. *Crop Protection*, *26*(11), 1667-1673. https://doi.org/10.1016/j.cropro.2007.02.008

Ambalavanan, A., Padma, C. P. S., Athira., & Ranjani, M. (2024). Post-harvest disease of fruits and vegetables. *Recent Innovations and Approaches in Plant Pathology (1st ed).* Stella International Publication, 221–253.

Bouhlali, E. D. T., Derouich, M., Meziani, R., & Essarioui, A. (2021). Antifungal potential of phytochemicals against *Mauginiella scaettae*, the plant pathogen causing inflorescence rot of date palm. *Scientifica*, *2021*(1), 1896015. https://doi.org/10.1155/2021/1896015

Cabañas, C. M., Hernández, A., Serradilla, M. J., Moraga, C., Martín, A., Córdoba, M. D. G., & Ruiz-Moyano, S. (2023). Improvement of shelf-life of cherry (*Prunus avium* L.) by combined application of modified-atmosphere packaging and antagonistic yeast for long-distance export. *Journal of the Science of Food and Agriculture*, *103*(9), 4592-4602. https://doi.org/10.1002/jsfa.12532

Camele, I., De Feo, V., Altieri, L., Mancini, E., De Martino, L., & Luigi Rana, G. (2010). An attempt of postharvest orange fruit rot control using essential oils from Mediterranean plants. *Journal of Medicinal Food*, *13*(6), 1515-1523. https://doi.org/10.1089/jmf.2009.0285

Fuchs, Y., Sandman, A., Ward, G., Kosto, I., Porat, R., & Weiss, B. (2007, December). Keeping quality of pomegranate fruit during prolonged storage and transport by MAP: new developments and commercial applications. In *Europe-Asia Symposium on Quality Management in Postharvest Systems-Eurasia, 2007, 804* (pp. 115-120). https://doi.org/10.17660/ActaHortic.2008.804.13

Guimarães, J. E., de la Fuente, B., Pérez-Gago, M. B., Andradas, C., Carbó, R., Mattiuz, B. H., & Palou, L. (2019). Antifungal activity of GRAS salts against *Lasiodiplodia theobromae* in vitro and as ingredients of hydroxypropyl methylcellulose-lipid composite edible coatings to control *Diplodia* stem-end rot and maintain postharvest quality of citrus fruit. *International Journal of Food Microbiology*, *301*, 9-18. https://doi.org/10.1016/j.ijfoodmicro.2019.04.008

Jadhav, R. R., Puri, S. G., & Rajput, M. O. (2023). Profile characteristics of pomegranate cultivators. *The Pharma Innovation 12*(2), 279-282. http://dx.doi.org/10.22271/tpi.2023.v12.i2d.18439

Kalupahana, K. I., Kuruppu, M., & Dissanayake, P. K. (2020). Effect of essential oils and GRAS compounds on postharvest disease control in mango (*Mangifera indica* L. cv Tom EJC). *Journal of Agricultural Sciences–Sri Lanka*, *15*(2), 207-221. https://doi.org/10.4038/jas.v15i2.8802

Kolaei, E. A., Tweddell, R. J., & Avis, T. J. (2012). Antifungal activity of sulfur-containing salts against the development of carrot cavity spot and potato dry rot. *Postharvest Biology and Technology*, *63*(1), 55-59. https://doi.org/10.1016/j.postharvbio.2011.09.006

Leck A. (1999). Preparation of lactophenol cotton blue slide mounts. *Community Eye Health*, *12*(30), 24.

Lyousfi, N., Legrifi, I., Ennahli, N., Blenzar, A., Amiri, S., Laasli, S. E., ... & Lahlali, R. (2023). Evaluating food additives based on organic and inorganic salts as antifungal agents against *Monilinia fructigena* and maintaining postharvest quality of apple fruit. *Journal of Fungi*, *9*(7), 762. https://doi.org/10.3390/jof9070762

Mahadevakumar, S., Shreenidhi, M., & Janardhana, G. R. (2019). First report of *Coniella granati* associated with dieback and fruit rot of pomegranate (*Punica granatum* L.) in India. *Journal of Plant Pathology*, *101*, 787-787. https://doi.org/10.1007/s42161-019-00256-z

Martínez-Blay, V., Pérez-Gago, M. B., de la Fuente, B., Carbó, R., & Palou, L. (2020). Edible coatings formulated with antifungal GRAS salts to control citrus anthracnose caused by

Colletotrichum gloeosporioides and preserve postharvest fruit quality. *Coatings*, *10*(8), 730. https://doi.org/10.3390/coatings10080730

Martínez-Blay, V., Taberner, V., Pérez-Gago, M. B., & Palou, L. (2021). Postharvest treatments with sulfur-containing food additives to control major fungal pathogens of stone fruits. *Foods*, *10*(9), 2115. https://doi.org/10.3390/foods10092115

Mincuzzi, A., Sanzani, S. M., Palou, L., Ragni, M., & Ippolito, A. (2022). Postharvest rot of pomegranate fruit in southern Italy: Characterization of the main pathogens. *Journal of Fungi*, *8*(5), 475. https://doi.org/10.3390/jof8050475

Mladenović, K. G., Muruzović, M. Ž., Stefanović, O. D., Žugić Petrović, T. D., & Čomić, L. R. (2018). Effects of some potassium preservatives on physiological activities of selected food borne bacteria. *Acta Alimentaria*, *47*(2), 171-180. https://doi.org/10.1556/066.2018.47.2.5

Montecalvo, M. P., Mendoza, M. J. C., & Dalisay, T. U. (2023). Disease-reducing Effect of Calcium Salts Against Postharvest Diseases of Mango (*Mangifera indica* L. cv. Carabao) Fruits. *The Philippine Agricultural Scientist*, *106*(3), 2. https://doi.org/10.62550/GH076022

Murthy, D. S., Gajanana, T. M., Sudha, M., & Dakshinamoorthy, V. (2009). Marketing and post-harvest losses in fruits: its implications on availability and economy. *Indian Journal of Agricultural Economics*, *64*(2), 259-275.

Palou, L. (2018). Postharvest treatments with GRAS salts to control fresh fruit decay. *Horticulturae*, *4*(4), 46. https://doi.org/10.3390/horticulturae4040046

Palou, L., & Taberner, V. (2022, May). Evaluation of hot water and GRAS salt solutions for the control of postharvest gray and green molds of pomegranate fruit. In *VI International Symposium on Postharvest Pathology: Innovation and Advanced Technologies for Managing Postharvest Pathogens 1363* (pp. 117-124). https://doi.org/10.17660/ActaHortic.2023.1363.17

Palou, L., Ali, A., Fallik, E., & Romanazzi, G. (2016). GRAS, plant-and animal-derived compounds as alternatives to conventional fungicides for the control of postharvest diseases of fresh horticultural produce. *Postharvest Biology and Technology*, *122*, 41-52. https://doi.org/10.1016/j.postharvbio.2016.04.017

Pedrozo, L. P., Kuchen, B., Flores, C. B., Rodríguez, L. A., Pesce, V. M., Maturano, Y. P., ... & Vazquez, F. (2024). Optimization of sustainable control strategies against blue rot in table grapes under cold storage conditions. *Postharvest Biology and Technology*, *213*, 112946. https://doi.org/10.1016/j.postharvbio.2024.112946

Ranjani, M., Shricharan, S., Nandhini, S., & Meichander, P. (2023). Revolutionizing fruit Preservation: 1-MCP's diverse applications and innovations. *Journal of Plant Development Sciences*, *15*(10), 525.

Romanazzi, G., Orçonneau, Y., Moumni, M., Davillerd, Y., & Marchand, P. A. (2022). Basic substances, a sustainable tool to complement and eventually replace synthetic pesticides in the management of pre and postharvest diseases: reviewed instructions for users. *Molecules*, *27*(11), 3484. https://doi.org/10.3390/molecules27113484

Romero-Cortes, T., López-Pérez, P. A., Pérez España, V. H., Medina-Toledo, A. K., Aparicio-Burgos, J. E., & Cuervo-Parra, J. A. (2019). Confrontation of *Trichoderma asperellum* VSL80 against *Aspergillus niger* via the effect of enzymatic production. *Chilean journal of Agricultural & Animal Sciences*, *35*(1) 68-80. https://doi.org/10.4067/S0719-38902019005000202

Saleem, A., & El-Shahir, A. A. (2022). Morphological and molecular characterization of some *Alternaria* species isolated from tomato fruits concerning mycotoxin production and polyketide synthase genes. *Plants*, *11*(9), 1168. https://doi.org/10.3390/plants11091168

Sgroppo, S. C., Vergara, L. E., & Tenev, M. D. (2010). Effects of sodium metabisulphite and citric acid on the shelf life of fresh cut sweet potatoes. *Spanish Journal of Agricultural Research*, *3*, 686-693. https://doi.org/10.5424/SJAR%2F2010083-1266

Shricharan, S., Mahalakshmi, S., & Kaviraj, S. (2020). In vitro evaluation of efficacy of botanicals against *Aspergillus niger* causing collar rot in groundnut. *Journal of Pharmacognosy and Phytochemistry*, *9*(4), 3177-3179. http://doi.org/10.13140/RG.2.2.11479.91040

Singh, V., Pathak, S., Pandey, K., & Singh, J. (2023). Effect of Low-Density Polyethylene on Shelf Life and Fruit Quality of Indian Gooseberry (*Emblica officinalis* Gaertn.). *International Journal of Plant & Soil Science*, *35*(18), 704-715. https://doi.org/10.9734/ijpss/2023/v35i183337

Soto-Muñoz, L., Taberner, V., de la Fuente, B., Jerbi, N., & Palou, L. (2020). Curative activity of postharvest GRAS salt treatments to control citrus sour rot caused by *Geotrichum citri-aurantii*. *International Journal of Food Microbiology*, *335*, 108860. https://doi.org/10.1016/j.ijfoodmicro.2020.108860

Türkkan, M., Özcan, M., & Erper, İ. (2017). Antifungal effect of carbonate and bicarbonate salts against *Botrytis cinerea*, the casual agent of grey mould of kiwifruit. *Akademik Ziraat Dergisi*, *6*(2), 107-114. https://doi.org/10.29278/azd.371066

Uysal, A., Kurt, Ş., Soylu, E. M., Kara, M., & Soylu, S. (2018). Morphology, pathogenicity and management of Coniella fruit rot (*Coniella granati*) on pomegranate. *Turkish Journal of Agriculture-Food Science and Technology*, *6*(4), 471-478. https://doi.org/10.24925/turjaf.v6i4.471-478.1787

Waskar, D. P., Khedkar, R. M., & Garande, V. K. (1999). Effect of post-harvest treatments on shelf life and quality of pomegranate in evaporative cool chamber and ambient conditions. *Journal of Food Science and Technology (Mysore)*, *36*(2), 114-117.

Yadav, A., Kumar, N., Upadhyay, A., Fawole, O. A., Mahawar, M. K., Jalgaonkar, K., ... & Mekhemar, M. (2022). Recent advances in novel packaging technologies for shelf-life extension of guava fruits for retaining health benefits for longer duration. *Plants*, *11*(4), 547. https://doi.org/10.3390/plants11040547

Youssef, K., & Roberto, S. R. (2014). Salt strategies to control *Botrytis* mold of 'Benitaka' table grapes and to maintain fruit quality during storage. *Postharvest Biology and Technology*, *95*, 95-102. https://doi.org/10.1016/j.postharvbio.2014.04.009

Youssef, K., Ligorio, A., Nigro, F., & Ippolito, A. (2012). Activity of salts incorporated in wax in controlling postharvest diseases of citrus fruit. *Postharvest Biology and Technology*, *65*, 39-43. https://doi.org/10.1016/j.postharvbio.2011.10.006

Youssef, K., Roberto, S. R., & de Oliveira, A. G. (2019). Ultra-structural alterations in *Botrytis cinerea*—the causal agent of gray mold—treated with salt solutions. *Biomolecules*, *9*(10), 582. https://doi.org/10.3390/biom9100582

Yu, Y., Zeng, L., Huang, L., Yan, Z., Sun, K., Zhu, T., & Zhu, A. (2016). First report of black leaf spot caused by *Alternaria alternata* on ramie in China. *Journal of Phytopathology*, *164*(5), 358-361. https://doi.org/10.1111/jph.12428

Zhao, J., Wang, Y., Liu, Q., Liu, S., Pan, H., Cheng, Y., & Long, C. (2023). The GRAS salts of Na2SiO3 and EDTA-Na2 control citrus postharvest pathogens by disrupting the cell membrane. *Foods*, *12*(12), 2368. https://doi.org/10.3390/foods12122368

JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH
2025, VOL. 8(4), 491-506

Journal of Horticulture and Postharvest Research

Journal homepage: www.jhpr.birjand.ac.ir

University of Birjand

Optimizing pineapple fruit production through growing media selection

Ebrahim Saboki[1] and Azam Jafari[2,*]

1, Agronomy and Horticulture Crops Research Department, Baluchestan Agricultural and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization (AREEO), Iranshahr, Iran
2, Department of Horticultural Sciences, Faculty of Agriculture & Natural Resources, Ardakan University, Ardakan, Iran

ARTICLE INFO

Original Article

Article history:
Received 12 December 2024
Revised 7 March 2025
Accepted 2 April 2025

Keywords:
Cocopeat
Growing media
Peat moss
Perlite
Pineapple

DOI: 10.22077/jhpr.2025.8557.1460
P-ISSN: 2588-4883
E-ISSN: 2588-6169

***Corresponding author:**
Department of Horticultural Sciences, Faculty of Agriculture & Natural Resources, Ardakan University, Ardakan, Iran.

Email: ajafari@ardakan.ac.ir

ABSTRACT

Purpose: Pineapple (*Ananas comosus* L.) is a tropical fruit of significant economic importance worldwide. The cultivation of this crop is influenced by various factors, including the growth media, which directly affect plant growth, yield, and fruit quality. Therefore, selecting the appropriate growth medium is crucial for achieving optimal pineapple production. **Research method:** This study was conducted over four years and evaluated five growing medium treatments: 1) a control consisting of field soil, sand, and animal manure; 2) peat moss and perlite; 3) peat moss, perlite, and sand; 4) cocopeat and perlite; and 5) cocopeat, perlite, and sand. Each treatment was replicated three times, with six pots per replicate, arranged in a randomized complete block design under a shade system with drip irrigation in Chabahar, located in Sistan and Baluchestan province, Iran. **Findings:** The results indicated that the cocopeat, perlite, and sand mixture significantly outperformed the other treatments in terms of vegetative growth and yield. The fruit weights with crown for the main plant and ratoon grown in this medium were 1208 and 851 g, respectively, with corresponding yields of 101,173 and 62,240 kg/ha, respectively. A combination of cocopeat, perlite, and sand has proven to be the optimal growth medium for pineapple cultivation. **Research limitations:** No limitations were found. **Originality/Value:** This study demonstrated that this specific mixture significantly enhanced vegetative growth, fruit yield, and overall plant health compared with the other tested media. These results suggest that this growing medium can be effectively used for pineapple production in a controlled environment.

INTRODUCTION

Pineapple (*Ananas comosus* (L.) Merr.) is a perennial herb belonging to the Bromeliaceae family and is native to the tropical regions of America. Its distinctive fruit, composed of fused berries, develops from a flowering plant. Costa Rica, the Philippines, and Brazil, which have favorable tropical climates, have become major pineapple-producing countries (Aruna, 2019). Indonesia, the Philippines, and Costa Rica were the top pineapple producers globally in 2022. Costa Rica's output alone reached 2.9 million metric tons each year. The world harvested approximately 29.4 million metric tons of pineapples worldwide during the same period (World Population Review, 2023). Pineapple, offers a combination of exceptional juiciness, vibrant flavor, and numerous health benefits. It's a source of essential nutrients, including calcium, potassium, vitamin C, carbohydrates, fiber, and various minerals. Low in fat and sodium (Sabahelkhier et al., 2010). Minerals present include calcium, chlorine, potassium, phosphorus, and sodium (Dull, 1971). Pineapple, a tropical species, thrives optimally within a temperature range of 22–32°C, with daily fluctuations of 8–14°C. Exceeding 32°C, particularly in conjunction with intense sunlight, can impair growth and cause sunburn during fruit maturation. Conversely, temperatures below 20°C hinder development and induce premature flowering, complicating cultivation, and increasing fruit loss (Bartholomew et al., 2003). Despite the limited tolerance to temperatures above freezing, the plant is highly susceptible to frost damage. Pineapple exhibits a remarkable tolerance to drought conditions. Its leaves possess specialized water-storage parenchyma cells, which enable them to retain moisture during periods of aridity. Moreover, a thick waxy cuticle covers the leaves, and the stomata are concentrated in sunken areas on the underside of the leaf (Nakasone & Paull, 1998). Although pineapple can thrive in regions with high annual rainfall (ideally 1500 mm), it can also be cultivated in areas receiving as little as 500 mm. In regions with less than 1500 mm of annual rainfall, supplemental irrigation, especially during the hot and dry seasons, is essential. Despite their drought resistance, prolonged dry spells can significantly harm pineapple plants. For commercial fruit production, pineapple requires 1000-1500 mm of annual rainfall supplemented by irrigation, and 70-80% relative humidity (Singh, 1995). Owing to its shallow root system, pineapple benefits from frequent, shallow irrigation. The optimal growth temperature for pineapple is reported to be 32°C during the day and 20°C at night. For every degree of Celsius deviation from these optimal temperatures, growth is reduced by approximately 6%. Intense sunlight and temperatures exceeding 35°C can lead to sunscald fruit (Anonymous, 2013). Cool temperatures delayed growth, resulting in smaller, more serrated leaves, increased sucker production, smaller fruits, cloudy fruit flesh, increased acidity, and reduced sugar content (Bartholomew & Kadzimin, 1977). Pineapples cannot tolerate low temperatures or prolonged exposure to temperatures below 7-10°C. Once a plant reaches a certain vegetative growth stage, cool nights can induce flowering. High temperatures (above 28°C) disrupt the formation of compounds necessary for flowering, thereby reducing flowering ability. Conversely, low temperatures, bright light, and complete shade are detrimental to plant growth (Bartholomew & Kadzimin, 1977). Fruit weight was directly correlated with light exposure during the growth period. Increased light intensity decreases fruit acidity but does not affect the total soluble solids content. Cloudy days reduced plant growth and fruit size. Some cultivars, such as those in the Queen group, are susceptible to sun scalding during fruit development when exposed to intense sunlight. Cultivars such as Smooth Cayenne and Cayenne are day neutral and can flower at any time of the year. However, pineapple can be considered as a short-day plant, although short days are not strictly required. Interrupting dark periods and providing supplementary light can inhibit flowering. Cool night temperatures enhance short-day effects (Nakasone & Paull, 1998). Optimal pineapple cultivation necessitates annual sunlight exposure between 2,500 and 3,000 h, equivalent to 7-8 hours daily. A minimum of 1,200-1,500 sunlight hours were required. Shade significantly influences growth and mandates meticulous site selection for solitary or companion planting (Reinhardt, 2001).

Agricultural production, particularly rain-fed cultivation, is highly susceptible to climate variability and change, which often adversely affects horticultural productivity. Meteorological assessments indicate a global trend towards rising surface temperatures, more intense and frequent extreme precipitation events, and elevated sea levels, especially in tropical regions (IPCC, 2014). A significant challenge posed to the horticulture industry is a temperature increase of up to 4°C. Regional shifts in rainfall patterns and temperatures are expected to have detrimental consequences on horticultural production (Deuter, 2008). For instance, untimely rainfall during drought periods or suboptimal temperatures during flowering and fruit development can diminish yields and induce physiological disorders (Datta, 2013). Empirical evidence from Uganda highlights the vulnerability of pineapple cultivation to climate extremes, with farmers reporting reduced yields and increased pest and disease prevalence in 2012, attributed to prolonged drought and elevated temperatures (Mugambwa, 2014). Protected cultivation systems, such as shade netting, have gained significant prominence in the face of contemporary climate change and the associated biotic stresses. Globally, diverse shade nets are employed to shield trees from adverse environmental conditions, including hail, wind, excessive solar radiation, and pests, thereby enhancing tree health and fruit quality (Manja & Aoun, 2019). Concurrently, modifying the composition of the growing media can improve plant growth and resilience to environmental stressors. Studies have demonstrated that a variety of growing media are widely used for cultivating horticultural crops in trays, bags, and pots. The combination of soil with well-rotted manure or vermicompost is a suitable growing medium and nutrient source for plants because it is readily available and economical. Furthermore, it was concluded that cocopeat mixed with any type of organic matter outperformed cocopeat alone, which may be attributed to the supply of nutrients to the growing medium by organic matter (Agarwal et al., 2021). To optimize the growth media, careful selection of components with optimal properties is imperative. Historically, sphagnum peat has been the predominant component because of its exceptional characteristics. However, increasing concerns about peatland degradation and the imperative for sustainable practices have stimulated research on alternative substrates. A wide range of materials, including compost, wood fiber, bark, and cocopeat, have been identified as potential substitutes. Although each material possesses unique advantages and limitations, peat continues to be a critical component in many growing media, especially as a diluent (Schmilewski, 2008). The growing global demand for pineapple has intensified the need for sustainable and efficient cultivation. The choice of growing medium is a key aspect of pineapple production. This study undertakes a comparative analysis of different growing media types, focusing on their impact on pineapple plant growth and fruit quality. By identifying the most suitable substrates, this study will contribute to the development of more sustainable and economically viable pineapple production systems.

MATERIALS AND METHODS

Location and treatments

This study was conducted at the Chabahar Agricultural Research Station, located at 60° 38' 19"″ E and 25° 10' 57″ North latitude, at an altitude of 10 m above sea level. The proximity of Chabahar to the sea, its location near the Tropic of Cancer, and its exposure to the Indian monsoon and equatorial fronts contribute to a relatively warm tropical climate characterized by high humidity. It is the warmest region in the country during winter and the coolest southern port of Iran during summer. The average maximum temperature (in June) over a seven-year period was 31°C, the average minimum temperature (in January) was 19°C, and the annual average temperature was 26°C. The minimum relative humidity was 60%, and the average relative humidity was 70%. The average annual rainfall is less than 200 mm, with 64% of the rainfall occurring in winter (Chabahar, 2022).

To investigate pineapple growth and adaptation, a 2.5-meter-high shade net was constructed using galvanized poles and a green shade net providing 40% shading. The shading net covered an area of 500 m^2. The surrounding walls were enclosed in similar nets. The following are the treatments.

SSM: Control treatment consisting of an equal-volume mixture of agricultural soil, washed sand (0.5-8 mm), and manure.

PP: Equal-volume mixture of peat moss and medium-grade perlite (2-5 mm). PPS: Equal-volume mixture of peat moss, perlite, and washed sand.

CP: Equal-volume mixture of cocopeat and perlite.

CPS: Equal-volume mixture of cocopeat, perlite, and washed sand.

Six 15-liter pots (25 cm diameter) were used for each treatment in each replicate. The substrate components were mixed volumetrically and filled into pots. One rooted MD2 pineapple plantlet produced through tissue culture was transplanted into each pot. Tissue culture has been demonstrated to be a reliable and efficient method for pineapple propagation, providing benefits such as increased productivity and disease-free planting material. Therefore, tissue-cultured pineapple plantlets were used in the present study (Jackson et al., 2016). At transplanting, the plantlets had an average of 15 leaves, a plant height of 7 cm, a stem diameter of 1.5 cm, 10 roots, and a root length of 12 cm. Daily drip irrigation was applied to maintain field capacity in the root zone. All the treatments received the same foliar fertilization. No cooling or heating system was used within the shade house during the experiment. Due to the unsuitable substrate, the growth of plants in the control treatment gradually ceased, and they completely dried up after nine months, then the experiment was conducted using four treatments.

In December 2017, the plants were sprayed with 25 ppm ethephon to induce flowering. Flowering was observed in December of the same year, and fruit growth continued until harvest the following spring. Harvesting began in early July and continued until the end of that month. The largest sucker closest to the soil surface (first ratoon) was retained for the next generation, whereas other plantlets on the fruit stem and suckers were removed. Ethephon spraying was repeated in December 2018 to induce flowering in the first ratoons, and harvesting was conducted in the summer of 2019. In the third year, second-generation ratoons were selected, but they exhibited poor vegetative growth, resulting in negligible fruit production.

Measurement of vegetative and reproductive traits

During flower emergence, various vegetative traits have been evaluated in pineapple plants. These included plant height (measured from the crown to the inflorescence), number of leaves per plant, leaf length, and the width of the largest leaf (leaf D) (Matos Viegas et al., 2014). At harvest, fruit characteristics were evaluated, including fresh weight (with and without crown), crown weight, crown weight percentage (calculated as crown weight divided by total fruit weight with crown multiplied by 100), fruit and crown length, fruit diameter (measured with Vernier calipers), number of reproductive organs, and fruit set percentage (proportion of plants bearing fruit per treatment). The yield was calculated based on the weight of the fruit (with and without crown) multiplied by the fruit set percentage, assuming 80,000 plants per hectare (Hung et al., 2024).

Statistical analysis

Data analysis was performed using SAS software (version 9.1.3). Mean comparisons were evaluated using Duncan's multiple range tests. A randomized complete block design (RCBD) was used in this experiment. Five treatments were evaluated, each with three replicates, with each replicate consisting of six pots.

RESULTS

Given the loss of all control plants, data analysis was conducted for the remaining four treatment groups. The results of these treatments are presented below.

Plant Height

The results revealed that the highest plant height (23.3 cm) was observed in plants grown in a cocopeat- perlite-sand mixture. This treatment did not differ significantly from the peat moss-perlite-sand mixture, but was significantly different from the other two treatments. The lowest plant height (21.5 cm) was recorded for plants grown in a peat moss-perlite mixture, which did not differ significantly from the cocopeat-perlite and peat moss-perlite-sand mixtures (Table 1).

Similarly, at the ratoon fruiting stage, the highest plant height (16.3 cm) was observed in plants grown in a cocopeat-perlite-sand mixture. This treatment did not differ significantly from the peat moss-perlite- sand mixture but was significantly different from the other two sand-free treatments. The peat moss- perlite and cocopeat-perlite treatments did not differ significantly and had plant heights of 13.4 cm and 14.3 cm, respectively (Table 2).

The results indicated an average decrease of 34.7% in ratoon plant height. The lowest reduction in plant height (30%) was observed in the cocopeat-perlite-sand treatment, whereas the highest reduction (38.6%) was observed in the cocopeat-perlite treatment.

Number of leaves

The results of this study revealed that the highest number of leaves (30) was observed in plants grown in a cocopeat-perlite-sand mixture. This treatment did not differ significantly from that of the peat moss- perlite-sand mixture. Additionally, the cocopeat-perlite and peat moss-perlite treatments, with 27 and 26 leaves, respectively, were grouped together without significant differences (Table 1).

Table 1. The effect of different growing media on plant height, number of leaves, leaf length and width, number of reproductive organs, and fruit weight with crown in the main fruit production stage.

Treatments	Plant height (cm)	Number of leaves	Leaf length (cm)	Leaf width (cm)	Number of reproductive organs	Fruit weight crown (g)
PP	21.5^{b*}	26.0^{b}	55.1^{d}	4.2^{c}	3.3^{c}	1095.0^{b}
PPS	22.6^{ab}	29.0^{a}	60.8^{b}	4.3^{b}	4.7^{b}	1168.7^{a}
CP	22.1^{b}	27.0^{b}	57.9^{c}	4.2^{c}	4.3^{b}	1125.3^{b}
CPS	23.3^{a}	30.0^{a}	63.7^{a}	4.5^{a}	5.3^{a}	1208.0^{a}

* Means with the same letters do not have significant differences based on Duncan's test at the 5% level.
PP: equal-volume mixture of peat moss and medium-grade perlite (2-5mm). PPS: equal-volume mixture of peat moss, perlite, and washed sand. CP: equal-volume mixture of cocopeat and perlite. CPS: equal-volume mixture of cocopeat, perlite, and washed sand.

Table 2. Effect of different growing media on plant height, number of leaves, leaf length and width, number of reproductive organs, and fruit weight with crown in the ratoon fruit production stage.

Treatments	Plant height (cm)	Number of leaves	Leaf length (cm)	Leaf width (cm)	Number of reproductive organs	Fruit weight crown (g)
PP	13.4^{b}	16.3^{d}	34.2^{d}	2.5^{b}	3.0^{b}	760.0^{c}
PPS	15.3^{a}	19.7^{b}	41.3^{b}	2.7^{b}	4.3^{a}	808.0^{ab}
CP	14.3^{b}	17.7^{c}	37.6^{c}	2.7^{b}	4.2^{a}	776.3^{bc}
CPS	16.3^{a}	21.3^{a}	45.2^{a}	3.0^{a}	4.6^{a}	851.0^{a}

* Means with the same letters do not have significant differences based on Duncan's test at the 5% level. PP: equal-volume mixture of peat moss and medium-grade perlite (2-5mm). PPS: equal-volume mixture of peat moss, perlite, and washed sand. CP: equal-volume mixture of cocopeat and perlite. CPS: equal-volume mixture of cocopeat, perlite, and washed sand.

At the ratoon fruiting stage, the highest number of leaves (21.3) was observed in the cocopeat-perlite-sand treatment. The peat moss-perlite-sand treatment ranked second with 19.7 leaves. The lowest number of leaves (16.3) was observed in the peat moss-perlite treatment, which differed significantly from the other treatments (Table 2).

On average, there was a 33.2% decrease in the number of leaves in ratoon plants compared with the main crop. The highest percentage decrease in leaf number (37.3%) was observed in the peat moss- perlite treatment, whereas the lowest decrease (29%) was observed in the cocopeat-perlite-sand treatment.

Leaf Length

The results of this study demonstrated that the type of growing medium had a significant impact on leaf length during the main fruiting stage. Plants grown in a cocopeat-perlite-sand mixture exhibited the longest leaf length (63.7 cm). The shortest leaf length (55.1 cm) was observed in plants grown in the peat moss-perlite mixture (Table 1).

During the ratoon fruiting stage, the type of growing medium also significantly influenced leaf length. The longest leaf length (45.2 cm) was found in plants grown in a cocopeat-perlite-sand mixture. The shortest leaf length (34.2 cm) was observed in plants grown in the peat moss-perlite mixture (Table 2).

On average, leaf length decreased by approximately 33.5% in ratoon plants compared with that in the main crop. The greatest reduction in leaf length (37.9%) was observed in plants grown in a peat moss- perlite mixture, whereas the least reduction (29%) was observed in plants grown in a cocopeat-perlite- sand mixture.

Leaf width

The results indicated a significant influence of growing medium type on leaf width during the main fruiting stage. Plants cultivated in a cocopeat-perlite-sand mixture exhibited the largest leaf width (4.5 cm), which was significantly different from that of the other treatments. The cocopeat-perlite and peat moss-perlite-sand mixtures were comparable, while the peat moss-perlite mixture displayed the smallest leaf width (4.2 cm), which was not significantly different from that of cocopeat-perlite (Table 1).

During ratoon fruiting, the growing medium continued to significantly affect leaf width. The cocopeat- perlite-sand mixture again yielded the widest leaves (3 cm), differing significantly from the other treatments. The remaining treatments exhibited similar leaf widths, ranging from 2.5 to 2.7 cm (Table 2).

On average, leaf width decreased by approximately 36.7% in ratoon plants compared with that in the main crop. The cocopeat-perlite-sand mixture demonstrated the smallest reduction in leaf width (33.3%), whereas the peat moss-perlite treatment exhibited the most pronounced decrease (40.5%).

Number of reproductive organs

The results of this study demonstrated that the type of growing medium significantly influenced the number of propagules in pineapple plants during the main fruiting stage. The highest number of propagules (5.3) was observed in plants cultivated in the cocopeat-perlite-sand mixture, which differed significantly from the other treatments. The peat moss-perlite-sand (4.7 propagules) and cocopeat-perlite (4.3 propagules) treatments were grouped together and showed no significant differences between them. The lowest number of propagules (3.3) was found in plants grown in peat moss-perlite, which differed significantly from the other treatments (Table 1).

During the ratoon fruiting stage, the type of growing medium significantly affected the number of propagules. The highest number of propagules (4.6) was observed in the cocopeat-perlite-sand mixture, which did not differ from the peat moss-perlite-sand or cocopeat-perlite treatments. The lowest number of propagules (3) was found in the peat moss-perlite treatment, which differed significantly from the other treatments (Table 2).

On average, the number of propagules in ratoon plants decreased by 8.3% compared with the main crop. This decrease ranged from 2.3% in the cocopeat-perlite treatment to 13.2% in the cocopeat-perlite- sand treatment.

Fruit weight with crown

The results of this study revealed that the type of growing medium significantly influenced the crown fruit weight of pineapple plants during the main fruiting stage. The highest crown fruit weight (1208 g) was observed in plants grown in a cocopeat-perlite-sand mixture, which did not significantly differ from plants grown in a peat moss-perlite-sand mixture (1168.7 g). The two treatments were then grouped together. Similarly, the cocopeat-perlite (1125.3 g) and peat moss-perlite (1095 g) treatments were grouped together and showed no significant difference between them (Table 1).

During the ratoon fruiting stage, the type of growing medium significantly affected crown fruit weight. The highest crown fruit weight (851 g) was observed in the cocopeat-perlite-sand mixture, which did not significantly differ from that of the peat moss-perlite-sand mixture (808 g). The lowest crown fruit weight (760 g) was observed in the peat moss-perlite treatment (Table 2).

Table 3. The effect of different growing media on fruit weight without crown, crown weight, crown weight percentage, fruit length with crown, fruit length without crown, and crown length in the main fruit production stage.

Treatments	Fruit weight without crown (g)	Crown weight (g)	Crown weight percentage	Fruit length with crown (cm)	Fruit length without crown (cm)	Crown length (cm)
PP	944.0d	206.0c	18.3 b	22.5d	11.3d	11.5c
PPS	981.0b	230.0a	18.5 b	25.1b	12.2b	12.3b
CP	956.7c	217.7b	18.4 b	24.1c	11.7c	11.8c
CPS	1009.0a	236.7a	18.7a	26.0a	12.8a	12.8a

* Means with the same letters do not have significant differences based on Duncan's test at the 5% level. PP: equal-volume mixture of peat moss and medium-grade perlite (2-5mm). PPS: equal-volume mixture of peat moss, perlite, and washed sand. CP: equal-volume mixture of cocopeat and perlite. CPS: equal-volume mixture of cocopeat, perlite, and washed sand.

On average, the crown fruit weight of ratoon plants decreased by 30.5% compared that with of the main crop. The variation in this decrease among the treatments was a maximum of 1.3%. The lowest decrease was observed in the cocopeat-perlite-sand treatment, whereas the highest decrease was observed in the peat moss-perlite-sand treatment.

Fruit weight without crown

The results of this study demonstrated that the type of growing medium significantly influenced the crownless fruit weight of pineapple plants during the main fruiting stage. The highest crownless fruit weight (1009 g) was observed in plants grown in the cocopeat-perlite-sand mixture, which differed significantly from the other treatments. The peat moss-perlite-sand mixture yielded a crownless fruit weight of 981 g, ranking second, and showing significant differences compared to other treatments. The lowest crownless fruit weight (944 g) was observed in the peat moss-perlite treatment, which differed significantly from the other treatments (Table 3).

During the ratoon fruiting stage, the type of growing medium significantly affected the crownless fruit weight. The highest crownless fruit weight (683 g) was observed in the cocopeat-perlite-sand mixture, which differed significantly from that of the other treatments. The peat moss-perlite-sand mixture yielded a crownless fruit weight of 647 g, and the lowest crownless fruit weight (602 g) was observed in the peat moss-perlite treatment. All treatments at the ratoon fruiting stage showed significant differences (Table 4).

On average, the crownless fruit weight of ratoon plants decreased by 34.3% compared with that of the main crop. The highest decrease in crownless fruit weight (36.2%) was observed in the peat moss-perlite treatment, whereas the lowest decrease (32.3%) was observed in the cocopeat-perlite-sand mixture.

Crown weight

The results of this study demonstrated that the type of growing medium significantly influenced the crown weight of pineapple plants during the main fruiting stage. The highest crown weight (236.7 g) was observed in plants grown in the cocopeat-perlite-sand mixture, which did not significantly differ from the peat moss-perlite-sand mixture. These two treatments were grouped together. The cocopeat-perlite treatment, with a crown weight of 217.7 g, was grouped separately and showed significant differences compared to the other treatments. The lowest crown weight (206 g) was observed in the peat moss-perlite treatment (Table 3).

During the ratoon fruiting stage, the type of growing medium also significantly affected the crown weight. The highest crown weight (164.7 g) was observed in the cocopeat-perlite-sand mixture, which differed significantly from the other treatments. The peat moss-perlite-sand mixture, with a crown weight of 148.3 g, ranked second and showed significant differences compared to the other treatments. The lowest crown weight (130.7 g) was observed in the peat moss-perlite treatment (Table 4).

On average, the crown weight of ratoon plants decreased by 34.7% compared to that of the main crop. The highest decrease in crown weight (36.6%) was observed in the peat moss-perlite treatment, whereas the lowest decrease (30.4%) was observed in the cocopeat-perlite-sand mixture.

Table 4. The effect of different growing media on fruit weight without crown, crown weight, crown weight percentage, fruit length with crown, fruit length without crown, and crown length in the ratoon fruit production stage.

Treatments	Fruit weight without crown (g)	Crown weight (g)	Crown weight percentage	Fruit length with crown (cm)	Fruit length without crown (cm)	Crown length (cm)
PP	602.0d	130.7d	18.4 b	13.6d	7.4d	7.5d
PPS	647.0b	148.3b	18.4 b	15.4b	8.3b	8.8b
CP	625.7c	138.7c	18.4 b	14.3c	7.9c	8.2c
CPS	683.0a	164.7a	18.7a	17.1a	9.1a	9.3a

* Means with the same letters do not have significant differences based on Duncan's test at the 5% level. PP: equal-volume mixture of peat moss and medium-grade perlite (2-5mm). PPS: equal-volume mixture of peat moss, perlite, and washed sand. CP: equal-volume mixture of cocopeat and perlite. CPS: equal-volume mixture of cocopeat, perlite, and washed sand.

Crown weight percentage

This study demonstrated a significant influence of the growing medium type on the percentage of crown weight to fruit weight in pineapple plants during the main fruiting stage. The cocopeat-perlite-sand mixture exhibited the highest percentage (18.7%), which differed significantly from the other treatments. The three other treatments, with percentages ranging from 18.3% to 18.5%, were statistically similar (Table 3).

During the ratoon fruiting stage, growing medium continued to significantly impact the percentage of crown weight to fruit weight. The cocopeat-perlite-sand mixture again yielded the highest percentage (18.7%), with other treatments showing no significant differences. All other treatments resulted in a crown weight percentage of 18.4% (Table 4).

Overall, the percentage of crown weight to fruit weight remained relatively consistent between the main crop and ratoon stages, averaging approximately 18.5%. Minor fluctuations were observed among the treatments, with a slight increase in the peat moss-perlite medium and a slight decrease in the cocopeat- perlite-sand mixture in ratoon plants.

Fruit length with crown

The results of this study indicated a significant influence of the growing medium on fruit length with the crown during the main fruiting stage. Each of the four growing medium treatments resulted in a distinct fruit length with a crown. The longest crown fruit (26 cm) was observed in the cocopeat-perlite-sand mixture. This was followed by peat moss-perlite-sand (25.1 cm) and cocopeat-sand (24.1 cm) mixtures. The shortest crown fruit (22.5 cm) was observed in the peat moss-perlite treatment (Table 3).

During the ratoon fruiting stage, the type of growing medium continued to significantly affect fruit length with the crown. The cocopeat-perlite-sand mixture again yielded the longest fruit length, with a crown (17.1 cm). Subsequently, peat moss-perlite-sand (15.4 cm) and cocopeat-sand (14.3 cm) mixtures were used. The shortest fruit length with the crown (13.6 cm) was observed in the peat moss-perlite treatment (Table 4).

On average, the fruit length with the crown of ratoon plants decreased by 38.3% compared that with of the main crop. The highest decrease (40.7%) was observed in the cocopeat-perlite treatment, whereas the lowest decrease (13.6%) was observed in the peat moss-perlite treatment.

Fruit length without crown

The results of this study demonstrated that the type of growing medium significantly influenced the crownless fruit length of pineapple plants during the main fruiting stage. Each of the four growing media treatments resulted in distinct crownless fruit lengths. The longest crownless fruit (12.8 cm) was observed in the cocopeat-perlite-sand mixture. This was followed by peat moss-perlite-sand (12.2 cm) and cocopeat-sand (11.7 cm) mixtures. The shortest crownless fruit (11.3 cm) was observed in the peat moss- perlite treatment (Table 3).

During the ratoon fruiting stage, the type of growing medium continued to significantly affect crownless fruit length. The cocopeat-perlite-sand mixture yielded the longest crownless fruit (9.1 cm), followed by the peat moss-perlite-sand (8.3 cm) and cocopeat-sand (7.9 cm) mixtures. The shortest crownless fruit (7.4 cm) was observed in the peat moss-perlite treatment (Table 4).

On average, the crownless fruit length of ratoon plants decreased by 32% compared with that of the main crop. The highest decrease (34.5%) was observed in the peat moss-perlite treatment, while the lowest decrease (28.9%) was found in the cocopeat-perlite-sand mixture.

Crown length

Statistical analysis revealed a significant impact of different growing media on crown length at both the primary and secondary fruiting stages. In the primary fruiting stage, the longest crown (12.8 cm) was observed in the treatments with cocopeat, perlite, and sand. Treatments with peat moss, perlite, and sand (12.3 cm), and cocopeat and sand (11.8 cm) followed, respectively, and were placed in the subsequent homogeneous statistical groups. The shortest crown (11.5 cm) was observed in the peat moss and perlite treatments (Table 3). In the secondary fruiting stage, the longest crown (9.3 cm) was observed in the treatment with cocopeat, perlite, and sand. Other treatments included peat moss with perlite and sand (8.8 cm) and cocopeat with sand (8.2 cm). Notably, crown length decreased by an average of 30.3% in the secondary fruiting stage. The highest decrease was observed in the treatment with peat moss and perlite (34.8%), whereas the lowest decrease was observed in the treatment with cocopeat, perlite, and sand (27.3%) (Table 4).

Fruit diameter

Statistical analysis revealed a significant impact of growing media on fruit diameter at both the primary and secondary fruiting stages. In the primary fruiting stage, the smallest fruit diameter (8.7 cm) was observed in the peat moss and perlite media. This medium, along with peat moss, perlite, sand, cocopeat, and sand (both 8.8 cm), was grouped into a homogeneous statistical group. However, cocopeat, perlite, and sand medium, with the largest fruit diameter (9.1 cm), were also included in this group, indicating no significant statistical difference between the three treatments (Table 5).

In the secondary fruiting stage, significant statistical differences were observed among the treatments. The cocopeat, perlite, and sand media exhibited the largest average fruit diameter (6.4 cm), whereas the smallest diameter (5.4 cm) was observed in the peat moss and perlite media (Table 6).

Table 5. The of different growing media on fruit diameter, fruit set, fruit yield with crown, and fruit yield without crown in the main fruit production stage.

Treatments	Fruit diameter (cm)	Fruit set (%)	Fruit yield with crown (g)	Fruit yield without crown (g)
PP	8.7^{b}	86.7^{a}	69547^{c}	56789^{c}
PPS	8.8 ^{ab}	100.0^{a}	94293^{a}	76880^{a}
CP	8.8 ab	93.3^{a}	81392^{b}	66411^{b}
CPS	9.1^{a}	100.0a	101173a	82240^{a}

* Means with the same letters do not have significant differences based on Duncan's test at the 5% level. PP: equal-volume mixture of peat moss and medium-grade perlite (2-5mm). PPS: equal-volume mixture of peat moss, perlite, and washed sand. CP: equal-volume mixture of cocopeat and perlite. CPS: equal-volume mixture of cocopeat, perlite, and washed sand.

Table 6. The of different growing media on fruit diameter, fruit set, fruit yield with crown, and fruit yield without crown in the ratoon fruit production stage.

Treatments	Fruit diameter (cm)	Fruit set (%)	Fruit yield with crown (g)	Fruit yield without crown (g)
PP	5.4^{d}	66.7^{b}	33163^{c}	27061^{c}
PPS	6.1^{b}	80.0^{ab}	51285^{b}	41835^{b}
CP	5.7^{c}	73.3^{ab}	41541^{bc}	33893^{bc}
CPS	6.4^{a}	86.7^{a}	62240^{a}	50592^{a}

* Means with the same letters do not have significant differences based on Duncan's test at the 5% level. PP: equal-volume mixture of peat moss and medium-grade perlite (2-5mm). PPS: equal-volume mixture of peat moss, perlite, and washed sand. CP: equal-volume mixture of cocopeat and perlite. CPS: equal-volume mixture of cocopeat, perlite, and washed sand.

Overall, the fruit diameter decreased by an average of 33.4% during the secondary fruiting stage. The greatest decrease (37.9%) was observed in the peat moss and perlite media, whereas the least decrease (29.7%) was observed in the cocopeat, perlite, and sand media.

Fruit set

Based on the results of this study, the type of growing medium did not have a significant effect on the fruit set percentage in the primary fruiting stage. All treatments were performed in a homogeneous statistical group. The highest fruit set percentage (100%) was observed in peat moss with perlite and sand and cocopeat with perlite and sand treatments, while the lowest percentage (86.7%) was observed in the peat moss with perlite treatment (Table 5).

In the secondary fruiting stage, significant differences were observed among the treatments. Peat moss with perlite and sand treatments had the highest fruit set percentage (86.7%). In contrast, peat moss with perlite and cocopeat with sand treatments had the lowest fruit set percentages (66.7%) and were placed in a homogeneous statistical group (Table 6).

Overall, the fruit set percentage decreased by an average of 19.5% during the secondary fruiting stage. The greatest decrease (23.1%) was observed in the peat moss with perlite treatment, whereas the lowest decrease (13.3%) was observed in the cocopeat with perlite and sand treatments.

Fruit yield with crown

Statistical analysis indicated that the type of growing medium had a significant impact on fruit yield per hectare during the initial production stage. As shown in Table 5, the treatments of cocopeat with perlite and sand and peat moss with perlite and sand yielded the highest fruit yields of 101,173 and 94,293 kg/ha, respectively, and there was no significant difference between them. The cocopeat with perlite treatment ranked next, with a yield of 81,392 kg/ha. The lowest yield (69,547 kg/ha) was observed in peat moss with perlite treatment, which differed significantly from the other treatments (Table 5).

In the ratooning fruit production stage, as shown in Table 6, the cocopeat with perlite and sand treatments had the highest yield of 62,240 kg/ha, which differed significantly from the other treatments. Peat moss with perlite and sand and cocopeat with perlite treatments were in the next group with yields of 51,285 and 41,541 kg/ha, respectively and there was no significant difference between them. Peat moss treated with perlite had the lowest yield (33,163 kg/ha) was observed in the peat moss with perlite treatment (Table 6).

Overall, the fruit yield decreased by an average of 46.4% during the ratooning stage. As shown in Tables 5 and 6, the highest yield reduction (52.3%) was observed in peat moss treated with perlite, and the lowest reduction.

Fruit yield without crown

Statistical analysis revealed that the type of growing medium had a significant impact on the yield. In the initial production stage, the combination of cocopeat with perlite and sand yielded the highest fruit yields (82, 240 and 76, 880 g/ha, respectively). However, the difference between the two combinations was not statistically significant. The cocopeat with perlite combination ranked next, with a yield of 66,411 kg/ha, while the lowest yield was observed in the peat moss with a perlite combination at 56,789 kg/ha (Table 5).

In the secondary (ratooning) production stage, the cocopeat with perlite and sand combination yielded the highest fruit yield at 50,592 kg/ha and showed a significant difference compared to other combinations.

Peat moss with perlite, sand, and cocopeat with perlite combinations had similar yields of 41,835 and 33,893 kg/ha, respectively (Table 6).

Overall, fruit yield decreased by an average of 46.4% in the secondary stage compared to that in the initial stage. The highest yield reduction (52.3%) was observed in the peat moss with perlite combination, whereas the lowest reduction (38.5%) was observed in the cocopeat with perlite and sand combination.

DISCUSSION

The findings of this study demonstrated that pineapple plants cultivated under shade conditions with drip irrigation exhibited superior vegetative and reproductive growth parameters during both the main crop and ratoon cycles. Specifically, these included increased plant height, leaf count, leaf dimensions, propagule number, fruit weight (including and excluding the crown), crown weight percentage, fruit length (including and excluding the crown), crown length, fruit diameter, fruit set, and total fruit yield when grown in a cocopeat-perlite-sand medium. The peat moss-perlite-sand medium yielded the second- best results.

Previous studies have consistently highlighted the significant impact of growing media on plant growth and development. In one study, the results indicated significant variations in the dry matter production of pineapple seedlings among the treatments. Compost-based media, particularly the 1:0 and 1:1 compost-to- topsoil ratios, demonstrated superior performance in terms of leaf and root dry weights, as well as total plant dry biomass. These findings underscore the importance of optimizing the composition of the growing media to enhance pineapple seedling growth and development (Ajema & Shewangizaw, 2021).

Another study confirmed the effect of growing media on the growth of young pineapple plants. Gebisa (2021) indicated significant differences among treatments with compost-based media, particularly 1:0 and 1:1ratios, promoting superior seedling growth. These findings suggest that compost-rich nursery media can effectively enhance pineapple seedling development and support subsequent field establishment and production (Gebisa, 2021). According to the research results of Lakho et al. (2023), the type of growing medium affects the adaptability of pineapple plantlets. Based on their results, while peat moss, a 1:1 bolhari-peat moss mixture, and river silt all supported high plantlet survival rates in the greenhouse (100%, 98.9%, and 95.1%, respectively), the survival rate in pure peat moss was significantly higher ($p < 0.05$).

A study examining the effects of various growing media on strawberry performance indicated that a bio- plus compost enriched with synthetic nutrients was the most effective substrate for enhancing strawberry growth and yield in a greenhouse environment (Madhavi et al., 2021). A study has shown that the type of growing medium affects the growth of basil plants. According to the results of this research, the cocopeat + perlite growing medium was the most effective in improving the physiological and phytochemical parameters of basil (Yonesi et al., 2025). Studies have shown that incorporating compounds like perlite or cocopeat into sand improves phytochemical traits (Agarwal et al., 2021).

Our results are consistent with those of Ilahi and Ahmad (2017), who showed that incorporating perlite into cocopeat enhanced the physical and hydraulic characteristics of the growth substrate. In line with our research, soilless substrates, such as sand, perlite, and cocopeat, outperform traditional soil in terms of Geranium oil production results in higher yields and enhanced oil quality (Rezaei Nejad & Ismaili, 2014). Our results, which showed that cocopeat had a positive effect, are consistent with a study that found that adding it to vermicompost had a positive effect on the growth of tomato plants (Erdal & Aktaş, 2025). Cocopeat's benefits for plant growth include its high water-holding capacity, antifungal properties, and significant phosphorus and potassium content. This contrasts with inorganic substrates such as pumice and sand, which generally have low nutrient levels (Chhetri et al., 2022)

Shade cultivation combined with drip irrigation has been shown to enhance various growth metrics of pineapple plants. This is consistent with the findings of Santos et al. (2020), who reported that partial shading improves pineapple growth and fruit quality. The observed increases in plant height, leaf count, and leaf dimensions under these conditions suggest an improved photosynthetic efficiency and overall plant vigor. These improvements in vegetative growth are crucial to the overall health and productivity of pineapple crops. Fruit characteristics were significantly improved, with larger fruit weights (including and excluding the crown), increased fruit length, and greater fruit diameter (Santos et al., 2020).

It's important to note that while these findings are promising; their applicability may vary depending on local climate conditions, soil types, and pineapple varieties. Farmers should consider conducting small- scale trials to determine the optimal combination of shade levels, irrigation schedules, and growing media for specific circumstances (Umi et al., 2020).

CONCLUSION

In summary, the addition of sand to growing media consisting of peat moss, perlite, cocopeat, and perlite when used in drip irrigation systems significantly enhanced plant growth and yield due to improved water-holding capacity and nutrient retention. At a planting density of 80,000 plants per hectare in greenhouses or shade houses, a total fruit yield of up to 101,173 g was obtained from the primary crop and 62,240 g from the ratoon crop.

Conflict of interest
No potential conflict of interest was reported by the authors.

Funding
No funding was received.

Data availability statement
All relevant data are included within the manuscript and its supplementary information files.

REFERENCES

Agarwal, P., Saha, S., & Hariprasad, P. (2021). Agro-industrial-residues as potting media: Physicochemical and biological characters and their influence on plant growth. *Biomass Conversion and Biorefinery*, *9*, 1-24. https://doi.org/10.1007/s13399-021-01998-6.

Ajema, L., & Shewangizaw, K. (2021). Dry matter production response of non-conventional pineapple (*Ananas comosus* L.) Seedling to Nursery Media Preparation. *European Journal of Biophysics*, *9*(1), 9-12. https://doi.org/ 10.11648/j.ejb.20210901.12.

Aruna, T. E. 2019. Production of value-added product from pineapple peels using solid state fermentation. *Innovative Food Science and Emerging Technologies*, *57*, 102193. https://doi.org/10.1016/j.ifset.2019.102193

Anonymous. (2013). Land requirements for growing pineapple. Queensland Government, Department of Agriculture and Fisheries. https://era.daf.qld.gov.au/id/eprint/8639/

Bartholomew, D. P., Malézieux, E., Sanewski, G. M., & Sinclair, E. (2003). Inflorescence and fruit development and yield. In D. P. Bartholomew, R. E. Paull, & K. G. Rohrbach (Eds.), The pineapple: Botany, production and uses (pp. 167-202). CABI Publishing.

Bartholomew, D. P., & Kadzimin, S. B. (1977). Pineapple. In *Ecophysiology of tropical crops* (pp. 113-156). Academic Press.

Chhetri, S., Dulal, S., Subba, S., & Gurung, K. (2022). Effect of different growing media on growth and yield of leafy vegetables in nutrient film technique hydroponics system. *Archives of Agriculture and Environmental Science*, *7*(1), 12-19. https://doi.org/10.26832/24566632.2022.070103.

Datta, S. (2013). Impact of climate change in Indian horticulture—A review. *International Journal of Science, Environment and Technology*, *2*(4), 661-671.

Deuter, P. (2008). Defining the impacts of climate change on horticulture in Australia, [Report prepared for the Garnaut Climate Change Review, Canberra].

Dull, G. G. (1971).The pineapple: general. In: A. C. Hulme (Ed.). The biochemistry of fruits and their products, Academic Press, New York. 2, 303-324.

Erdal, İ., & Aktaş, H. (2025). Comparison of the perlite, leonardite, vermicompost and peat moss and their combinations with cocopeat as tomato growing media. *Journal of Soil Science and Plant Nutrition*, 1-16. https://doi.org/10.1007/s42729-025-02294-2.

Gebisa, L. A. (2021). Determination of appropriate compost based nursery media preparation for pineapple (*Annanas comosus* MERR L.) seedling growth at South Ethiopia. *International Journal of Bioorganic Chemistry*, *6*(2), 14-20. https://doi.org/ 10.11648/j.ijbc.20210602.11.

Hung, N. Q., Ha, L. T. M., Lien, D. T., Nga, N. T. T., & Lam, V. P. (2024). Optimal shoot mass for propagation to increase the yield and quality of pineapple. *Sustainability*, *16*(13), 5729. https://doi.org/10.3390/su16135729.

Ilahi, W., & Ahmad, D. (2017). A study on the physical and hydraulic characteristics of cocopeat perlite mixture as a growing media in containerized plant production. *Sains Malaysiana*, *46*(6), 975-980. https://doi.org/10.17576/JSM-2017-4606-17.

Intergovernmental Panel on Climate Change (IPCC). (2014). Climate change 2014: synthesis report. In: Core Writing Team, Pachauri, R. K., Meyer, L. A. (Eds.). Contribution of working groups I, II and III to the fifth assessment report of the Intergovernmental Panel on Climate Change (pp. 151). Geneva, Switzerland: IPCC.

Jackson, D., Williams, I., Newby, D., Hall, S., Higgins, S., Francis, R., & Smith, A. (2016). Tissue cultured versus traditionally grown pineapples: Growth and nutrient profile. *Journal of Biotechnology & Biomaterials*, *6*, 1-5. https://doi.org/10.4172/2155-952X.1000237.

Lakho, M. A., Jatoi, M. A., Solangi, N., Abul-Soad, A. A., Qazi, M. A., & Abdi, G. (2023). Optimizing in vitro nutrient and ex vitro soil mediums-driven responses for multiplication, rooting, and acclimatization of pineapple. *Scientific Reports*, *13*(1), 1275. https://doi.org/10.1038/s41598-023-28359-9.

Madhavi, B., Khan, F., Bhujel, A., Jaihuni, M., Kim, N., Moon, B., & Kim, H. (2021). Influence of different growing media on the growth and development of strawberry plants. *Heliyon*, 7(6). https://doi.org/10.1016/j.heliyon.2021.e07170.

Manja, K., & Aoun, M. (2019). The use of nets for tree fruit crops and their impact on the production: A review. *Scientia Horticulturae*, *246*, 110-122. https://doi.org/10.1016/j.scienta.2018.10.050.

Matos Viegas, I. J., Paes da Silva, R. N., Seabra Silva, D. A., De Oliveira Neto, C. F., Oliveira da Conceição, H. E., Mascarenhas, G. S., Okumura, R. S., Monfort, L. E. F. & Lima da Silva, R. T. (2014). Mineral composition and visual symptoms of nutrients deficiencies in Curaua plants (*Ananas comosus* var. erectifolius). *Australian Journal of Crop Science*, *8*(5), 747-753.

Mugambwa, E. K. (2014). Effects of climatic variability on pineapple growing in Uganda: A case study of pineapple growers in Kangulumira sub-county, Kayunga District (LAP Lambert Academic Publishing). OmniScriptum GmbH & Co. KG.

Nakasone, H. Y., & Paull, R. E. (1998). Tropical fruits. CAB Publishing.

Reinhardt, D. H. (2001). Clima. In D. H. Reinhardt, L. F. da S. Souza, J. R. S. Cabral (Eds.), Abacaxi irrigado em condições semi-áridas (p. 11). Embrapa Mandioca e Fruticultura.

Rezaei Nejad, A., & Ismaili, A. (2014). Changes in growth, essential oil yield and composition of geranium (*Pelargonium graveolens* L.) as affected by growing media. *Journal of the Science of Food and Agriculture*, *94*(5), 905-910. https://doi.org/10.1002/jsfa.6334.

Sabahelkhier, K. M., Hussain, A. S., & Ishag, K. E. A. (2010). Effect of maturity stage on protein fractionation, in vitro protein digestibility and anti-nutrition factors in pineapple (*Ananas comosus*) fruit grown in Southern Sudan. *African Journal of Food Science*, 4(8), 550 -552.

Santos, M. D. S., Bomfim, G. V. D., Azevedo, B. M. D., Carvalho, A. C. P. P. D., & Fernandes, C. N. V. (2020). The production of ornamental pineapple in pots under different drip-irrigation depths. *Revista Ceres*, *67*, 111-118.

Schmilewski, G. (2008). The role of peat in assuring the quality of growing media. *Mires & Peat*, 3, 1-8. Singh, S. P. (1995). Commercial fruits. Kalyani publishers.

Umi, H. N., Tricahya, R. A., & Farid, A. M. (2020). Performance analysis of drip and sprinkler irrigation on pineapple cultivation. *In IOP Conference Series: Earth and Environmental Science*, *451*(1), 012034. IOP Publishing.

World Population Review. (n.d.). (2023). Pineapple production by country. Retrieved October 26, from https://worldpopulationreview.com/country-rankings/pineapple-production-by-country.

Yonesi, S., Hemmati, K., Moradi, P., & Khorasaninejad, S. (2025). Evaluation of hydroponic growth media on phytochemical performances of two basil genotypes. *International Journal of Horticultural Science and Technology*, 191-206. https://doi.org/10.22059/IJHST.2024.373388.793.

JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH
2025, VOL. 8(4), 507-520

Journal of Horticulture and Postharvest Research

Journal homepage: www.jhpr.birjand.ac.ir

University of Birjand

The optimal storage temperature for Ngoc Linh ginseng (*Panax Vietnamensis* Ha et Gush.)

Nhi Yen Dinh[1, 2], Da Uyen Tran Dao[3], Loan Thi Thanh Cao[4], Phu Hong Le [1,2] and Diep Thi Ngoc Tran[1,2,5,*]

1, School of Biotechnology, International University, Ho Chi Minh City, Vietnam; 2, Vietnam National University, Ho Chi Minh City, Vietnam; 3, Research Institute for Biotechnology and Environment, Nong Lam University, Ho Chi Minh City, Vietnam; 4, Faculty of Biological Sciences, Nong Lam University, Ho Chi Minh City, Vietnam; 5, Center for Innovation and Technology Transfer, International University, Ho Chi Minh City, Vietnam.

ARTICLE INFO

Original Article

Article history:
Received 1 January 2025
Revised 2 March 2025
Accepted 2 April 2025

Keywords:
Cold storage
Phytochemicals
Sensory evaluation
Shelf life

DOI: 10.22077/jhpr.2025.8570.1461
P-ISSN: 2588-4883
E-ISSN: 2588-6169

***Corresponding author:**
School of Biotechnology, International University, Ho Chi Minh City, Vietnam.

Email: ttndiep@hcmiu.edu.vn

ABSTRACT

Purpose: This study aimed to determine optimal storage conditions for preserving ten-year-old fresh Ngoc Linh ginseng (*Panax vietnamensis* Ha et Grushv.), a highly valued medicinal plant grown in the Ngoc Linh mountain region of Vietnam. Effective postharvest storage methods are important to maintain Ngoc Linh ginseng's quality and phytochemical integrity. **Research Method:** Ethylene production and respiration rates of Ngoc Linh ginseng were evaluated, followed by a preliminary investigation in the dry season to understand the impact of temperature on its quality and shelf life. Finally, subsequent experiments were implemented in both seasons to determine the optimal storage temperature. **Findings:** Ethylene production and respiration rates of the Ngoc Linh ginseng were consistently low in both the dry and rainy seasons. The ginseng experienced severe dehydration and fungal decay at room temperature, while storage at 0 °C led to chilling injuries. Higher temperatures of 10 °C and 15 °C accelerated the deterioration of the ginseng. In contrast, storage at 3 °C and 6 °C significantly extended the ginseng's shelf life. A follow-up experiment confirmed that 3 °C was the most effective for retaining freshness, skin brightness, visual sensory attributes, and total saponin content in Ngoc Linh ginseng in both seasons over 35 days. **Research limitations:** No limitations were identified. **Originality/Value:** This is the first study on extending the fresh storage of Ngoc Linh ginseng, a highly valuable herb of Vietnam. Identifying 3 °C as the optimal storage temperature provides a potential standard for fresh ginseng preservation and supports future research and commercial applications.

INTRODUCTION

Ngoc Linh ginseng (*Panax vietnamensis* Ha et Grushv.), an herbaceous perennial plant, was initially discovered in 1975 Ngoc Linh mountain of Vietnam, ranging from 14° 44' to 15° 13' latitude north and from 107° 45' to 108° 10' longitude east (Duc et al., 1996; Vu-Huynh et al., 2020). Ginseng contains numerous bioactive compounds standing out for their complex pharmacological properties attributed to their steroid-like structure (Ratan et al., 2021; Titova et al., 2024). Recent studies on Ngoc Linh ginseng have unveiled 52 types of distinct ginsenosides (Nguyen & Phuong, 2021; Tien et al., 2021). Ginsenosides might provide anticancer, immune-boosting, anti-inflammatory, antiallergenic, antiatherosclerosis, blood pressure-regulating, stress-relieving, and blood sugar-lowering effects, and positive impacts on metabolism and the central nervous system (Christensen, 2009; Tien et al., 2021), although clinical data are lacking.

Postharvest technology for Ngoc Linh ginseng has not been investigated. In the cultivation regions of Kon Tum and Quang Nam provinces, traditional methods involve drying Ngoc Linh ginseng to prevent spoilage. While drying extends shelf life and facilitates processing and commercialization, it also reduces water content, potentially changing the morphological, physiological, biochemical, and sensory qualities of the ginseng. This emphasizes the importance of developing postharvest methods that extend shelf life while maintaining product quality.

The lack of published literature providing guidelines for the fresh preservation of Ngoc Linh ginseng urges the need for research in this area. Critical physiological factors influencing postharvest quality and extending the shelf life of horticulture crops are respiration and ethylene rates (Dhall & Dhall, 2013; Wills & Golding, 2016; Kahramanoglu, 2023; Ali et al., 2024). Ethylene is a plant hormone central to the ripening and senescence of many fruits and vegetables (Saltveit, 1999; Pech et al., 2012; Kandasamy, 2022). However, ginseng is not a high ethylene-producing crop (Park et al., 2013), thus its physiological response to ethylene exposure might differ from climacteric fruits. Research on postharvest ethylene management in ginseng has shown that treatments such as 1-methylcyclopropene (1-MCP) effectively delay senescence and preserve freshness (Park et al., 2013). Similarly, using ethylene absorbents in packaging resulted in reducing sprouting and rotting in fresh ginger rhizomes while preserving sensory quality (Chung et al., 2010). Respiration has a significant impact on shelf life of harvested crops by determining the rate at which stored carbohydrates are converted into energy (Kahramanoglu, 2023; Ali et al., 2024). Since the respiration rate is affected by temperature, lowering the temperature reduces metabolic activity, respiration rate, and microbial growth, thereby extending shelf life and preventing spoilage (Eriko et al., 2001; Kandasamy, 2022). However, cold storage may result in chilling injuries, while higher temperatures accelerate the rate of spoilage in perishable commodities (Wills & Scott, 1971). Optimal temperature management is therefore essential to maintain postharvest quality.

The significance of temperature regulation in postharvest quality preservation has been extensively studied in various horticultural commodities. Research demonstrates that maintaining optimal low temperatures slows deterioration and extends shelf life (Mahangade et al., 2000). For instance, the optimal storage temperatures for Korean and American ginseng have been identified as 0 °C (Hu et al., 2014), 2 °C (Jeon & Lee, 1999; Gao et al., 2019), 4 °C (Jin et al., 2016), and 10 °C (Whang et al., 2008). Effective regulation of temperature and humidity is vital for maintaining the quality and prolonging the shelf life of perishable crops. While controlling humidity helps prevent moisture loss, temperature is the key factor due to its significant impact on metabolism, respiration, and microbial activity (Eriko et al., 2001; Ambuko et al., 2018; Cheng et al., 2023; Wu et al., 2024). Simultaneously conducting experiments with multiple treatment factors would have required substantial quantities of Ngoc Linh ginseng, specialized equipment, and extensive analytical time. Therefore, determination of the optimal storage temperature was prioritized before implementing additional preservation methods, such as modified atmosphere packaging or controlled atmosphere storage.

In this study, the respiration rates, reflected by CO_2 production, and ethylene production rates of Ngoc Linh ginseng harvested in the dry season was first measured to evaluate its metabolic activity. Then, the effects of various storage temperatures, based on the studies on Korean and American ginseng was investigated. The study examined how low temperatures influence key quality parameters of Ngoc Linh ginseng, including skin color, freshness, fresh weight, and contents of phytochemicals. These preservation methods are critical for ensuring the quality and shelf life of Ngoc Linh ginseng, thereby supporting its value as a medicinal herb in Vietnam.

MATERIALS AND METHODS

Materials

Ngoc Linh ginseng materials

Ten-year-old Ngoc Linh ginseng, supplied by Dak To Forestry Co., Ltd in Kon Tum province, Vietnam, was cultivated on Ngoc Linh mountain (14°59'16" - 14°59'39" N, 107°54'25" - 107°54'52" E). The ginseng showed approximately 10 scars on the rhizome indicating its age, appearing fresh with green stems. Fresh weight correlated positively with diameter but not length, with rainy-season ginseng exhibiting higher fresh weight and larger middle rhizome diameters (Fig. 1). Harvested ginseng was packed in moistened, ice-lined boxes (~12 °C, ~90% RH) to prevent damage during transport to Ho Chi Minh city. At the laboratory, the ginseng was cleaned, dried with cotton linen, and prepared for experiments.

Chemicals

All chemicals used were of analytical grade and bought from the following companies: vanillin, Folin–Denis, 2,2-diphenyl-1-picrylhydrazyl-hydrate (DPPH), oleanolic acid, gallic acid from Sigma Aldrich (Germany); L-Ascorbic acid from Biobasic (Canada); sodium carbonate (Na_2CO_3), sodium chloride (NaCl), sulfuric acid (H_2SO_4, ACS reagent, 97%), ethanol (EtOH, 99.8%), methanol (MeOH, 99.9%), and acetic acid (CH_3COOH) by Xilong Chemical Co., Ltd (China). Deionized water was produced by automatic water stills (Aquatron, A4000D, UK) at The International University.

The equipment used for the experiments were F-950 three gas analyzer (Felix Instrument Applied Food Science, USA), three Aqua 90- liter AQR-D99FA BS fridges (Aqua, Vietnam), and 50-L SR-5KR fridge (Sanyo, Vietnam), the Elitech Temperature Data Logger - Version 6.2.0 (Elitech, USA), the JZ-300 Universal Color Meter (Shenzhen King Well Instrument, China), UV-Vis spectrophotometer (UVD-3500, Labomed, Los Angeles, CA, USA), a water bath shaker (MaXturdy 18 Daihan, Korea), a rotary evaporator (STRIKE 300), miller (IKA A11 Basic), a freeze dryer (FreeZone 6 Liter Benchtop System, Labconco Corporation, USA). They are in the Food Technology Lab and Biomedical Engineering Lab at The International University.

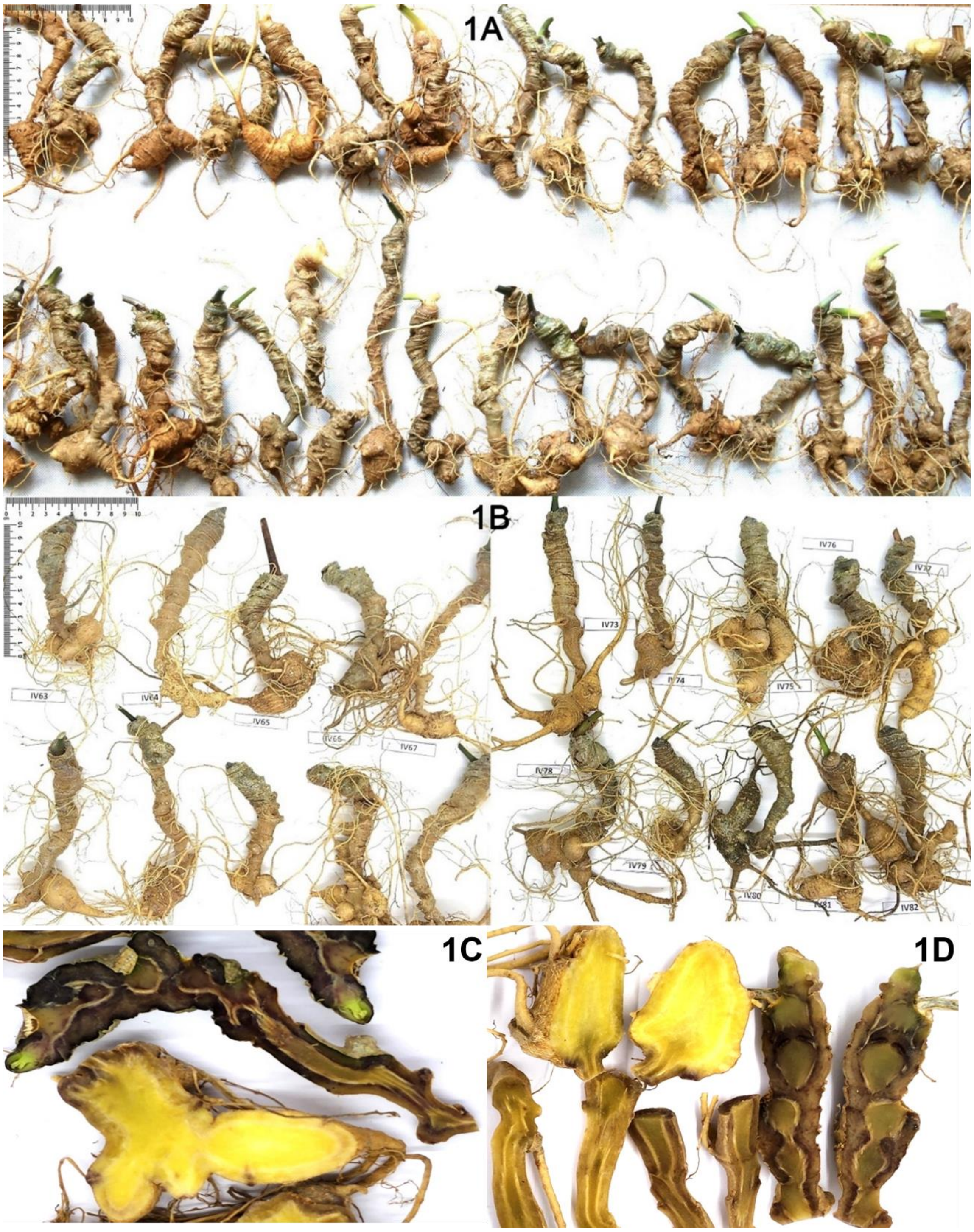


Fig. 1. Ngoc Linh ginseng one day after harvest in the dry season (1A) and in the rainy season (1B), used as materials for the experiments, has an average fresh weight, length, and middle rhizome diameter of 36.67 ± 7.9 g, 161.25 ± 24.33 mm, and 59.53 ± 9.16 mm (n = 32) in the dry season; and 43.93 ± 13.5 g, 164.08 ± 15.2 mm, and 68.58 ± 9.83 mm (n = 12) in the rainy season. The cross-sections of dry-season (1C) and rainy-season (1D) samples show the initial internal appearance.

Table 1. Temperature and relative humidity (RH) measurements at target temperature treatments.

Temperature (°C)	Replicate 1[a]		Replicate 2[a]		Replicate 3[a]		Average	
	T (°C)	RH (%)	T (°C)	RH (%)	T (°C)	RH (%)	T (°C)	RH (%)
0	0.03 ± 0.4^{a}	23.01 ± 5.4^{a}	0.02 ± 0.4^{a}	23.72 ± 5.0^{a}	0.01 ± 0.4^{a}	23.78 ± 4.4^{a}	0.02 ± 0.4^{a}	23.5 ± 4.9^{a}
3	2.9 ± 0.4^{b}	28.6 ± 3.8^{b}	2.9 ± 0.4^{b}	29.0 ± 5.6^{b}	3.1 ± 0.5^{b}	29.2 ± 4.5^{b}	3.0 ± 0.4^{b}	28.9 ± 4.7^{b}
6	5.9 ± 0.6^{c}	37.2 ± 4.3^{c}	5.9 ± 0.7^{c}	37.9 ± 6.4^{c}	5.8 ± 0.5^{c}	38.8 ± 4.5^{c}	5.9 ± 0.6^{c}	38.0 ± 5.2^{c}
10	9.8 ± 0.7^{d}	41.7 ± 10.8^{d}	9.9 ± 0.7^{d}	40.01 ± 5.1^{d}	9.8 ± 0.7^{d}	41.9 ± 6.6^{d}	9.8 ± 0.7^{d}	41.2 ± 7.9^{d}
15	15.4 ± 0.4^{e}	46.2 ± 7.8^{e}	15.5 ± 0.5^{e}	47.5 ± 6.1^{e}	15.5 ± 0.5^{e}	47.9 ± 2.4^{e}	15.4 ± 0.5^{e}	47.2 ± 5.9^{e}
RT	29.8 ± 0.8^{f}	74.4 ± 4.8^{f}	29.8 ± 0.7^{f}	73.0 ± 6.3^{f}	30.0 ± 0.9^{f}	73.8 ± 2.9^{f}	29.9 ± 0.8^{f}	73.7 ± 4.9^{f}

[a] Within three replicates, no statistical differences (P<0.05) were observed in any of the evaluated temperature and relative humidity values according to The ordinary one-way ANOVA test. The results of each replicate were expressed at the mean and standard deviation of 150 – 200 times of measurement (1 hour each). Within each column, means followed by different superscript letters account for statistical differences (P < 0.05).

Methods

Determination of ethylene production and respiration rates

The experiments, adapted from Kandasamy (2022), measured respiration and ethylene evolution rates in Ngoc Linh ginseng using the F-950 Three Gas Analyzer. Three ginseng samples were placed in three separate 2-liter airtight jars while the control consisted of the empty sealed one. The measurements were taken three times a day at 7:00 AM, 12:00 PM, and 7:00 PM, following the F-950 trigger mode. Each experiment was replicated three times for consistency.

The preliminary investigation into the impact of various storage temperatures on the quality of Ngoc Linh ginseng in the dry season

As no prior documentation on the storage temperature for Ngoc Linh ginseng, a preliminary experiment was conducted using Ngoc Linh ginseng harvested during the dry season. The temperature range selected, from 0 to 15 °C, was based on published data for ginseng varieties (Jeon & Lee, 1999; Whang et al., 2008; Hu et al., 2014; Jin et al., 2016; Gao et al., 2019). The ginseng samples were stored in 50-L SR-5KR fridge set at 15°C and in Aqua 90 – L refrigerators at 10 °C, 6 °C, 3 °C, and 0 °C for which the corresponding relative humidity levels were maintained at about 47.2%, 41.2%, 38%, 28.9%, and 23.5%, respectively (Table 1). The Elitech Temperature Data Loggers - Version 6.2.0 were utilized to monitor and adjust the level of each refrigerator to achieve the desired temperatures. Weight loss, skin brightness, and visual sensory attributes of the ginseng samples were weekly recorded over a 28-day period.

Determination of the optimal storage temperature for Ngoc Linh ginseng in both seasons

Ngoc Linh ginseng, obtained from both the dry and rainy seasons, was subjected to storage in refrigerators at 3 °C and 6 °C, as identified from the above experiment. The control treatment involved storing the ginseng at room temperature (~30 °C, ~73.7% RH) (Table 1). The measurement of the fresh weight loss, the visual sensory evaluation, and total phytochemical components, including the total saponin content (TSC), the total polyphenol content (TPC), and the total antioxidant capacity (TAC) were conducted on Ngoc Linh ginseng stored at 3 °C, 6 °C, and room temperature after 7, 14, and 35 days of storage.

The loss rate of fresh weight in Ngoc Linh ginseng was recorded using an electronic scale, while sensory evaluation values, reflecting overall visual quality, were rated on a 1 to 9 scale, assessing freshness and smoothness of rhizome skin, stem and root. Color values (L, a, b) of the three parts of ginseng's rhizome (the top, the middle, and the base), were measured using a colorimeter. Methods for determining total phytochemical components (TSC, TPC, and TAC) and calculating quality parameters are detailed in our previous publication (Nguyen et al., 2023). The experiment was replicated four times.

All the experiments will be performed in replicates and listed details for each method. The statistical analysis was performed, and graphs were drawn using GraphPad Prism 9.5. The results are reported as mean ± standard deviation, and a P-value of <0.05 was considered statistically significant.

RESULTS AND DISCUSSIONS

Ethylene production and respiration rates of Ngoc Linh ginsengs

The ethylene production rate of Ngoc Linh ginseng one day after harvest was consistently low, ranging from 0.03 to 0.07 mg·kg·h^{-1}, with no significant variations observed at specific times of the day or between harvests during the dry and rainy seasons (Fig. 2). There has been limited published research on ethylene production in ginseng with the exception of one study on four-year-old fresh Korean ginseng (*Panax ginseng* C.A. Meyer), which found that ethylene production one day after harvest remained relatively low and stable, ranging from 0.3 to 0.6 mL kg.h^{-1} (approximately 0.34 mg kg.h^{-1} to 0.69 mg kg.h^{-1}) (Park et al., 2013). Compared to high ethylene-producing fruits like apples (*Malus domestica*) or tomatoes (*Solanum lycopersicum*) (Dhall & Dhall, 2013; Keller et al., 2013), Ngoc Linh ginseng exhibits minimal ethylene activity, suggesting that its postharvest quality may depend more on other storage factors.

Similarly, the respiration rate of Ngoc Linh ginseng, measured as CO_2 production, ranged from 24.6 to 34.8 mg CO_2·kg.h^{-1}, with no significant differences between seasons. Lower respiration rates in the early morning and late afternoon compared to midday were observed (Fig. 2). These findings are consistent with the respiration rates of other ginseng varieties. In particular, American ginseng had a respiration rate of around 23.4 mL kg.h^{-1}, equivalent to approximately 42.1 mg kg.h^{-1} (Jeon & Lee, 1999). Ngoc Linh ginseng's respiration rate, being lower than those of fruits and vegetables like tomatoes 120 mg·kg^{-1}·h^{-1}; (Kandasamy, 2022), reflects its inherently slower metabolic activity post-harvest. This characteristic could be advantageous for extended storage for Ngoc Linh ginseng, as slower metabolic activity generally leads to reduced deterioration rates in harvested crops (Eriko et al., 2001; Kandasamy, 2022; Kahramanoglu, 2023; Ali et al., 2024).

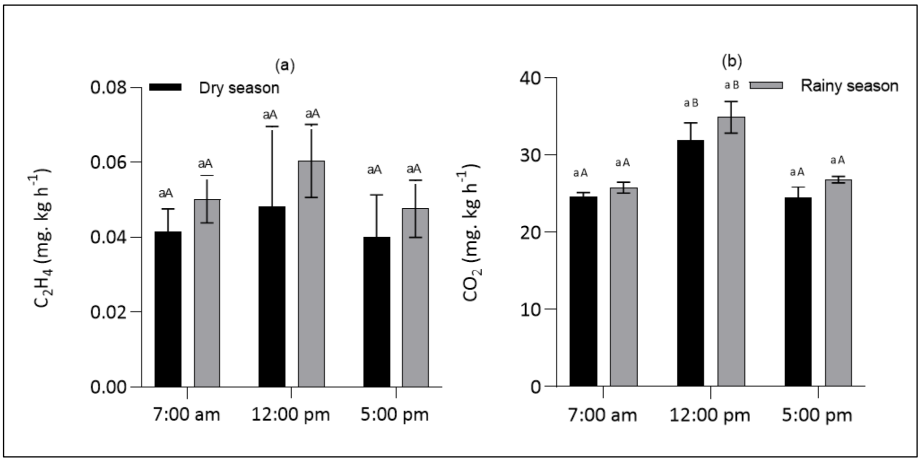


Fig. 2. Changes in the ethylene production rate (a), the respiration rate as CO_2 production (b) of Ngoc Linh ginseng harvested in the dry and the rainy seasons and assessed at specific times of one day after harvest. At each sampling time, means followed by the same letter within a season treatment are not significantly different ($P < 0.05$, n=3).

Preliminary investigation of the effect of different storage temperatures on the quality of Ngoc Linh ginseng in the dry season

The initial color values (L, a, and b) of the three parts of Ngoc Linh ginseng's rhizome (the top, the middle, and the base) were recorded to assess visual quality. Through observation and analysis, the a and b values of Ngoc Linh ginseng remained unchanged significantly across all conditions (data not shown). Therefore, the average L values of the three parts of ginseng's rhizome showing the brightness of Ngoc Linh ginseng's skin was the focus for analysis and depicted in Table 2. Initially, the L values averaged 46.9, representing a fresh appearance. By day 28, brightness had diminished at all storage temperatures and shown no significant differences among temperatures (Table 2).

Fresh weight loss rate increased steadily over the 28 days. Storage at 3 °C and 6 °C mitigated weight loss (13.8% and 19.1%, respectively) compared to higher temperatures (10 °C and 15 °C) and the lowest one (0 °C), which ranged from 25% to 28% (Table 2). Sensory evaluations corroborated these findings, as shown in Table 2. Specifically, ginseng stored at 3 °C and 6 °C maintained high scores, exhibiting minimal wrinkling and visual defects. In contrast, samples stored at 0 °C with the lowest score exhibited dryness, shriveling, and skin darkening (Fig. 3G). Lower storage temperatures typically decrease metabolic activity (Silip et al., 2022; Wu et al., 2024), but excessively low temperatures can cause chilling injury, as observed at 0 °C, emphasizing the sensitivity of Ngoc Linh ginseng (Fig. 3G). Strategies to extend the shelf life of horticultural crops often involve manipulating storage conditions to slow metabolism. Controlling temperature levels can significantly impact respiration rates, extending crops' post-harvest shelf life (Watkins, 2007; Kahramanoglu, 2023; Ali et al., 2024). For instance, fresh Korean ginseng (*Panax ginseng* C.A. Meyer) exhibited reduced respiration rates and ethylene production when stored at 4 °C (Park et al., 2013).

Determination of the optimal storage temperature for Ngoc Linh ginseng in both seasons

Room temperature storage (~30 °C) caused rapid quality deterioration for Ngoc Linh ginseng harvested in both seasons. By day 7, significant dehydration and fungal growth were observed, leading to complete shriveling and flesh rot by day 14 (Fig. 3A and 3B). In contrast, ginseng stored at 6 °C remained fresh for up to 14 days, with only minor stem browning. By day 35, moderate shriveling occurred in the top and middle rhizome sections, while the base remained relatively fresh (Fig. 3C and 3D). Storage at 3 °C yielded the best results. After 14 days, ginseng across all rhizome parts remained fresh, with green stems and no fungal symptoms. Even by day 35, minor shriveling was limited to the top and middle sections, while the base retained its firmness and closely resembled freshly harvested ginseng (Fig. 3E and 3F). This superior preservation at 3 °C is attributed to the suppression of metabolic activity without causing significant quality loss.

Fresh weight loss patterns of the samples supported these observations. Room temperature storage resulted in rapid weight loss, reaching 21.5% by day 14 and 37.2% by day 35 (Fig. 4). In comparison, ginseng stored at 3 °C and 6 °C exhibited significantly lower weight loss rates of 14.3% and 23.1%, respectively, underscoring the effectiveness of cold storage in minimizing moisture loss and maintaining product integrity. Skin brightness (L values) also declined over time but was better preserved at lower temperatures. By day 35, room temperature storage led to a 63% reduction in brightness, while samples stored at 6 °C and 3 °C showed reductions of only 16% and 5%, respectively (Fig. 4). Sensory evaluation scores aligned with these findings; with ginseng stored at 3 °C achieving the highest scores (7 to 8 points) after 14 days, compared to 3 points for room temperature storage. Even after 35 days, sensory scores for ginseng stored at 3 °C remained acceptable (6 points), whereas scores for 6 °C and room temperature dropped to 3 and 2, respectively.

Fig. 3. Skin and flesh changes in Ngoc Linh ginseng harvested in the dry (A, C, E, G) and rainy (B, D, F) seasons and stored at different temperatures: room temperature (~30 °C) for 14 days (3A and 3B); 6 °C for 35 days (3C and 3D); 3 °C for 35 days (3E and 3F); and Ngoc Linh ginseng harvested in the dry season and stored at 0 °C for 28 days (3G). The initial appearance of all ginseng samples was shown in **Figure 1**.

Prolonged exposure to low humidity exacerbates issues such as dry rhizome skin and tissue damage (Couey, 1982; Marangoni et al., 1996). Storage at 3 °C and 6 °C significantly reduced moisture loss and maintained skin brightness and visual sensory quality. These findings align with studies on Korean and American ginseng, which also demonstrate optimal storage between 3 °C and 6 °C (Jeon & Lee, 1999; Park et al., 2013; Hu et al., 2014; Jin et al., 2016; Gao et al., 2019).

Phytochemical components, including total saponin content (TSC), total polyphenol content (TPC), and total antioxidant capacity (TAC), were analyzed to assess the impact of storage temperatures on ginseng's medicinal value. Initially, the TSC of Ngoc Linh ginseng was approximately 168.45 mg/g dry weight (dw). Over 35 days, TSC decreased significantly at room temperature (51% reduction), while losses at 6 °C and 3 °C were limited to 24.2% and 18.8%, respectively (Fig. 5). Similar trends were observed for TPC, which decreased by 42.1%, 25.4%, and 22.7% at room temperature, 6 °C, and 3 °C, respectively. Although TAC decreased across all treatments, reductions were less observed at 3 °C (63.1%) compared to room temperature (68.5%) (Fig. 5).

Table 2. Changes in fresh weight loss rate, skin brightness, and visual sensory evaluation of Ngoc Linh ginseng stored at different cold temperatures after 28 days.

Storage temperature (°C)	Weight loss rate (%) [a]	Skin brightness (L)[b]	Sensory evaluation (Score)[c]
15	28.1 ± 1.9[a]	37.0 ±3.1[a]	2.2 ± 1.0[b]
10	26.2 ± 2.5[b]	34.9 ± 0.9[a]	4.2 ± 1.0[ad]
6	19.1 ± 1.4[c]	35.7 ± 2.7[a]	5.4 ± 0.2[a]
3	13.8 ± 3.1[d]	37.0 ± 1.8[a]	6.5 ± 0.2[ac]
0	25.2 ± 2.1[e]	33.4 ± 0.5[a]	3.3 ± 0.6[b]

At each index, means followed by the same letter within a temperature treatment are not significantly different ($P < 0.05$, n=4).
[a] Initial average fresh weight (g) = 37.07g ± 6.2 (n=20)
[b] Initial average skin brightness (L) = 46.9 ± 3 (n=20). L: 0 (dark) – 100 (brightness)
[c] Initial sensory average skin brightness expressed by the overall visual quality (score) = 8.8 ± 0.2. Score: 9 (excellent), 5 (fair), 1 (extremely poor).

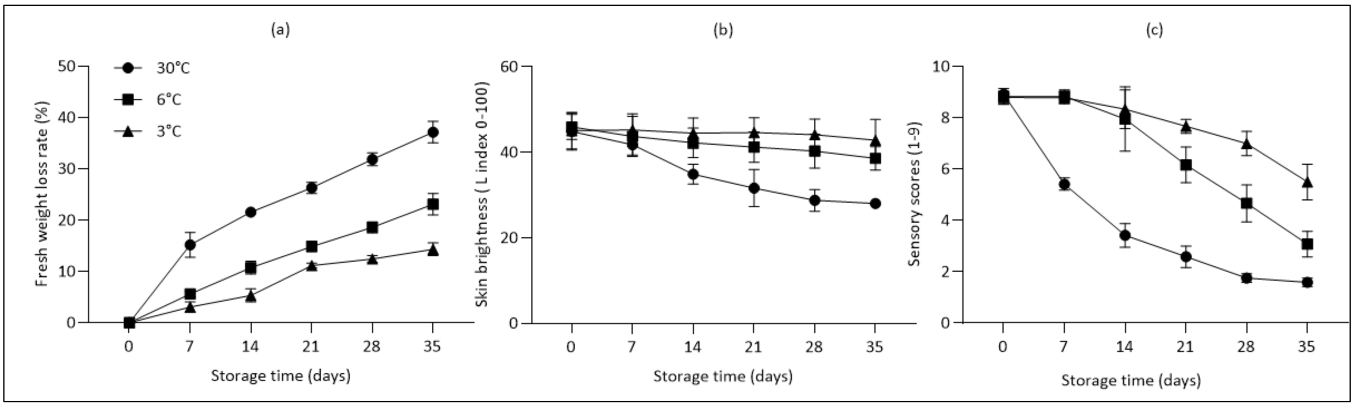


Fig. 4. Changes in fresh weight (a), skin brightness (b), and visual sensory evaluation (c) of Ngoc Linh ginseng stored at 3 °C, 6 °C, and room temperature (~30 °C) (n=8 on the first 28 storage days and n=4 on the day 35[th]).

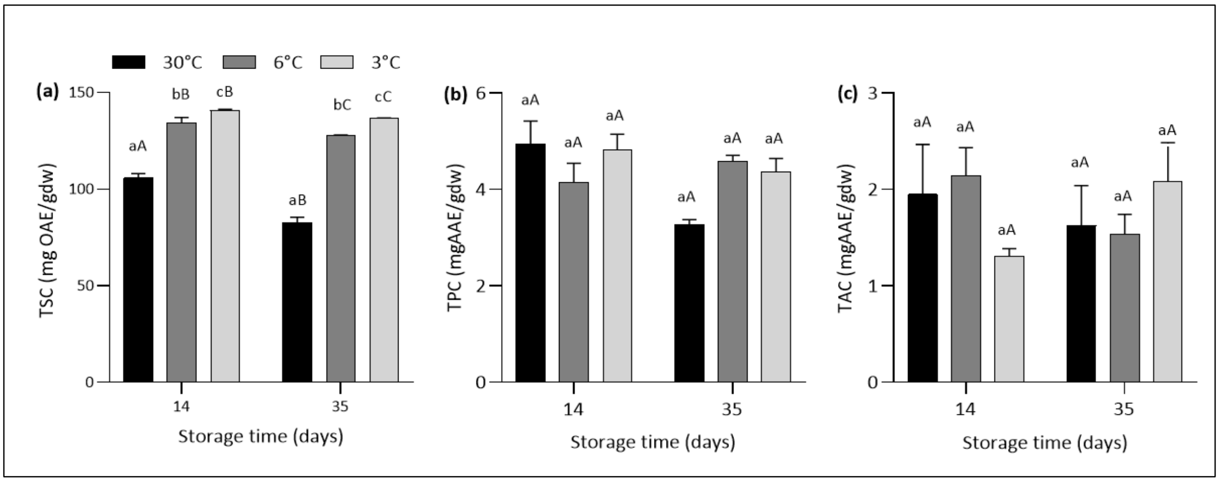


Fig. 5. Changes in total phytochemical components, including the total saponin content (TSC) (a), the total polyphenol content (TPC) (b), and the total antioxidant capacity (TAC) (c) of Ngoc Linh ginseng stored at room temperatures (~30 °C), 6 °C, and 3 °C. The initial values of TSC, TPC, and TAC were 168.45 mg/g dw, 5.66 mg/g dw, and 4.88 mg/g dw, respectively. The small letter shows the statistical difference in temperature factor while the capital letter shows the statistical difference in storage time factor ($P < 0.05$, n =4).

Preservation of phytochemicals at 3 °C is essential for maintaining the therapeutic efficacy of Ngoc Linh ginseng. Saponins and polyphenols contribute significantly to their antioxidant properties, critical for medicinal applications (Nguyen & Phuong, 2021; Tien et al., 2021). These findings are consistent with studies on Korean ginseng, where higher storage temperatures accelerated bioactive compound degradation (Park et al., 2013). The results demonstrate that storage at 3 °C effectively preserves the visual, sensory, and phytochemical qualities of Ngoc Linh ginseng. Compared to room temperature, which leads to rapid quality deterioration, 3 °C minimizes weight loss, maintains skin brightness and retains key bioactive compounds. Similar findings in other medicinal roots reinforce the importance of low-temperature storage (Chung et al., 2010).

CONCLUSION

This study emphasizes the importance of storing Ngoc Linh ginseng at an optimal cold temperature to minimize weight loss, maintain visual sensory quality, and avoid chilling injuries at 0 °C or rapid deterioration at higher temperatures. While humidity also plays a role in mitigating moisture loss, it was not included in this research because temperature regulation was prioritized as the fundamental step before integrating additional preservation methods, in which humidity will be a key focus.

Based on evaluations of phytochemical components, fresh weight loss, skin brightness, and sensory quality in both dry and rainy seasons, 3 °C is recommended as the optimal storage temperature. This conclusion was achieved through a stepwise experimental approach, beginning with a preliminary study, followed by experiments conducted across two harvest seasons, establishing a strong foundation for further postharvest research. However, weight loss averaged 14.3%, and saponin content declined by 18.8% after 35 days, with sensory scores around 6 points, indicating good but suboptimal market quality. Therefore, a combination of low storage temperature at 3 °C with other postharvest technologies, such as high humidity or modified atmosphere packaging, is recommended for future studies to improve the preservation and market readiness of Ngoc Linh ginseng.

Conflict of interest
No potential conflict of interest was reported by the authors.

Acknowledgments
The authors thank the International University and Research Institute for Biotechnology and Environment of Nong Lam University - Ho Chi Minh City for their valuable support in facilities during the accomplishment of this project. The author is deeply grateful to Dr. Randolph Beaudry, Professor in the Department of Horticulture at Michigan State University, for his valuable and insightful comments on the manuscript. Appreciation is also extended to Mr. Hoang Nhat Truong, PhD candidate at Chulalongkorn University, for capturing and editing the photographs of Ngoc Linh ginseng.

This work was supported by The Department of Science and Technology of Kon Tum Province in Vietnam under grant number 09/2020/HD-KHCN.

REFERENCES

Ali, S., Mir, S. A., Dar, B. N., & Ejaz, S. (Eds.). (2024). *Sustainable postharvest technologies for fruits and vegetables*. CRC Press. https://doi.org/10.1201/9781003370376

Ambuko, J., Wanjiru, F., Karithi, E., Hutchinson, M., Chemining'wa, G., Mwachoni, E., Hansen, B., Wasilwa, L., Owino, W., & Nenguwo, N. (2018). Cold chain management in horticultural crops value chains: Options for smallholder farmers in Africa. *Acta Horticulturae*, *1225*, 85–91. https://doi.org/10.17660/ActaHortic.2018.1225.9

Cheng, Y., Gao, C., Luo, S., Yao, Z., Ye, Q., Wan, H., Zhou, G., & Liu, C. (2023). Effects of storage temperature at the early postharvest stage on the firmness, bioactive substances, and amino acid compositions of chili pepper (*Capsicum annuum* L.). *Metabolites*, *13*(7), 820. https://doi.org/10.3390/metabo13070820

Christensen, L. P. (2009). Ginsenosides: Chemistry, biosynthesis, analysis, and potential health effects. *Advances in Food and Nutrition Research*, *55*, 1–99. https://doi.org/10.1016/S1043-4526(08)00401-4

Chung, H. S., Lee, H. J., & Moon, K. (2010). Effects of ethylene absorbent on quality changes of fresh ginger rhizomes during modified atmosphere storage. *Korean Journal of Horticultural Science and Technology*, *28*(1), 82–88. https://koreascience.kr/article/JAKO201010102405064.page

Couey, H. M. (1982). Chilling injury of crops of tropical and subtropical origin. *HortScience*, *17*(2), 162–165. https://doi.org/10.21273/HORTSCI.17.2.162

Dhall, R. K., & Dhall, R. K. (2013). Ethylene in post-harvest quality management of horticultural crops: a review. *Research & Reviews: A Journal of Crop Science and Technology*, *2*(2), 9–25. Retrieved from https://www.researchgate.net/publication/292151893

Duc, N. M., Kasai, R., Ohtani, K., Ito, A., Yamasaki, K., Nham, N. T., & Tanaka, O. (1996). New saponins from Vietnamese ginseng: Highlights on biogenesis of dammarane triterpenoids. In Waller, G. R., & Yamasaki, K. (Eds.), *Saponins used in traditional and modern medicine*. *Advances in Experimental Medicine and Biology* (Vol. 404, pp. 129–149). Springer, Boston, MA. https://doi.org/10.1007/978-1-4899-1367-8_13

Eriko, Y., Keiko, T., Daisuke, H., Wenzhong, H., & Toshitaka, U. (2001, October). Effect of temperature on the respiration rate of some vegetables. *In IFAC Proceedings Volumes*, *34*(28) Tokyo, Japan. (pp. 205–210). Elsevier. https://doi.org/10.1016/s1474-6670(17)32849-5

Fugate, K. K., Suttle, J. C., & Campbell, L. G. (2010). Ethylene production and ethylene effects on respiration rate of postharvest sugarbeet roots. *Postharvest Biology and Technology*, *56*(1), 71–76. https://doi.org/10.1016/j.postharvbio.2009.12.004

Gao, K., Liu, Z., Chen, J., Chen, L., Qi, Y., Wang, Z., & Sun, Y. (2019). Effects of different substrates on low-temperature storage of fresh ginseng. *Journal of the Science of Food and Agriculture*, *99*(14), 6258–6266. https://doi.org/10.1002/jsfa.9899

Hu, W. Z., Jiang, A. L., & Qi, H. P. (2014). Physiological behavior and quality of fresh ginseng stored in modified atmospheres generated by several package films. *Journal of Food Science and Technology, 51*(12), 3862–3869. https://doi.org/10.1007/s13197-012-0922-6

Jeon, B. S., & Lee, C. Y. (1999). Shelf-life extension of American fresh ginseng by controlled atmosphere storage and modified atmosphere packaging. *Journal of Food Science, 64*(2), 328–331. https://doi.org/10.1111/j.1365-2621.1999.tb15893.x

Jin, T. Z., Huang, M., Niemira, B. A., & Cheng, L. (2016). Shelf life extension of fresh ginseng roots using sanitizer washing, edible antimicrobial coating, and modified atmosphere packaging. *International Journal of Food Science and Technology, 51*(9), 2132–2139. https://doi.org/10.1111/ijfs.13201

Kahramanoglu, I. (Ed.). (2023). *Postharvest physiology and handling of horticultural crops* (1st ed.). Boca Raton: CRC Press. https://doi.org/10.1201/9781003452355

Kandasamy, P. (2022). Respiration rate of fruits and vegetables for modified atmosphere packaging: A mathematical approach. *Journal of Postharvest Technology*, *10*(1), 88–102. Retrieved from https://www.researchgate.net/publication/358532606

Keller, N., Ducamp, M. N., Robert, D., & Keller, V. (2013). Ethylene removal and fresh product storage: A challenge at the frontiers of chemistry. Toward an approach by photocatalytic oxidation. *Chemical Reviews*, *113*(7), 5029–5070. https://doi.org/10.1021/cr900398v

Mahangade, P. S., Mani, I., Beaudry, R., Müller, N., & Chopra, S. (2020). Using amaranth as a model plant for evaluating imperfect storages: Assessment of solar-refrigerated and evaporatively-cooled structures in India. *HortScience*, *55*(11), 1759–1765. https://doi.org/10.21273/HORTSCI15249-20

Marangoni, A. G., Palma, T., & Stanley, D. W. (1996). Membrane effects in postharvest physiology. *Postharvest Biology and Technology*, *7*(3), 193–217. https://doi.org/10.1016/0925-5214(95)00042-9

Nguyen, T. H., & Phuong, T. T. (2021). Vietnamese ginseng (*Panax vietnamensis* Ha and Grushv.): Phylogenetic, phytochemical, and pharmacological profiles. *Pharmacognosy Reviews*, *13*(26), 59–62. https://doi.org/10.5530/phrev.2019.2.5

Nguyen, N., Nguyen, T., Le Hong, P., Ta, T. K. H., Phan, B. T., Ngoc, H. N. T., Bich, H. P. T., Yen, N. D., Van, T. V., Nguyen, H. T., & others. (2023). Application of coating chitosan derivatives (N,O–Carboxymethyl chitosan/chitosan oligomer saccharide) in combination with polyvinyl alcohol solutions to preserve fresh Ngoc Linh ginseng quality. *Foods*, *12*(21), 1–22. https://doi.org/10.3390/foods12214012

Park, M. H., Shin, Y. S., Kim, S. J., & Kim, J. G. (2013). Effect of 1-methylcyclopropene treatment on extension of freshness and storage potential of fresh ginseng. *Korean Journal of Horticultural Science and Technology*, *31*(3), 308–316. http://dx.doi.org/10.7235/hort.2013.12212

Pech, J. C., Purgatto, E., Bouzayen, M., & Latché, A. (2012). Ethylene and fruit ripening. In *Annual Plant Reviews Volume 44: The Plant Hormone Ethylene* (McManus M. T. Ed.), Wiley-Blackwell, Oxford, UK, 275–304. https://doi.org/10.1002/9781118223086.ch11

Ratan, Z. A., Haidere, M. F., Hong, Y. H., Park, S. H., Lee, J. O., Lee, J., & Cho, J. Y. (2021). Pharmacological potential of ginseng and its major component ginsenosides. *Journal of Ginseng Research*, *45*(2), 199–210. https://doi.org/10.1016/j.jgr.2020.02.004

Rosenfeld, H. J., Røed Meberg, K., Haffner, K., & Sundell, H. A. (1999). MAP of highbush blueberries: Sensory quality in relation to storage temperature, film type, and initial high oxygen atmosphere. *Postharvest Biology and Technology*, *16*(1), 27–36. https://doi.org/10.1016/S0925-5214(98)00102-1

Saftner, R., Polashock, J., Ehlenfeldt, M., & Vinyard, B. (2008). Instrumental and sensory quality characteristics of blueberry fruit from twelve cultivars. *Postharvest Biology and Technology*, *49*(1), 19–26. https://doi.org/10.1016/j.postharvbio.2008.01.008

Saltveit, M. E. (1999). Effect of ethylene on quality of fresh fruits and vegetables. *Postharvest Biology and Technology*, *15*(3), 279–292. https://doi.org/10.1016/S0925-5214(98)00091-X

Silip, J. J., Supramaniam, J., Mijan, S., Gobilik, J., & Elsabagh, A. S. (2022). The effect of hydro cooling time, storage temperature, and storage duration on Saba banana. *Journal of Physics: Conference Series*, *2314*(1). https://doi.org/10.1088/1742-6596/2314/1/012015

Tien, N. Q. D., Ma, X., Man, L. Q., Chi, D. T. K., Huy, N. X., Nhut, D. T., Rombauts, S., Ut, T., & Loc, N. H. (2021). De novo whole-genome assembly and discovery of genes involved in triterpenoid saponin biosynthesis of Vietnamese ginseng (*Panax vietnamensis* Ha et Grushv.). *Physiology and Molecular Biology of Plants*, *27*, 2215–2229. https://doi.org/10.1007/s12298-021-01076-1

Titova, M. V., Lunkova, M. K., Tyurina, T. M., Prudnikova, O. N., Popova, E. V., Klychnikov, O. I., Metalnikov, P. S., Ikhalaynen, Y. A., Vasileva, E. N., Rodin, I. A., & others. (2024). Suspension cell cultures of *Panax vietnamensis* as a biotechnological source of ginsenosides: Growth, cytology, and ginsenoside profile assessment. *Frontiers in Plant Science*, *15*, 1–15. https://doi.org/10.3389/fpls.2024.1349494

Vu-Huynh, K. L., Nguyen, H. T., van Le, T. H., Ma, C. T., Lee, G. J., Kwon, S. W., Park, J. H., & Nguyen, M. D. (2020). Accumulation of saponins in underground parts of *Panax vietnamensis* at different ages analyzed by HPLC-UV/ELSD. *Molecules*, *25*(13), 3086. https://doi.org/10.3390/molecules25133086

Watkins, C. B. (2007). The effect of 1-MCP on the development of physiological storage disorders in horticultural crops. *Stewart Postharvest Review*, *3*(2), 11. https://doi.org/10.2212/spr.2007.2.11

Whang, J. H., Yu, K. W., Park, S. S., Koh, J. H., Oh, S. H., Suh, H. J., & Lee, S. H. (2008). Prevention of quality changes in the cultured wild ginseng during storage. *Journal of the Korean Society of Food Science and Nutrition*, *37*(10), 1312–1317. https://doi.org/10.3746/jkfn.2008.37.10.1312

Wills, R. B. H., & Golding, J. (Eds.). (2016). *Advances in postharvest fruit and vegetable technology*. Boca Raton, Florida: CRC Press. https://doi.org/10.1201/b18489

Wills, R. B. H., & Scott, K. J. (1971). Chemical induction of low temperature breakdown in apples. *Phytochemistry*, *10*(8), 1783–1785. https://doi.org/10.1016/S0031-9422(00)86438-9

Wu, J., Tang, R., & Fan, K. (2024). Recent advances in postharvest technologies for reducing chilling injury symptoms of fruits and vegetables: A review. *Food Chemistry: X*, *21*, 101080. https://doi.org/10.1016/j.fochx.2023.101080

Zhang, J., Cheng, D., Wang, B., Khan, I., & Ni, Y. (2017). Ethylene control technologies in extending postharvest shelf life of climacteric fruit. *Journal of Agricultural and Food Chemistry*, *65*(34), 7308–7319. https://doi.org/10.1021/acs.jafc.7b02616

JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH
2025, VOL. 8(4), 521-538

Journal of Horticulture and Postharvest Research

Journal homepage: www.jhpr.birjand.ac.ir

University of Birjand

Evaluation of microbiological safety and antioxidant activity of stored seedless barberry fruit from the main production regions of South Khorasan province, Iran

Farid Moradinezhad[1,*], Maryam Dorostkar[1], Razieh Niazmand[2] and Gholamreza Doraki[3]

1, Department of Horticultural Science, Faculty of Agriculture, University of Birjand, Birjand, Iran
2, Department of Food Chemistry, Research Institute of Food Science and Technology (RIFST), Mashhad, Iran
3, Department of Agronomy, Faculty of Agriculture, University of Birjand, Birjand, Iran

ARTICLE INFO

Original Article

Article history:
Received 23 November 2024
Revised 30 May 2025
Accepted 20 June 2025

Keywords:
Antioxidant activity
Coliform
E. coli
Seedless barberry
Yeast/Mold

DOI: 10.22077/jhpr.2025.7955.1402
P-ISSN: 2588-4883
E-ISSN: 2588-6169

***Corresponding author:**
Department of Horticultural Science, Faculty of Agriculture, University of Birjand, Birjand, Iran.

Email: fmoradinezhad@birjand.ac.ir

ABSTRACT

Purpose: The microbial load and nutritional value of products have always been important issues in human nutrition. This research aimed to identify and determine the microbial levels and total antioxidant contents of dry, seedless barberry fruits collected from different regions of South Khorasan Province, which is the main production area in Iran. **Research Method**: Fruit samples were prepared from four regions, including the Birjand region, Zirkoh region, Darmian region, and Qaen region. Chemical traits and microbial analysis were evaluated. The microbial load was determined and compared with the maximum limit (ML) standards. **Findings:** Microbiological analyses of fruits from the Birjand region, Zirkoh region, Darmian region and Qaen region revealed that, fortunately, *E. coli* bacteria were not found in any of the studied regions. However, the highest amounts of total aerobic bacteria (4.60 $\log_{10}$ CFU.g^{-1}) and yeast/mold bacteria (4.17 $\log_{10}$ CFU.g^{-1}) were obtained from fruits prepared from the Darmian region, which was higher than the standard defined by the Food and Drug Organization of Iran. The highest coliform level was related to the fruits of the Darmian region (1.69 $\log_{10}$ CFU.g^{-1}) and the Qaen region (1.69 $\log_{10}$ CFU.g^{-1}), which were lower than those of the MLs. Considering the standards defined with the MLs in Iran, the microbial load regulations in Iran exhibit a higher level of stringency than those in other nations do. Additionally, chemical analyses revealed that the highest amount of total soluble solids and the lowest pH of fruit juice were from the fruits of the Birjand region. The highest amount of titratable acidity and the highest antioxidant activity were related to the fruits of the Qaen region. **Research limitations:** No limitations were found. **Originality/Value:** The nutritional value of barberry fruit is the highest in the Qaen region, followed by the Birjand region. Although the pre- and postharvest stages can affect the microbial load of products, storage conditions during the drying period of seedless barberry fruits play a crucial role in determining the microbial load.

INTRODUCTION

Seedless barberry (*Berberis vulgaris* L.) is an important medicinal and small fruit of the *Berberidaceae* family that is native to Iran (Behrad et al., 2023). Iran is the main seedless barberry fruit producer in the world. Its annual production is more than 22,322 tons of dried berries on approximately 19,220 hectares in Iran. More than 85% of seedless barberry fruits are produced in South Khorasan Province, which is located in East Iran (Javadzadeh, 2013). Most of the consumed seedless barberry is in the form of dried berries. The medicinal properties of barberry fruit, such as anticancer, antimicrobial (Och & Nowak, 2021), reducing morphine addiction and dependence (Sobhani et al., 2021), rehabilitating, hypoglycemic, and antidiabetes (Shidfar et al., 2012), and reducing LDL and total cholesterol (Ardestani et al., 2013), have been investigated.

Fresh and processed fruits, particularly small fruits such as barberries with high nutritional value, play important roles in the growth and health of the human body (Mathur et al., 2014); these products are rich and excellent sources of natural antioxidants that help prevent various diseases, such as cancer and cardiovascular diseases, in our body. Food insecurity, malnutrition, and lifestyle diseases such as obesity, hypertension, carcinogenesis, and diabetes are among the most important global issues that have increased the demand for healthy foods, especially fruits and vegetables (Gogo et al., 2017). It is approximated that up to one-third of the population in developed countries contracts foodborne diseases annually, and this is likely to be even more widespread in developing countries. Presently, individuals are cognizant that adhering to a nutritious diet comprising salads, vegetables, and fruits is crucial for enhancing their quality of life. Nevertheless, it is imperative to acknowledge that the intake of raw fruits and vegetables has the potential to lead to bacterial and infectious ailments owing to the presence of microorganisms such as *Escherichia coli* and Salmonella (Mritunjay & Kumar, 2015). Raw fruits and vegetables may be contaminated with fungi, molds, or various pathogenic microorganisms, such as *Salmonella* spp. and *Escherichia coli* (Alp & Bulantekin, 2021). The postharvest loss of fresh fruits and vegetables in developed countries is approximately 20% due to spoilage. Fruits and vegetables can sometimes be associated with harmful bacteria and viruses known as pathogens. In most developing countries, there is little information about food safety, and there are no strict measures in the production cycle of products (Abdel-Moneim et al., 2014). Factors related to the safety of fruits and vegetables include natural pesticides, contaminants such as chemical waste, heavy metals, and microbial contamination of fresh produce, which is highly susceptible to fungi. Contamination with pathogenic or corrosive microorganisms as a result of contact with soil, wastewater and dust during the pre- and postharvest stages is significant for fruits and vegetables, as it may cause various diseases, such as cramps, diarrhea, and even death (Alp & Bulantekin, 2021).

Fresh, seedless barberry fruit has a high water content (75–80%), and the majority of the produced fruit is dried via different methods to reduce the moisture content and microbial load and extend its storage life (Alavi and Mazloumzadeh, 2012; Moradinezhad et al., 2024). To maintain visual quality and nutritional value and minimize organoleptic changes during the drying process, conditions are kept as mild as possible, which enables many microorganisms to function under these conditions (Morgan et al., 2006; Jayaraman and Das Gupta, 2020). Although microbial growth is partially prevented or delayed in dried products due to severe water loss, when sufficient numbers of pathogenic microorganisms are present after the drying process, it may pose a risk to the consumer (Bourdoux et al., 2016). Although microbial growth is inhibited in the desiccated state, vegetative cells and spores can survive for months (Beuchat et al., 2013). In addition, when dried products are exposed to water during food preparation and absorb water, the growth of viable microorganisms may be enhanced (Bourdoux et al., 2016;

Saad et al., 2021). This leads to rapid spoilage of products and increases the risk to consumer health. However, microbial contaminants occur during both the pre- and postharvest stages (Zhao et al., 2021; El-Araby et al., 2023; Adewoyin, 2023). However, storage conditions are also crucial. One of the main issues in terms of storage conditions during product drying is controlling the temperature and humidity of the storage location (Mongi et al., 2023; Firdous et al., 2023). If optimal storage conditions are provided, drying the products may be the only way to reduce the existing microbiota (Moradinezhad et al., 2019).

The literature shows that little information is available about the microbial load of dried, seedless barberry fruit, as mentioned in a recent study (Bideli et al., 2022). In addition, no information has been found regarding the microbial load and antioxidant activity of seedless barberry fruits cultivated and dried from significant producing regions in Iran. The purpose of this research was therefore to determine the microbial load and chemical properties of four major regions of seedless barberry cultivation in Iran.

MATERIALS AND METHODS

In the present study, the microbial load and several biochemical traits of four major barberry regions in South Khorasan Province, Iran, were compared. Dried, seedless barberry fruit samples were prepared from four regions, including the Birjand region, Zirkoh region, Darmian region, and Qaen region. Fruit were dried traditionally in a shaded house during autumn and winter. The date of barberry collection from different regions ranged from the 1st to the 20th of May 2022. They were then transferred to the Horticulture Laboratory, Faculty of Agriculture, University of Birjand, South Khorasan Province, Iran. The methods used for fruit picking, storage conditions and the monthly average temperature and relative humidity of the shade houses used in all the studied regions are presented in Tables 1 and 2.

Sample preparation

Two kilos of dried, seedless barberry fruit were prepared from each region. Four replicates were prepared from each region, and 500 grams of barberry were used for each replicate. A total of 8 kilos of dried barberry were purchased from prominent producers in the four regions. These regions were considered fully representative. For analysis, berries were sorted, and berries with abnormal color and visible contamination (such as leaves, twigs, and soil) were excluded.

Table 1. The type of fruit picking, height of branches mass, and storage conditions in the four studied regions of South Khorasan province, Iran.

Regions	Fruit picking	Height of fruit branches mass (cm)	Storage conditions
Birjand	With branches	30	One-row metal drying rack, with ventilation
Zirkoh	With branches	40	Double-row wooden drying rack, with ventilation
Darmian	With branches	10	On the cement floor, no ventilation
Qaen	With branches	40	One-row metal drying rack, with ventilation

Table 2. Monthly average of temperature and relative humidity (RH) of shade-house in different regions during 2022-2023.

Regions/Months	November 2022	December 2022	January 2023	February 2023	March 2023	April 2023
	Temperature (°C)					
Birjand	10	9.3	8.2	6.8	15.9	17.3
Zirkoh	7.6	6.8	5.2	5	10.2	13.3
Darmian	11	10.1	8.6	7.3	17.4	18.5
Qaen	9.8	8.4	6.2	5.7	13.5	16.9
	Relative humidity (RH%)					
Birjand	48	52	67	41	32	28
Zirkoh	59	63	58	49	40	33
Darmian	45	49	60	37	28	25
Qaen	52	68	65	52	43	30

Microbiological analyses

The microbiological analyses included total aerobic bacteria (TAB), coliforms, *E. coli* and molds/yeasts from dried, seedless barberry fruit, which were carried out at the Microbiology Laboratory of the Research Institute of Food Science and Technology (RIFST), Mashhad, Iran. Dried fruit samples weighing 25 g were subjected to homogenization for 120 seconds in 1:5 dilutions of 1% sterile peptone water via a stomacher apparatus (HG400 pro stomacher). The use of filtered stomacher bags aimed to eradicate soil particles from the homogenates, and tenfold dilution were subsequently prepared in peptone-buffered water for plating as necessary. Total aerobic bacteria (TAB) were quantified through spread-plating on plate count agar (Merck) and incubated at 30°C for 48 hours; coliforms and *E. coli* on Coli-ID (Biomérieux) were quantified via double layer inclusion and incubation at 37°C for 48 hours; and molds and yeasts on glucose yeast extract agar with a 10 mg/ml solution of oxytetracycline chlorhydrate were assessed via pour plating and incubation at 25°C for five days.

Chemical properties

The chemical properties determined were titratable acidity (TA), total soluble solids (TSS), juice acidity (pH), and total antioxidant activity (TAA).

The method of Talebzadeh et al. (2022) was used to prepare dried barberry extracts. First, 20 grams of dried fruit was weighed and turned into powder through a mortar. Then, 20 ml of water was added, and the mixture was placed in a shaker (orbital shaker made by IKA, Germany - KS260 digital) for 25 minutes at a speed of 50 rpm. The solution was then passed through filter paper. This prepared extract was used to determine the biochemical characteristics of dried barberry fruit.

To evaluate the total soluble solids, a few drops of the prepared extract were poured onto the prism of an optical refractometer (RF 10, Brix, 0–32%, Extech Co., USA). The TSS data are presented in °Brix (Hosseini et al., 2021).

The titratable acidity of the barberries was determined via the titration method with 0.1 standard NaOH and with a desktop pH meter (AZ 86502 model, made in Taiwan). For this purpose, 10 ml of the extract was diluted with 100 ml of distilled water. The solution was then titrated with 0.1% NaOH until it reached pH 8.23. Finally, the results were calculated as a percentage via formula (1) (Saebi et al., 2023).

$$\text{TA (\%)}= \frac{(\text{ml base titrant})\times(\text{N of base in}\frac{\text{mol}}{\text{L}})\times\text{equivalent weight of acid}}{\text{sample volume in ml}} \quad (1)$$

The pH of the fruit extract was measured via a digital desktop pH meter (AZ 86502 model, made in Taiwan) with a measurement accuracy of 0.01.

The ability of the extracts to scavenge the DPPH radical was determined via the method of Blois (1985), with slight modifications. Fifty microliters of fruit extract was added to 2 mL of methanol, and then the DPPH compound (24 µg/mL) was added. The mixture was kept in the dark at room temperature for 60 min, and the absorbance was measured via a spectrophotometer (model Novaspec II; Pharmacia LKB, Uppsala, Sweden) at 517 nm. The percentage of DPPH radical scavenging activity was calculated via the following equation (2):

$$\text{DPPH radical scavenging activity (\%)} = (A_{blank} - A_{sample})/A_{blank} \times 100 \qquad (2)$$

where A_{blank} is the absorbance of the blank (containing all the reagents except the test compound) and A_{sample} is the absorbance of the test compound.

Statistical analysis

Analyses were performed via JMP Statistical Discovery Pro v13.2.1, and Excel Ver. 2019 software was used to draw graphs. Additionally, the comparison of the means was performed via the least significant difference (LSD) test at the 5% probability level. The statistical plan used for this experiment was a completely randomized design (CRD) with four replications.

RESULTS AND DISCUSSION

Microbiological analysis

Table 3 presents the mean levels of total aerobic bacteria (TAB), coliform bacteria, *E. coli* and mold/yeast in dried, seedless barberry fruits selected from four regions.

The mean TAB in the samples obtained from the Darmian region was 4.60±0.37 $\log_{10}$ CFU.g^{-1}, which was the highest TAB level. Although the lowest TAB level was related to the samples from the Birjand region (2.30±0.12 $\log_{10}$ CFU.g^{-1}), it was not significantly different from the samples from the Qaen region (2.47±0.21 $\log_{10}$ CFU.g^{-1}) and Zirkoh region (2.47±0.24 $\log_{10}$ CFU.g^{-1}) ($p<0.05$). The results revealed no *E. coli* bacteria in any of the samples selected from the four regions. According to the data presented in Table 3, the mean level of coliform bacteria in the samples significantly varied from 0.00 $\log_{10}$ CFU.g^{-1} to 1.69 $\log_{10}$ CFU.g^{-1}. The highest overall level of the forms was 1.69 $\log_{10}$ CFU.g^{-1}, which was related to the samples prepared from the Darmian region (1.69±0.11 $\log_{10}$ CFU.g^{-1}) and the Qaen region (1.69±0.12 $\log_{10}$ CFU.g^{-1}). The lowest total form was observed in the samples from the Zirkoh region (0.00), followed by the samples from the Birjand region (1.32±0.10 $\log_{10}$ CFU.g^{-1}), with the lowest level of the overall form. The mean amount of yeast mold in dry, seedless barberry samples from the four regions significantly varied between 1.77 $\log_{10}$ CFU.g^{-1} and 4.17 $\log_{10}$ CFU.g^{-1}. The highest amount was obtained from the samples prepared from the Darmian region (4.17±0.28 $\log_{10}$ CFU.g^{-1}). Additionally, the lowest overall level of forms was related to the Birjand region (1.77±0.13 $\log_{10}$ CFU.g^{-1}), followed by the Zirkoh region (2.30±0.20 $\log_{10}$ CFU.g^{-1}).

Table 3. Total aerobic bacteria, *E. coli*, coliforms, and molds/yeasts isolated from dried seedless barberry fruit selected from different regions of South Khorasan province, Iran.

Regions	Total aerobic bacteria	*E. coli*	Coliform	Yeast/Mold
Birjand	$2.30^{b}\pm0.12$	ND	$1.32^{b}\pm0.10$	$1.77^{c}\pm0.13$
Zirkoh	$2.47^{b}\pm0.24$	ND	ND ^{c}	$2.30^{b}\pm0.20$
Darmian	$4.60^{a}\pm0.37$	ND	$1.69^{a}\pm0.11$	$4.17^{a}\pm0.28$
Qaen	$2.47^{b}\pm0.21$	ND	$1.69^{a}\pm0.12$	$2.60^{b}\pm0.19$

Means ± SEs (n=4) followed by different letters in the same column for the same evaluated parameter are significantly different ($P \leq 0.05$) according to the LSD test. Quantitative ($\log_{10}$ CFU. g^{-1}). ND: not detected. CFU: colony-forming unit.

Plate counting of aerobic mesophilic microorganisms in products is one of the key microbiological tests used to evaluate the quality of products (Aycicek et al., 2006). The number of aerobic mesophilic organisms in products indicates their exposure to pollution and favorable conditions for the proliferation of microorganisms (Alp & Bulantekin, 2021). In other words, the determination of this microbiological parameter helps us to understand whether cleaning, disinfection, and temperature control during processing, transportation and storage of products are sufficient and valuable (Lambert, 1995). According to Iranian standards, the ML of total aerobic bacteria is less than 4.30 $\log_{10}$ CFU.g^{-1} (standard number 5752-1). Therefore, according to Iran's food standards, the food standards of the Birjand region, Zirkoh region and Qaen region are defined as being lower than the ML. However, the results obtained from the samples from the Darmian region were not satisfactory, and the total number of aerobic bacteria was greater than that in the ML. Some researchers have reported that the expiration date of products is generally considered inappropriate when the TAB population reaches 7 $\log_{10}$ CFU.g^{-1} (Gómez-López et al., 2008). In the present study, the TAB level in all dried, seedless barberry fruit samples from different regions was less than 7 $\log_{10}$ CFU.g^{-1}. The total number of aerobic bacteria detected in the present study ranged from 2 $\log_{10}$ CFU.g^{-}1 to 5 $\log_{10}$ CFU.g^{-1}. Similarly, Artimová et al. (2023), in their study on leafy vegetables and berries, reported that the range of the microbial load varied between 5 $\log_{10}$ CFU.g^{-1} and 8 $\log_{10}$ CFU.g^{-1}. Additionally, the total number of aerobic bacteria has been reported to be 2 $\log_{10}$ CFU.g^{-1} to 5 $\log_{10}$ CFU.g^{-}1 (Quansah et al., 2019; Kuźniar et al., 2020). Macori et al. (2018) reported slightly lower values (mean 3.89 $\log_{10}$ CFU.g^{-1}) in berry fruit samples obtained directly from 50 growers and reported the highest microbial load in currants and blueberries. Nizam et al. (2019) investigated the microbiological quality and sensory evaluation of semidried mango for fruit salad. They reported that increasing the percentage of active water in semidried mango fruit slices increased the activity of anaerobic bacteria. The total number of microorganisms in mango slices ranged from 3 $\log_{10}$ CFU.g^{-1} to 6.5 $\log_{10}$ CFU.g^{-1}, which is consistent with the results of the present study (from 2.30 $\log_{10}$ CFU.g^{-1} to 4.60 $\log_{10}$ CFU.g^{-1}).

Escherichia coli (*E. coli*) are a bacterium that is commonly found in the intestines of warm-blooded organisms (Allocati et al., 2013). Most *E. coli* strains are harmless, but some can cause severe food poisoning. Shiga toxin-producing *E. coli* (STEC) is a bacterium that can cause severe foodborne disease (Smith et al., 2014; WHO, 2018). In most cases, the illness is self-limiting, but it may lead to a life-threatening disease, including hemolytic uremic syndrome (HUS), especially in young children and elderly individuals (Smith et al., 2014). The STEC is sensitive to heat. When preparing food at home, the basic principles of food hygiene, such as "cooking thoroughly", must be observed (WHO, 2018). One of the factors that prevent the growth of *Escherichia coli* is the pH of the product. Owing to the presence of acids such as citric acid, malic acid, ascorbic acid, etc., fruits have a pH less than neutral or acidic. It has been reported that *E. coli* bacteria can survive in highly acidic environments and resist and tolerate them (Lu et al., 2011). Brown (1991) reported that *E. coli* secretes carboxylase enzymes preferentially at very acidic pH values. The role of these enzymes is to increase the external pH value and thus induce acid tolerance under certain conditions (Rowbury, 1997). However, Ndjomgoue-Yossa et al. (2022) focused on removing *E. coli* via pH changes. They reported that a slightly acidic pH (5.5) has antibacterial effects and high inactivation efficiency of *E. coli* at this pH. The results of the present study revealed that *E. coli* bacteria were not present in the samples from all four studied regions. Therefore, this was highly likely due to the lower pH range (<3.5) of the fruit samples, which did not allow the growth and proliferation of *E. coli* bacteria in the dried, seedless barberry. Similarly, Hyun et al. (2019), who evaluated the microbial load of dried persimmons during 70 days of storage, reported that *E. coli* was not present in the samples. They reported that *Escherichia coli* cannot be detected when the value is less than 0.48 log_{10} CFU.g^{-1}. Another noteworthy point in the findings of Hyun et al. (2019) was that the different storage temperatures (-20, 5, 12, and 25°C) did not significantly affect the microbial load value of the samples. Under conditions of low acidic pH, undigested weak acids have the ability to freely diffuse through the cytoplasmic membrane. Upon entry into the cytoplasm, these weak acids undergo degradation, thereby increasing the internal pH of *E. coli* (Li et al., 2015; Beales, 2004). Consequently, the capacity to maintain the intracellular pH at a nearly neutral level (pH homeostasis) becomes compromised, resulting in cellular death. As explicated by Wiggins (1975), alterations in pH within the cell can instigate chemical modifications in crucial compounds such as DNA or adenosine triphosphate. Furthermore, oxidative and reductive reactions may transpire within the cellular framework (Gilliland & Speck, 1967), culminating in cellular disintegration and death, hence impeding the growth and persistence of *E. coli* bacteria.

Coliform bacteria are widely found in the natural environment, and the feces of animals and humans do not necessarily cause disease. However, its high levels can indicate the presence of other, more dangerous pathogens. This makes the coliform bacteria test a useful "indicator" for detecting bacterial contamination in products. A simple coliform count test can be a good indication of the safety and hygiene of a product. The results of the present research revealed that all the studied regions presented coliform values less than 2 log_{10} CFU.g^{-1}. According to the National Standard Organization of Iran under numbers 11166 and 9263, the acceptable ML of total forms in products is less than 2 log_{10} CFU.g^{-1}. Therefore, on the basis of the Iranian standard, dried, seedless barberry fruits prepared from different regions have acceptable coliform limits. The mean coliform count in this study was lower than the value reported by Tango et al. (2018). They reported mean coliform counts ranging from 2.2 log_{10} CFU.g^{-1} to 7.9 log_{10} CFU.g^{-1} on apple, mandarin, and cherry tomato fruits. Our results are also acceptable regarding hygiene criteria for vegetables, fruits and products set by the European Union (Tango et al., 2018). In a study on dried persimmon fruit (Hyun et al., 2019), the mean number of total forms during 70 days of storage was 1.92 log_{10} CFU.g^{-1}, which was greater than the coliform concentration recorded in barberries from different regions in our research. In general, dried fruits and vegetables have lower coliform counts than fresh fruits do (Alp & Bulantekin, 2021).

Like bacteria, yeasts are unicellular organisms that are typically larger in size than bacteria. Most yeasts encounter growth inhibition beyond 100°F (Silva et al., 2019). A substantial water supply is usually imperative for the growth of common yeasts; nevertheless, numerous yeasts can thrive in environments with elevated sugar or salt concentrations. In general, yeasts require less water than bacteria do but more water than molds do (Babič et al., 2016). Sugars and acids are good food sources for yeasts. Growth is abundant in food substances containing carbohydrates (sugars or starch) and varying amounts of acid (Stratford, et al., 2019). The growth of most yeast is favored by an acid reaction. Yeasts grow well at a pH of 4–4.5 (Bărbulescu et al., 2022). Their growth on food or other nutrient sources results in a mass of loosely entrained filaments collectively called mycelia. Individual filaments are called hyphae (Padmavathi et al., 2020). According to standard No. 10899-3 (Iranian National Standard Organization) (ISIRI, 2013), the acceptable amount of mold/yeast in products is 3 log10 CFU.g-1. According to this standard, the barberry samples prepared from the Birjand, Qaen, and Zirkoh regions are below the ML. However, the samples from the Darmian region presented more yeast mold (4.17 log10 CFU.g-1) than the ML samples did. However, the results obtained from the counting of coliform bacteria were in accordance with the guidelines of Gilbert et al. (2000), who reported that a count below 5 log_{10} CFU.g^{-1} for coliform bacteria in products is acceptable. However, considering that there is little information about the ML of the microbial load on dried fruits, it is challenging to evaluate and compare the results. Therefore, one can infer that the microbial load regulations in Iran are more stringent than those in other nations.

Victor et al. (2017), in a study on dried fruits and vegetables, reported that the amount of yeast in vegetables is very high compared with that in fruits; for example, the number of molds and yeasts counted in dried spinach leaves is 6.74 log_{10} CFU.g^{-1}. Victor et al. (2017) reported that fruits are more acidic than other fresh produce and that the combination of low pH and low temperature during storage also inhibits yeast/mold growth. The acidic environment during refrigeration (5°C) is the inhibitory factor for the growth of mold and yeasts. The role of yeasts in the spoilage of fresh vegetables has not been well studied, although yeasts have been implicated in the spoilage of fermented vegetable products and the development of soft rot (Fleet, 2003). Ragaert et al. (2006) noted that some volatiles (rotting odor) associated with molds and yeasts were detected when populations were 5.0 log_{10} CFU.g^{-1} or greater. In a study on dried mango, the yeast and mold counts ranged from 4.18 log_{10} CFU.g^{-1} to 6.90 log_{10} CFU.g^{-1} (Nizam et al., 2019).

Microbial contamination in vegetables is attributed to sources such as soil, manure, and water and poor handling and storage after harvest (Halablab et al., 2011). Notably, in dried products, fertilizers, harvesting, drying and storage have a direct effect on increasing or decreasing the microbial load (Kadam et al., 2009; El-Dengawy et al., 2018; Mongi, 2023). In the present research, the highest amounts of mold and yeast in the samples were observed in the Darmian region. The drying and storage facilities in this region were unfavorable. Therefore, the barberry fruit dried on the ground, which probably caused an increase in the microbial load in the samples from this region.

Biochemical properties

An evaluation of the biochemical properties of dried, seedless barberry fruit revealed significant differences in the biochemical traits of the fruit selected from the four regions.

A mean comparison (Table 4) revealed that the Birjand region had the highest content of TSS among the other studied regions (6.33±0.22 °Brix), followed by the Zirkoh region (5.00±0.21 °Brix), the Darmian region (4.00±0.19 °Brix) and the Qaen region (3.31±0.10 °Brix). Additionally, the highest content of TA was obtained from the barberry of the Qaen region (3.10±0.11%), and the lowest value was related to the barberry of the Darmian region (2.12±0.15%). The pH analysis revealed that the Darmian region (3.44±0.24) had the highest pH value among the studied regions. However, there was no statistically significant difference in the pH of dried barberry between the Darmian and Qena regions (3.28±0.20). The lowest pH value was also obtained from the samples from the Birjand (3.05±0.29) and Zirkoh (3.19±0.27) regions.

The highest total antioxidant activity (TAA) in dried seedless barberry was obtained from the samples prepared from the Qaen region (71.03±5.88 mg. g^{-1}), and the lowest amount with a significant difference was related to the barberry from the Darmian region (30.02±3.09 mg. g^{-1}) (Table 4).

Table 4. Total soluble solids (TSS), titratable acidity (TA), pH, and total antioxidant activity (TAA) of dried seedless barberry fruit selected from different regions of South Khorasan Province, Iran.

Regions	TSS (°Brix)	TA (%)	pH	TAA
Birjand	6.33^{a}±0.22	2.54^{b}±0.11	3.05^{c}±0.29	61.19^{b}±4.41
Zirkoh	5.00^{ab}±0.21	2.51^{b}±0.17	3.19^{bc}±0.27	62.16^{b}±3.92
Darmian	4.00^{b}±0.19	2.12^{c}±0.15	3.44^{a}±0.24	30.02^{c}±3.09
Qaen	3.31^{b}±0.10	3.10^{a}±0.11	3.28^{ab}±0.20	71.03^{a}±5.88

Means ± SEs (n=4) followed by different letters in the same column for the same evaluated parameter are significantly different ($P \leq 0.05$) according to the LSD test.

The TSS content of the fruit is usually obtained from assessing the °Brix of the fruit. At the beginning of the ripening process, the sugar/acid ratio is low because of the low sugar content and high fruit acid content, which makes the fruit taste sour. During the ripening process, the fruit acids are degraded, the sugar content increases, and the sugar/acid ratio increases. The results of the present research revealed that the TSS contents in different studied regions differed from each other. The highest amount of TSS was observed in barberries from the Birjand region, and the lowest amount was obtained from samples from the Qaen region. The concentration of soluble solids can vary due to soil composition, weather conditions, fruit yield, and ripeness. In regions of southern Ukraine, superior sweet cherry varieties present soluble solids contents ranging from 12.1--19.9 °Brix, whereas in the central part of the country, this measure ranges from 11.3--12.8 °Brix (Slavin and Lloyd, 2012; Bublyk et al., 2014; Ivanova et al., 2021). Climatic factors have been shown to have a decisive effect on TSS accumulation compared with other factors (such as cultivar) (Sansavini & Lugli, 2008; Serdyuk et al., 2020). As mentioned, weather has a direct effect on TSS accumulation; however, more precisely, among weather factors, the difference between day and night temperatures has a positive effect on the accumulation of dry solids and the sugar content in fruits. However, the amount of rainfall and daily temperature do not significantly affect the accumulation of TSS in fruit (Sheiko et al., 2019; Caprio & Quamme, 2006). The range of data obtained from the TSS of dried, seedless barberry was consistent with those of Balandari et al. (2023) and Moradinezhad et al. (2018).

Titratable acidity indicates the amount of organic acids present in a fruit (Etienne et al., 2013). Titratable acidity represents the organic acids that affect overall fruit quality and flavor. The berry family is rich in organic acids and has useful benefits (Ardestani et al., 2015). The most important organic acids in berries are malic, citric, tartaric, oxalic and fumaric acids (Koyuncu, 2004). In the present research, significant differences in the amount of TA in seedless barberries were detected between the studied regions. The highest amount of TA was obtained from the Qaen region, and the lowest amount was obtained from the Darmian region. Many factors influence the amount of TA in fruits. The literature shows that the plant source–sink ratio, mineral fertilization, water supply, and temperature are the agro-environmental factors that have the greatest impact on fruit acidity (Anthon & Barrett, 2012; Famiani et al., 2015). Orchard management practices such as fruit thinning, plant pruning, or defoliation affect the source–sink ratio of the plant, which usually results in altered sugar supply and fruit growth. Water stress tends to increase the organic acid content and TA content in ripe fruits through a simple dilution/dehydration effect (Lez-Altozano & Castel, 1999). An increase in temperature during fruit growth or storage decreases fruit TA (Wang and Camp, 2000; Gautier et al., 2005). Changes in organic acid metabolism in response to temperature are likely due to the effects of temperature on the reaction rates of glycolysis and the TCA cycle (Araujo et al., 2012). Therefore, the differences in the TA and pH of dried, seedless barberries in the different studied regions are likely due to differences in climate and agricultural operations. In line with the results of the present research, Moradinezhad et al. (2018) reported that barberries prepared from two different regions presented different pH and TA values.

If the fruit juice is acidic, the concentration of hydrogen ions increases, which increases the acidity and decreases the pH of the solution. This increase in acidity and decrease in the pH of fruit juice are related primarily to the presence of organic acids such as citric acid, malic acid and tartaric acid. Other studies have shown that fruits exhibit a decrease in pH during ten days of storage (Suriati et al., 2020; Gao et al., 2018). Carbohydrates in fruits undergo conversion during storage, producing organic acids that help lower the pH (Dorostkar et al., 2022; Saki et al., 2019). In an experiment, Vwioko et al. (2013) investigated the effects of preservatives on the pH and microbial load of *Annona muricata* water. They reported that preservatives that reduce the pH of fruit juice (garlic and ginger) significantly reduce the microbial load of fruit juice. They identified pH as an important microbial growth factor (Ukwo et al., 2010). Additionally, the results of this research revealed that, compared with those in other regions, the microbial load of barberry fruits in the Birjand region and Zirkoh region was lower. Our results are consistent with the findings of other researchers (Ekanem and Ekanem, 2019; Fleet, 2003).

In addition, the terms "antioxidants," as well as the concepts of these compounds, are different in the food and biomedical sciences, and the antioxidant indices obtained via chemical assays often cannot be generalized to studies in vivo (López-Alarcón and Denicola, 2013; Yang et al., 2018). Fruits have been proven to be excellent sources of natural antioxidants (Carlsen et al., 2010; Kusano and Ferrari, 2008). These compounds protect cells from the damaging effects of reactive oxygen species (ROS) and neutralize the mechanisms of active free radicals such as hydrogen peroxide, superoxide radicals, and hydroxyl radicals or disrupt their destructive reactions. These events are related to the chemical composition of antioxidants (Rekha et al., 2012; Wang and Jiao, 2000; Lee et al., 2009). These plant compounds have several biological functions, such as anti-inflammatory, anticancer, antidegenerative, antiaging, and antidiabetes properties, among others (Jideani et al., 2021; Lu et al., 2021; Pérez-Lamela et al., 2021). One of the most important properties of antioxidant compounds (such as phenolic compounds) is their antimicrobial and antibacterial properties (Suriyaprom et al., 2022). The mechanisms of these compounds in bacterial cells are diverse. Antioxidants can cause deterioration of the cell wall, destabilize the cytoplasmic membrane, deactivate the enzymes that exist inside the cell and are responsible for the metabolic processes of the cell, and, in turn, through interactions with nucleic acids, inhibit reproduction processes. And transcription (Henie et al., 2009; Cendrowski et al., 2020). These actions may damage the structure and function of pathogenic microorganisms. Phenolic compounds cause protein denaturation in microbial cells (Cowan, 1999). Previous reports suggest that phenolic compounds are likely to have toxic effects at the membrane surface. Phenols affect membrane function by changing the ratio of proteins to lipids in the membrane and causing potassium ion leakage and cell death (Negi, 2012). Additionally, flavonoids, another class of antioxidant compounds, damage bacteria and cause the accumulation of bacterial cells, which may lead to disruption of bacterial growth (Tarahovsky et al., 2014). In this context, the results of the present study revealed that the lowest amount of antioxidant activity in dried barberry was obtained from the Darmian region. Additionally, considering the microbial load data, the low antioxidant activity in the Darmian region likely caused the increase in the barberry microbial load in this region. A comparison of the microbial load and antioxidant activity results revealed that a lack of compliance with health protocols likely increased contamination of the shadehouse environment of barberry fruits. On the other hand, the antioxidant compounds of fruits have been used to fight against pathogenic microorganisms and reduce their effects. This probably caused the reduction in the antioxidant activity of fruits in the Darmian region. Our results are consistent with those of other researchers (Cendrowski et al., 2020; Aslam et al., 2023).

Correlation coefficients of traits

Significant strong correlations revealed among investigated variables in this experiment. The results showed positive significant correlations between TAB and yeast/mold ($r = 0.96$), TAB and pH ($r = 0.85$). Also negative significant correlations between TAB and TA ($r = -0.71$), and TAB and TAA ($r = -0.95$) were observed. There was also a positive significant correlation between yeast/mold and pH ($r = 0.96$), and a negative significant correlation between yeast/mold and TAA ($r = -0.84$). Some of chemical traits had strong correlations too. TA had a positive significant correlation with TAA (r = 0.88), and TSS showed a negative significant correlation with pH (r = 0.80).

In line with the Pokhrel et al. (2022), the results of correlations revealed that fruit from Darmian region, which had lower TAA and higher pH compared to other regions showed the higher TAB and yeast/mold. Zitouni et al. (2020) reported significant strong correlations among pH, TSS and TAA of strawberry. Higher levels of microbial inactivation at lower pH values could be due to the changes within the cell membrane allowing the diffusion of acids from the environment to the cytoplasm, which decreases the internal pH of the cell. Therefore, bacterial inactivation can be enhanced in acidic fruit with lower pH as we observed in seedless barberry fruit selected from all regions than Darmian.

CONCLUSION

This study investigated the effects of different regions on the microbiological analysis and chemical characteristics, especially the antioxidant activity, of barberry fruits. The results briefly revealed high amounts of total aerobic bacteria, mold and yeast in barberry fruits selected from the Darmian region shadehouse. However, other regions were at an acceptable level regarding the microbial load, which shows that the health protocols in these regions (the Birjand, Zirkoh, and Qaen regions) have been carried out effectively. Notably, this comparison was made with the standards of the Food and Drug Organization of Iran. However, according to other standards, the levels of total aerobic bacteria, mold and yeast may be at acceptable levels. The fruits of the Birjand region had the highest TSS content and the most acidic pH value of the fruit juice, which suggests that the fruits of this region have better taste quality than those of other regions. Additionally, fruits prepared from the Qaen region presented the highest amount of TA and total antioxidant activity. This finding highlights the high nutritional value of the fruit of the Qaen region compared with that of other regions, which is highly likely due to the higher altitude of the Qaen region compared with other regions. Therefore, to reduce the risk of microbial contamination and maintain the nutritional value of dried, seedless barberry fruit, it is necessary to provide particular standards for shadehouses.

Funding: Authors would like to thank gratefully the University of Birjand for providing the financial support of this project, Grant No. 93034.

Conflict of interest

The authors declare no competing interests.

REFERENCES

Abdel-Moneim, A., Ceuppens, S., El-Tahan, F., & Uyttendaele, M. (2014). Microbiological safety of strawberries and lettuce for domestic consumption in Egypt. *Journal of Food Processing & Technology*, *5*(3), 1-7. http://dx.doi.org/10.4172/2157-7110.1000308

Adewoyin, O. B. (2023). Pre-harvest and postharvest factors affecting quality and shelf life of harvested produce. *In* new Advances in Postharvest Technology. *IntechOpen.* 1-20. http://dx.doi.org/10.5772/intechopen.111649

Alavi, N., & Mazloumzadeh, S. M. (2012). Effect of harvesting and drying methods of seedless barberry on some fruit quality. *Journal of the Saudi Society of Agricultural Sciences*, *11*(1), 51-55. http://dx.doi.org/10.1016/j.jssas.2011.08.003

Allocati, N., Masulli, M., Alexeyev, M. F., & Di Ilio, C. (2013). *Escherichia coli* in Europe: an overview. *International Journal of Environmental Research and Public Health*, *10*(12), 6235-6254. http://dx.doi.org/10.3390/ijerph10126235

Alp, D., & Bulantekin, Ö. (2021). The microbiological quality of various foods dried by applying different drying methods: a review. *European Food Research and Technology, 247*(6), 1333-1343. http://dx.doi.org/10.1007/s00217-021-03731-z

Anthon, G. E., & Barrett, D. M. (2012). Pectin methylesterase activity and other factors affecting pH and titratable acidity in processing tomatoes. *Food Chemistry, 132*(2), 915-920. http://dx.doi.org/10.1016/j.foodchem.2011.11.066

Araujo, W. L., NUNES-NESI, A. D. R. I. A. N. O., Nikoloski, Z., Sweetlove, L. J., & Fernie, A. R. (2012). Metabolic control and regulation of the tricarboxylic acid cycle in photosynthetic and heterotrophic plant tissues. *Plant, Cell & Environment, 35*(1), 1-21. http://dx.doi.org/10.1111/j.1365-3040.2011.02332.x

Ardestani, S. B., Sahari, M. A., & Barzegar, M. (2015). Effect of extraction and processing conditions on organic acids of barberry fruits. *Journal of Food Biochemistry, 39*(5), 554-565. http://dx.doi.org/10.1111/jfbc.12158

Ardestani, S. B., Sahari, M. A., Barzegar, M., & Abbasi, S. (2013). Some physicochemical properties of Iranian native barberry fruits (abi and poloei): *Berberis integerrima* and *Berberis vulgaris*. *Journal of Food and Pharmaceutical Sciences, 1*(3), 60-67. https://doi.org/10.14499/jfps.

Artimová, R., Játiová, M., Baumgartnerová, J., Lipková, N., Petrová, J., Maková, J., & Medo, J. (2023). Microbial communities on samples of commercially available fresh-consumed leafy vegetables and small berries. *Horticulturae, 9*(2), 150. http://dx.doi.org/10.3390/horticulturae9020150

Aslam, H., Nadeem, M., Shahid, U., Ranjha, M. M. A. N., Khalid, W., Qureshi, T. M., & Awuchi, C. G. (2023). Physicochemical characteristics, antioxidant potential, and shelf stability of developed roselle–fig fruit bar. *Food Science & Nutrition, 11*(7), 4219-4232. http://dx.doi.org/10.1002/fsn3.3436

Aycicek, H., Oguz, U., & Karci, K. (2006). Determination of total aerobic and indicator bacteria on some raw eaten vegetables from wholesalers in Ankara, Turkey. *International Journal of Hygiene and Environmental Health, 209*(2), 197-201. http://dx.doi.org/10.1016/j.ijheh.2005.07.006

Babič, M. N., Zalar, P., Ženko, B., Džeroski, S., & Gunde-Cimerman, N. (2016). Yeasts and yeast-like fungi in tap water and groundwater, and their transmission to household appliances. *Fungal Ecology, 20*, 30-39. http://dx.doi.org/10.1016/j.funeco.2015.10.001

Balandari, A., Azizi, M., & Khodabandeh, M. (2023). Biochemical properties of twelve indigenous barberry (*Berberis spp.*) genotypes. *Journal of Horticultural Science, 37*(2), 293-306.

Bărbulescu, I. D., Dumitrache, C., DIGUȚĂ, C. F., Begea, M., MATEI, P. M., Frîncu, M., & Teodorescu, R. I. (2022). Evolution at the microfermenter level of the growth dynamics of Saccharomyces cerevisiae and Starmella bacillaris yeasts with potential for use in winemaking at the pietroasa winery. *AgroLife Scientific Journal, 11*(2), 9-16. http://dx.doi.org/10.17930/agl202221

Beales, N. (2004). Adaptation of microorganisms to cold temperatures, weak acid preservatives, low pH, and osmotic stress: a review. *Comprehensive Reviews in Food Science and Food Safety, 3*(1), 1-20. http://dx.doi.org/10.1111/j.1541-4337.2004.tb00057.x

Behrad, Z., Sefidkon, F., Ghasemzadeh, H., Rezadoost, H., & Balandary, A. (2023). Determination of phenolic compounds and antioxidant activities of 55 Iranian Berberis genotypes. *Journal of Medicinal Plants and By-product, 12*(2), 181-189.

Berthold-Pluta, A., Garbowska, M., Stefańska, I., Stasiak-Różańska, L., Aleksandrzak-Piekarczyk, T., & Pluta, A. (2021). Microbiological quality of nuts, dried and candied fruits, including the prevalence of *Cronobacter* spp. *Pathogens, 10*(7), 900. http://dx.doi.org/10.3390/pathogens10070900

Beuchat, L. R., Komitopoulou, E., Beckers, H., Betts, R. P., Bourdichon, F., Fanning, S., & Ter Kuile, B. H. (2013). Low--water activity foods: increased concern as vehicles of foodborne pathogens. *Journal of Food Protection, 76*(1), 150-172. http://dx.doi.org/10.4315/0362-028x.jfp-12-211

Bideli, N., Ahmadi-Roshan, M., & Berenji Ardestani, S. (2022). Effects of gamma irradiation, osmotic and freezing processes on chemical, microbial, and pest characteristics of dried Iranian barberry fruit during storage. *Acta Alimentaria*, *51*(4), 523-533. http://dx.doi.org/10.1556/066.2022.00141

Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature, 181*(4617), 1199-1200. http://dx.doi.org/10.1038/1811199a0

Bourdoux, S., Li, D., Rajkovic, A., Devlieghere, F., & Uyttendaele, M. (2016). Performance of drying technologies to ensure microbial safety of dried fruits and vegetables. *Comprehensive Reviews in Food Science and Food Safety*, *15*(6), 1056-1066. http://dx.doi.org/10.1111/1541-4337.12224

Brown, M. H. (1991). Acidulants and low pH. Food preservatives. *Journal of Antibacterial and Antifungal Agents*, *23*(4), 241-250.

Bublyk, M. O., Fryziuk, L. A., & Levchuk, L. M. (2014). Fruit crop production distribution in Ukraine: A research note. *Chemistry and Chemical Biology: Methodologies and Applications/ed. R. Joswik, AA Dalinkevich. Toronto*, 207-214. http://dx.doi.org/10.1201/b17413-27

Caprio, J. M., & Quamme, H. A. (2006). Influence of weather on apricot, peach and sweet cherry production in the Okanagan Valley of British Columbia. *Canadian Journal of Plant Science, 86*(1), 259-267. http://dx.doi.org/10.4141/p05-032

Carlsen, M. H., Halvorsen, B. L., Holte, K., Bøhn, S. K., Dragland, S., Sampson, L., & Blomhoff, R. (2010). The total antioxidant content of more than 3100 foods, beverages, spices, herbs and supplements used worldwide. *Nutrition Journal*, 9, 1-11. http://dx.doi.org/10.1186/1475-2891-9-3

Cendrowski, A., Kraśniewska, K., Przybył, J. L., Zielińska, A., & Kalisz, S. (2020). Antibacterial and antioxidant activity of extracts from rose fruits (*Rosa rugosa*). *Molecules*, *25*(6), 1365. http://dx.doi.org/10.3390/molecules25061365

Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews, 12*(4), 564-582. http://dx.doi.org/10.1128/cmr.12.4.564

Dorostkar, M., Moradinezhad, F., & Ansarifar, E. (2022). Influence of active modified atmosphere packaging pre-treatment on shelf life and quality attributes of cold stored apricot fruit. *International Journal of Fruit Science, 22*(1), 402-413. http://dx.doi.org/10.1080/15538362.2022.2047137

Ekanem, J. O., & Ekanem, O. O. (2019). The effect of natural and artificial preservatives and storage temperature on the pH and microbial load of freshly produced apple (*Malus domestica*) juice. *Agro-Science, 18*(1), 16-21. http://dx.doi.org/10.4314/as.v18i1.3

El-Araby, A., Azzouzi, A., Ayam, I. M., Samouh, K. F., & Errachidi, F. (2023). Survey on technical management of strawberries in Morocco and evaluation of their post-harvest microbial load. *Frontiers in Microbiology, 13*, 1115340. http://dx.doi.org/10.3389/fmicb.2022.1115340

El-Dengawy, E. F. A., Samaan, L. G., El-Shobaky, M. A., El-Kadi, S. M., & Saleh, M. A. A. (2018). Evaluation of rutability, quality and microbial load in Hayani date palm fruits during cold storage as affected by applying some safe postharvest treatments. *Journal of Plant Production, 9*(10), 805-813. http://dx.doi.org/10.21608/jpp.2018.36436

Etienne, A., Génard, M., Lobit, P., Mbeguié-A-Mbéguié, D., & Bugaud, C. (2013). What controls fleshy fruit acidity? A review of malate and citrate accumulation in fruit cells. *Journal of Experimental Botany, 64*(6), 1451-1469. http://dx.doi.org/10.1093/jxb/ert035

Famiani, F., Battistelli, A., Moscatello, S., Cruz-Castillo, J. G., & Walker, R. P. (2015). The organic acids that are accumulated in the flesh of fruits: occurrence, metabolism and factors affecting their contents-a review. *Revista Chapingo. Serie Horticultura, 21*(2), 97-128. http://dx.doi.org/10.5154/r.rchsh.2015.01.004

Feng, P., Weagant, S. D., Grant, M. A., Burkhardt, W., Shellfish, M., & Water, B. (2002). BAM: Enumeration of *Escherichia coli* and the *Coliform* Bacteria. *Bacteriological Analytical Manual, 13*(9), 1-13

Firdous, N., Moradinezhad, F., Farooq, F., & Dorostkar, M. (2023). Advances in formulation, functionality, and application of edible coatings on fresh produce and fresh-cut products: A review. *Food Chemistry*, *407*, 135186. http://dx.doi.org/10.1016/j.foodchem.2022.135186

Fleet, G. H. (2003). Yeast interactions and wine flavour. *International Journal of Food Microbiology, 86*(1-2), 11-22. http://dx.doi.org/10.1016/s0168-1605(03)00245-9

Gao, Y., Kan, C., Wan, C., Chen, C., Chen, M., & Chen, J. (2018). Quality and biochemical changes of navel orange fruits during storage as affected by cinnamaldehyde-chitosan coating. *Scientia Horticulturae*, *239*, 80-86. http://dx.doi.org/10.1016/j.scienta.2018.05.012

Gautier, H., Rocci, A., Buret, M., Grasselly, D., & Causse, M. (2005). Fruit load or fruit position alters response to temperature and subsequently cherry tomato quality. *Journal of the Science of Food and Agriculture*, *85*(6), 1009-1016. http://dx.doi.org/10.1002/jsfa.2060

Gilbert, R. J., De Louvois, J., Donovan, T., Little, C., Nye, K., Ribeiro, C. D., & Bolton, F. J. (2000). Guidelines for the microbiological quality of some ready-to-eat foods sampled at the point of sale. PHLS Advisory Committee for Food and Dairy Products. *Communicable Disease and Public Health*, *3*(3), 163-167.

Gilliland, S. E., & Speck, M. L. (1967). Mechanism of the bactericidal action produced by electrohydraulic shock. *Applied Microbiology*, *15*(5), 1038-1044. http://dx.doi.org/10.1128/aem.15.5.1038-1044.1967

Gogo, E. O., Opiyo, A. M., Hassenberg, K., Ulrichs, C., & Huyskens-Keil, S. (2017). Postharvest UV-C treatment for extending shelf life and improving nutritional quality of African indigenous leafy vegetables. *Postharvest Biology and Technology*, *129*, 107-117. http://dx.doi.org/10.1016/j.postharvbio.2017.03.019

Gómez-López, V. M., Ragaert, P., Jeyachchandran, V., Debevere, J., & Devlieghere, F. (2008). Shelf-life of minimally processed lettuce and cabbage treated with gaseous chlorine dioxide and cysteine. *International Journal of Food Microbiology*, *121*(1), 74-83. http://dx.doi.org/10.1016/j.ijfoodmicro.2007.11.036

Halablab, M. A., Sheet, I. H., & Holail, H. M. (2011). Microbiological quality of raw vegetables grown in Bekaa Valley, Lebanon. *American Journal of Food Technology*, *6*(2), 129-139. http://dx.doi.org/10.3923/ajft.2011.129.139

Henie, E. F. P., Zaiton, H., & Suhaila, M. (2009). Bacterial membrane disruption in food pathogens by Psidium guajava leaf extracts. *International Food Research Journal*, *16*(3), 297-311.

Hosseini, A., Moradinezhad, F., Khayyat, M., & Aminifard, M. H. (2021). Influence of foliar application of calcium nitrate and potassium nitrate on qualitative and quantitative traits of seedless barberry (*Berberis vulgaris* L.). *Erwerbs-Obstbau*, *63*(2), 151-161. http://dx.doi.org/10.1007/s10341-021-00553-x

Hyun, J. E., Kim, J. Y., Kim, E. M., Kim, J. C., & Lee, S. Y. (2019). Changes in microbiological and physicochemical quality of dried persimmons (*Diospyros kaki* Thunb.) stored at various temperatures. *Journal of Food Quality*, *2019*(1), 6256409. http://dx.doi.org/10.1155/2019/6256409

ISIRI. (2013). Institute of Standards and Industrial Research of Iran, Microbioligy of food and animal feeding stuffs - Enumeration of Yeast and mould -Colony count techni in products with water activity Less than or equal to 0.6. ISIRI no 10899 -3. 1st Edition, ISIRI: 2013 [in Persian]. http://dx.doi.org/10.3403/30151271u

Ivanova, I., Serdiuk, M., Malkina, V., Bandura, I., Kovalenko, I., Tymoshchuk, T., & Omelian, A. (2021). The study of soluble solids content accumulation dynamics under the influence of weather factors in the fruits of cherries. *Slovak Journal of Food Sciences, 15,* 350-359. http://dx.doi.org/10.5219/1554

Jang, A. R., Han, A., Lee, S., Jo, S., Song, H., Kim, D., & Lee, S. Y. (2021). Evaluation of microbiological quality and safety of fresh-cut fruit products at retail levels in Korea. *Food Science and Biotechnology*, *30*(10), 1393-1401. http://dx.doi.org/10.1007/s10068-021-00974-0

Javadzadeh, S. M. (2013). Effect of different methods of harvesting, drying and time on losses seedless barberry (*Berberis vulgaris* L). *International Journal of Agronomy and Plant Production*, *4*(2), 254-260.

Jayaraman, K. S., & Gupta, D. D. (2020). Drying of fruits and vegetables. *In Handbook of industrial drying* (pp. 643-690). CRC Press. http://dx.doi.org/10.1201/9780429289774-21

Jideani, A. I., Silungwe, H., Takalani, T., Omolola, A. O., Udeh, H. O., & Anyasi, T. A. (2021). Antioxidant-rich natural fruit and vegetable products and human health. *International Journal of Food Properties*, *24*(1), 41-67. http://dx.doi.org/10.1080/10942912.2020.1866597

Kadam, D. M., Nangare, D. D., & Oberoi, H. S. (2009). Influence of pre-treatments on microbial load of stored dehydrated onion slices. *International Journal of Food Science & Technology*, *44*(10), 1902-1908. http://dx.doi.org/10.1111/j.1365-2621.2009.01980.x

Koyuncu, F. (2004). Organic acid composition of native black mulberry fruit. *Chemistry of Natural Compounds*, *40*, 367-369. http://dx.doi.org/10.1023/b:conc.0000048249.44206.e2

Kusano, C., & Ferrari, B. (2008). Total antioxidant capacity: a biomarker in biomedical and nutritional studies. *Journal of Molecular Cell Biology*, *7*(1), 1-15.

Kuźniar, P., Belcar, J., Zardzewiały, M., Basara, O., & Gorzelany, J. (2022). Effect of Ozonation on the Mechanical, Chemical, and Microbiological Properties of Organically Grown Red Currant (*Ribes rubrum* L.) *Fruit. Molecules*, *27*(23), 8231. http://dx.doi.org/10.3390/molecules27238231

Lambert, P. A. (1995). Introductory Microbiology-by T. Gross, J. Faull, S. Ketteridge and D. Springham Chapman & Hall, 1995. &19. 99 pbk (xiv+ 414 pages) ISBN 0 412 45300 2. *Trends in Microbiology*, *3*(7), 288-288. http://dx.doi.org/10.1007/978-1-4899-7194-4

Lee, J., Whang, J. B., Youn, N. R., Lee, S. Y., Lee, H. J., Kim, Y. J., & Koh, K. H. (2009). Antioxidant and oxygen radical scavenging capacities of the extracts of pear cactus, mulberry and Korean black raspberry fruits. *Journal of Food Science and Nutrition*, *14*(3), 188-194. http://dx.doi.org/10.3746/jfn.2009.14.3.188

Lez-Altozano, P. G., & Castel, J. R. (1999). Regulated deficit irrigation inClementina de Nules' citrus trees. I. Yield and fruit quality effects. *The Journal of Horticultural Science and Biotechnology*, *74*(6), 706-713. http://dx.doi.org/10.1080/14620316.1999.11511177

Li, Y., Wu, M., Zhao, D., Wei, Z., Zhong, W., Wang, X., & Li, Z. (2015). Electroporation on microchips: the harmful effects of pH changes and scaling down. *Scientific Reports*, *5*(1), 17817. http://dx.doi.org/10.1038/srep17817

López-Alarcón, C., & Denicola, A. (2013). Evaluating the antioxidant capacity of natural products: A review on chemical and cellular-based assays. *Analytica Chimica Acta*, *763*, 1-10. http://dx.doi.org/10.1016/j.aca.2012.11.051

Lu, H. J., Breidt Jr, F., Pérez-Díaz, I. M., & Osborne, J. A. (2011). Antimicrobial effects of weak acids on the survival of *Escherichia coli* O157: H7 under anaerobic conditions. *Journal of Food Protection*, *74*(6), 893-898. http://dx.doi.org/10.4315/0362-028x.jfp-10-404

Lu, W., Shi, Y., Wang, R., Su, D., Tang, M., Liu, Y., & Li, Z. (2021). Antioxidant activity and healthy benefits of natural pigments in fruits: A review. *International Journal of Molecular Sciences*, *22*(9), 4945. http://dx.doi.org/10.3390/ijms22094945

Macori, G., Gilardi, G., Bellio, A., Bianchi, D. M., Gallina, S., Vitale, N., & Decastelli, L. (2018). Microbiological parameters in the primary production of berries: a pilot study. *Foods*, *7*(7), 105. http://dx.doi.org/10.3390/foods7070105

Mathur, A., Joshi, A., & Harwani, D. (2014). Microbial contamination of raw fruits and vegetables. *Internet Journal of Food Safety*, *16*, 26-28.

Mongi, R. J. (2023). Physicochemical properties, microbial loads and shelf life prediction of solar dried mango (*Mangifera indica*) and pineapple (*Ananas comosus*) in Tanzania. *Journal of Agriculture and Food Research*, *11*, 100522. http://dx.doi.org/10.1016/j.jafr.2023.100522

Moradinezhad, F., Khayyat, M., & Maraki, Z. (2018). Changes in anthocyanin and fruit quality attributes of barberry (*Berberis vulgaris* L.) grown in different altitude during growth and maturation. *Journal of Agricultural Sciences–Sri Lanka, 13*(3). http://dx.doi.org/10.4038/jas.v13i3.8396

Moradinezhad, F., Mehregan, M., & Jahani, M. (2019). Physicochemical traits of seedless barberry (*Berberis vulgaris* L.) fruits stored under refrigeration as affected by heat and calcium chloride treatments. *Agronomical Research in Moldavia, 51*(4), 73-86. http://dx.doi.org/10.2478/cerce-2018-0037

Moradinezhad, F., Dorostkar, M., Niazmand, R., & Doraki, G. (2024). A comprehensive study of qualitative and biochemical characteristics of dried seedless barberry fruits from different regions of South Khorasan Province, Iran. *Journal of Horticulture and Postharvest Research*, *7*(4), 345-360. https://doi.org/10.22077/jhpr.2024.7912.1399

Morgan, C. A., Herman, N., White, P. A., & Vesey, G. (2006). Preservation of micro-organisms by drying; a review. *Journal of Microbiological Methods, 66*(2), 183-193. http://dx.doi.org/10.1016/j.mimet.2006.02.017

Mritunjay, S. K., & Kumar, V. (2015). Fresh farm produce as a source of pathogens: a review. Research *Journal of Environmental Toxicology, 9*(2), 59-70. http://dx.doi.org/10.3923/rjet.2015.59.70

Ndjomgoue-Yossa, A. C., Nanseu-Njiki, C. P., & Ngameni, E. (2022). Effect of pH on Escherichia coli removal by electrocoagulation and elimination kinetics after treatment. *Journal of Chemistry, 2022*(1), 5249368. http://dx.doi.org/10.1155/2022/5249368

Negi, P. S. (2012). Plant extracts for the control of bacterial growth: Efficacy, stability and safety issues for food application. *International Journal of Food Microbiology, 156*(1), 7-17. http://dx.doi.org/10.1016/j.ijfoodmicro.2012.03.006

Nizam, L. M., Ardawati, A. N., Nurmahani, M. M., Roshita, I., & Zaiton, H. (2019). Microbiological quality and sensory evaluation of partially dried mango for fruit salad, Kerabu Mangga. Asian *Journal of Agriculture and Biology*. *7*(1), 103-115.

Och, A., & Nowak, R. (2021). Barberry (*Berberis vulgaris*)-Traditional and Contemporary Use. *In Medicinal Plants: Domestication, Biotechnology and Regional Importance* (pp. 797-825). Cham: Springer International Publishing. http://dx.doi.org/10.1007/978-3-030-74779-4_24

Padmavathi, A. R., P, S. M., Das, A., Priya, A., Sushmitha, T. J., Pandian, S. K., & Toleti, S. R. (2020). Impediment to growth and yeast-to-hyphae transition in Candida albicans by copper oxide nanoparticles. *Biofouling, 36*(1), 56-72. http://dx.doi.org/10.1080/08927014.2020.1715371

Pérez-Lamela, C., Franco, I., & Falqué, E. (2021). Impact of high-pressure processing on antioxidant activity during storage of fruits and fruit products: A review. *Molecules, 26*(17), 5265. http://dx.doi.org/10.3390/molecules26175265

Pokhrel, P. R., Boulet, C., Yildiz, S., Sablani, S., Tang, J., & Barbosa-Cánovas, G. V. (2022). Effect of high hydrostatic pressure on microbial inactivation and quality changes in carrot-orange juice blends at varying pH. *LWT*, *159*, 113219. https://doi.org/10.1016/j.lwt.2022.113219

Quansah, J. K., Gazula, H., Holland, R., Scherm, H., Li, C., Takeda, F., & Chen, J. (2019). Microbial quality of blueberries for the fresh market. *Food Control*, *100*, 92-96. http://dx.doi.org/10.1016/j.foodcont.2018.12.034

Ragaert, P., Devlieghere, F., Devuyst, E., Dewulf, J., Van Langenhove, H., & Debevere, J. (2006). Volatile metabolite production of spoilage micro-organisms on a mixed-lettuce agar during storage at 7 C in air and low oxygen atmosphere. *International Journal of Food Microbiology, 112*(2), 162-170. http://dx.doi.org/10.1016/j.ijfoodmicro.2006.06.018

Rekha, C., Poornima, G., Manasa, M., Abhipsa, V., Devi, J. P., Kumar, H. T. V., & Kekuda, T. P. (2012). Ascorbic acid, total phenol content and antioxidant activity of fresh juices of four ripe and unripe citrus fruits. *Chemical Science Transactions, 1*(2), 303-310. http://dx.doi.org/10.7598/cst2012.182

Rowbury, R. J. (1997). Regulatory components, including integration host factor, CysB and H-NS, that influence pH responses in Escherichia coli. *Letters in Applied Microbiology, 24*(5), 319-328. http://dx.doi.org/10.1046/j.1472-765x.1997.00065.x

Saad, A. M., El-Saadony, M. T., Mohamed, A. S., Ahmed, A. I., & Sitohy, M. Z. (2021). Impact of cucumber pomace fortification on the nutritional, sensorial and technological quality of soft wheat flour-based noodles. *International Journal of Food Science & Technology, 56*(7), 3255-3268. http://dx.doi.org/10.1111/ijfs.14970

Saebi, M. R., Moradinezhad, F., & Ansarifar, E. (2023). Quality preservation and decay reduction of minimally processed seedless barberry fruit via postharvest ultrasonic treatment. *Food Science & Nutrition, 11*(12), 7816-7825. http://dx.doi.org/10.1002/fsn3.3698

Saki, M., ValizadehKaji, B., Abbasifar, A., & Shahrjerdi, I. (2019). Effect of chitosan coating combined with thymol essential oil on physicochemical and qualitative properties of fresh fig (*Ficus carica* L.) fruit during cold storage. *Journal of Food Measurement and Characterization, 13*, 1147-1158. http://dx.doi.org/10.1007/s11694-019-00030-w

Sansavini, S., & Lugli, S. (2005, June). Sweet cherry breeding programs in Europe and Asia. *In V International Cherry Symposium 795* (pp. 41-58).http://dx.doi.org/10.17660/actahortic.2008.795.1

Serdyuk, M., Ivanova, I., Malkina, V., Kryvonos, I., Tymoshchuk, T., & Ievstafiieva, K. (2020). The formation of dry soluble substances in sweet cherry fruits under the influence of abiotic factors. *Scientific Horizons, 3*(88), 127-135. http://dx.doi.org/10.33249/2663-2144-2020-88-3-127-135

Sheiko, T., Tkachenko, S., Mushtruk, M., Vasyliv, V., Deviatko, O., Mukoid, R., & Bondar, N. (2019). Studying the processing of food dye from beet juice. *Potravinarstvo Slovak Journal of Food Sciences, 13*(1), 688-694. http://dx.doi.org/10.5219/1152

Shidfar, F., Ebrahimi, S. S., Hosseini, S., Heydari, I., Shidfar, S., & Hajhassani, G. (2012). The effects of Berberis vulgaris fruit extract on serum lipoproteins, apoB, apoA-I, homocysteine, glycemic control and total antioxidant capacity in type 2 diabetic patients. *Iranian journal of pharmaceutical research: IJPR, 11*(2), 643-652

Silva, F. V. (2019). Heat assisted HPP for the inactivation of bacteria, moulds and yeasts spores in foods: Log reductions and mathematical models. *Trends in Food Science & Technology, 88*, 143-156. http://dx.doi.org/10.1016/j.tifs.2019.03.016

Slavin, J. L., & Lloyd, B. (2012). Health benefits of fruits and vegetables. *Advances in nutrition, 3*(4), 506-516. http://dx.doi.org/10.3945/an.112.002154

Smith, J. L., Fratamico, P. M., & Gunther IV, N. W. (2014). Shiga toxin-producing *Escherichia coli*. *Advances in Applied Microbiology, 86*, 145-197. http://dx.doi.org/10.1016/b978-0-12-800262-9.00003-2

Stratford, M., Steels, H., Novodvorska, M., Archer, D. B., & Avery, S. V. (2019). Extreme osmotolerance and halotolerance in food-relevant yeasts and the role of glycerol-dependent cell individuality. *Frontiers in Microbiology, 9*, 3238. http://dx.doi.org/10.3389/fmicb.2018.03238

Suriati, L., Utama, I., Harjosuwono, B. A., & Gunam, I. B. W. (2020). Physicochemical characteristics of fresh-cut tropical fruit during storage. *International journal on advance science Engineering Information Technology, 10*(4), 1731-1736. http://dx.doi.org/10.18517/ijaseit.10.4.10857

Suriyaprom, S., Mosoni, P., Leroy, S., Kaewkod, T., Desvaux, M., & Tragoolpua, Y. (2022). Antioxidants of fruit extracts as antimicrobial agents against pathogenic bacteria. *Antioxidants, 11*(3), 602. http://dx.doi.org/10.3390/antiox11030602

Talebzadeh, S. L., Fatemi, H., Azizi, M., Kaveh, M., Salavati Nik, A., Szymanek, M., & Kulig, R. (2022). Interaction of different drying methods and storage on appearance, surface structure, energy, and quality of *Berberis vulgaris* var. asperma. *Foods, 11*(19), 3003. http://dx.doi.org/10.3390/foods11193003

Tango, C. N., Wei, S., Khan, I., Hussain, M. S., Kounkeu, P. F. N., Park, J. H., & Oh, D. H. (2018). Microbiological quality and safety of fresh fruits and vegetables at retail levels in Korea. *Journal of Food Science, 83*(2), 386-392. http://dx.doi.org/10.1111/1750-3841.13992

Tarahovsky, Y. S., Kim, Y. A., Yagolnik, E. A., & Muzafarov, E. N. (2014). Flavonoid–membrane interactions: Involvement of flavonoid–metal complexes in raft signaling. *Biochimica et Biophysica Acta (BBA)-Biomembranes, 1838*(5), 1235-1246. http://dx.doi.org/10.1016/j.bbamem.2014.01.021

Ukwo, S. P., Ezeama, C. F., & Ndaeyo, N. U. (2010). Growth of different yeast strains during fermentation of soursop (*Annona muricata*) juice as influenced by acetic acid bacteria (*Acetobacter aceti*). *Nature and Science, 8*(10), 285-291.

Victor, N., Peter, C., Raphael, K., Tendekayi, G. H., Jephris, G., Taole, M., & Portia, P. R. (2017). Microbiological quality of selected dried fruits and vegetables in Maseru, Lesotho. *African Journal of Microbiology Research, 11*(5), 185-193. http://dx.doi.org/10.5897/ajmr2016.8130

Vwioko, D. E., Osemwegie, O. O., & Akawe, J. N. (2013). The effect of garlic and ginger phytogenics on the shelf life and microbial contents of homemade soursop (*Annona muricata* L) fruit juice. *Biokemistri*, *25*(2), 31-38.

Wang, S. Y., & Camp, M. J. (2000). Temperatures after bloom affect plant growth and fruit quality of strawberry. *Scientia Horticulturae*, *85*(3), 183-199. http://dx.doi.org/10.1016/s0304-4238(99)00143-0

Wang, S. Y., & Jiao, H. (2000). Scavenging capacity of berry crops on superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen. *Journal of Agricultural and Food Chemistry, 48*(11), 5677-5684. http://dx.doi.org/10.1021/jf000766i

WHO (World Health Organization). (2008). *E. coli*. 7 February 2018. https://www.who.int/news-room/fact-sheets/detail/e-coli.

Wiggins, P. M. (1975). Cellular functions of a cell in a metastable equilibrium state. *Journal of Theoretical Biology*, *52*(1), 99-111. http://dx.doi.org/10.1016/0022-5193(75)90042-9

Yang, C. S., Ho, C. T., Zhang, J., Wan, X., Zhang, K., & Lim, J. (2018). Antioxidants: Differing meanings in food science and health science. *Journal of Agricultural and Food Chemistry*, *66*(12), 3063-3068. http://dx.doi.org/10.1021/acs.jafc.7b05830

Zhao, T., Ji, P., & Kumar, G. D. (2021). Pre-harvest treatment for reduction of foodborne pathogens and microbial load on tomatoes. *Food Control*, *119*, 107469. http://dx.doi.org/10.1016/j.foodcont.2020.107469

Zitouni, H., Hssaini, L., Ouaabou, R., Viuda-Martos, M., Hernández, F., Ercisli, S., ... & Hanine, H. (2020). Exploring antioxidant activity, organic acid, and phenolic composition in strawberry tree fruits (*Arbutus unedo* L.) growing in Morocco. *Plants*, *9*(12), 1677. https://doi.org/10.3390/plants9121677

JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH
2025, VOL. 8(4), 539-552

Journal of Horticulture and Postharvest Research

Journal homepage: www.jhpr.birjand.ac.ir

University of Birjand

Chitin nanofiber coating retains postharvest quality and extends shelf life of mango

Shirazoom Munira[1], Md. Yamin Kabir[1,*], Shamim Ahmed Kamal Uddin Khan[2], and Md. Iftekhar Shams[3]

1, Agrotechnology Discipline, Khulna University, Khulna 9208, Bangladesh
2, Faculty of Agriculture, Khulna Agricultural University, Khulna 9100, Bangladesh
3, Forestry and Wood Technology Discipline, Khulna University, Khulna 9208, Bangladesh

ARTICLE INFO

Original Article

Article history:
Received 20 February 2025
Revised 17 May 2025
Accepted 24 May 2025

Keywords:
Chemical attributes
Edible coating
Fruit firmness
Nanofiber
Vitamin C

DOI: 10.22077/jhpr.2025.8984.1479
P-ISSN: 2588-4883
E-ISSN: 2588-6169

***Corresponding author:**
Agrotechnology Discipline, Khulna University, Khulna 9208, Bangladesh.

Email: yaminkabir@at.ku.ac.bd

ABSTRACT

Purpose: Mango is a well-known fruit in tropical and sub-tropical countries, including Bangladesh. However, mangoes are climacteric fruits and exhibit limited storage life due to a high respiration rate. Mangoes are also susceptible to particular storage pathogens at postharvest and thus possess a short shelf life. **Research Method**: Mature mangoes were treated with different concentrations of chitin nanofiber (CNF) and stored in ambient conditions to evaluate the effect of CNF (0.1%, 0.3%, 0.5%) on postharvest quality and shelf life. Disease-free and physiologically mature mangoes were collected from an orchard. The experiment was devised following a Completely Randomized Design with three replications, and each replication consisted of 10 fruits. Fruits were evaluated for physical quality (weight loss, fruit firmness, and color changes), chemical attributes [changes in total soluble solids (TSS), titratable acidity (TA), vitamin C content], microbial (disease incidence and disease severity), and shelf life. **Findings**: The application of 0.3% CNF maintains fruit color (6.33 vs. 4), decreases disease incidence (62.5% vs. 100%) and prolongs the shelf life (8.5 days vs. 6.02 days) of mangoes than the control. Similarly, 0.1% of CNF retains vitamin C (24.33 mg/100g vs. 12.33 mg/100g), decreases disease severity (62% vs. 85.68%), and 0.5% of CNF reduces weight loss (19.34% vs. 31.4%) than the control. **Research Limitations**: CNF preparation requires lab facilities and technical expertise, and it is costly. **Originality/Value**: CNF 0.3% has the potential to maintain postharvest quality and extend the shelf life of mangoes. However, more research is needed to make the final recommendation at the farmers' level.

INTRODUCTION

Mango (*Mangifera indica* L.) – the 'King of fruits' from Anacardiaceae – is a familiar fruit in the tropics and subtropics (Bini et al., 2024), and is widely cultivated in Bangladesh (Sampa et al., 2019). In Bangladesh, it occupies more than121 thousand ha of land having 1.95 million metric tons of an annual production, and the contribution of mango to the total fruit crop production is 26.38% (BBS, 2022). Mango is very popular in Bangladesh because of its excellent taste, amazing flavor, rich nutritive value, wide adaptability, and diversity. However, mangoes are highly perishable, ripe quickly after harvest, and, thus, susceptible to various postharvest diseases (Giovannoni et al., 2017). These problems restricted long-distance handling, storage, and transportation for marketing and consumption, which impacted the world mango trade. As a result, a considerable number of mangoes waste every year (Parvin et al., 2023). In addition, lack of postharvest management ultimately reduces the shelf life, and deteriorates the overall quality of mango.

Shelf life of mangoes and other perishable fruits can be extended by modified atmosphere (MA) packaging, controlled atmosphere (CA) storage (Thakur et al., 2022), edible coatings (Camatari et al., 2017), chemical treatments including calcium chloride (Lekhon et al., 2024; Shao et al., 2019), botanical extracts (Kabir & Hossain, 2024), and growth regulators (Naleo & Maiti, 2018). However, CA and MA storage is very costly, and the use of chemical fungicides for preventing storage decay in mangoes is no longer preferred due to the detrimental effects on the environment, the creation of chemical residue, and the development of resistance in the pathogen population (Sellitto et al., 2021). In this context, the necessity of searching ecofriendly and hazard free alternatives are of getting importance. Recently, fresh fruits have been coated with coating materials like chitin nanofiber (CNF), a natural substance, to increase shelf life and preserve quality after harvest. CNF is the nanofibrilation of chitin, diameters smaller than 100 nm, and a high aspect ratio more than 100 (Xia et al., 2003). Apart from its biomedical and mechanical uses (Ifuku, 2014), it is used as an environmentally friendly substance to prolong the storage life and preserving fruit quality (Zhang et al., 2011). Although the chitin is insoluble, CNF which is readily dissolves in water, is used in agricultural applications (Shamshina et al., 2019; Shams et al., 2025). Though the several studies have been conducted on the postharvest quality of dragon fruits, strawberries, and tomatoes (Fisk et al., 2008), studies on the potentiality of CNF for postharvest quality and storage life of mangoes are scanty. Therefore, the purpose is to evaluate how the chitin nanofiber treatment affects the quality and shelf life of mangoes at postharvest.

MATERIALS AND METHODS

Experimental site and materials

On a bright sunny day, visually blemish-free, fully matured uniform-sized mango fruits (var. Neelambari) were collected from a commercial farmer's orchard (Satkhira, Bangladesh, 88°40' to 89°50' east longitude and 22°47' to 23°47' north latitudes), and immediately transported to Horticulture Laboratory, Khulna University through an air-cooled vehicle. It is a medium-sized mango with 9.26 cm length and the breadth is about 6.18 cm, with an average weight of 350 g. The flesh thickness is 2.10 cm while an edible portion is about 87.26%, and the average yield is 10.15 kg plant^{-1} (Rahman & Akter, 2019).

Experimental design, preparation and application of treatments

The experiment was conducted following a Completely Randomized Design with four concentrations of CNF (0%, 0.1%, 0.3%, 0.5%) and replicated three times. CNF was extracted from the shells tiger shrimp (*Peneus monodon*). Shrimp shells were meticulously cleaned in water to wash the clinging dust, dirt, and other debris. Before letting them dry in the air, the shrimp shells were repeatedly cleaned with tap water. Using a typical grinder, the dry shells were reduced in size to particles between 2 and 4 mm. About 300 g dried, crushed shrimp shells were demineralized, deproteinized, and depigmented using HCI (37%), NaOH (99.9%), and Ethanol 50%, respectively (Ifuku et al., 2011, Ifuku, 2014). The suspension was blended with a super-speed blender (Vita-Mix Blender, Osaka Chem. Co. Ltd.) and a normal-speed blender (Panasonic MX Blender, Panasonic Holdings Corporation), and transferred to the super colloidal machine. The CNF was mechanically processed by passing it through a Super Masscolloider (MKCA6-51, Saitama-ken, Japan). The grinding stone clearance was set to -0.15 with a 1500 rpm rotating stone speed. After going through a super-speed blender for 10, 15, and 20 milling cycles, the CNF was extracted. The required concentrations (0.1%, 0.3%. and 0.5%) of CNF were prepared by adding more distilled water.

For each replication, 10 mangoes with similar physiological maturity were selected. Each set of mangoes was placed separately on the newspaper. The CNF were sprayed on the fruits uniformly through a hand sprayer, except for the control. On each date [4, 6, 8, and 10 days after treatment (DAT)] of chemical measurements, 12 fruits were sampled from four treatments (i.e., three fruits per treatment). However, data on physical parameters were collected on every day.

Firmness determination

Firmness of mango was estimated hedonically as stated by Hassan (2006) following scale of rating 1-6, where 1 is hard green, 2 represents sprung, 3 means between sprung and eating ripe, 4 indicates eating ripe, 5 is Overripe, and 6 means decayed.

Observation of changes in fruit color

Visual assessment

Mango color changes were visually assessed using a numerical rating system of 1–7, where 1 stands for green, 2 for breaker, 3 for up to 25% yellow, 4 for 25–50% yellow, 5 for 50–75% yellow, 6 for 75–100% yellow, and 7 for blackened (Hassan, 2006).

Assessment of fruit color by using a chromameter

Mango skin color was measured on color coordinates as L*, a*, b*, Chroma (C*), and hue angle (h°) from opposite positions of each fruit in Chroma Meter CR-410. Chromaticity L* means the lightness of the fruit color ranging between 0 (black) to 100 (white). Chromaticity a* denotes the redness (+a*) or greenness (-a*), and chromaticity b* signifies the yellow (+b*) or blue (-b*) color skin of fruit. The intensity of color saturation from dull to bright (low-to-high values, respectively) was referred to as chroma (C*). In terms of color interpretation, the angles of red, yellow, green, and blue were 0°, 360°, 90°, and 180°, respectively. Absorbance or reflection is indicated by the hue angle (h°), and the values relate to the intrinsic luminosity.

Determination of weight loss (%)

The weight loss of mango was calculated according to the technique stated by AOAC (2012). Throughout storage, the samples were weighed every day with a digital balance. The findings were expressed using the following formula to get the percentage weight loss (1):

$$\text{Weight loss (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100 \qquad (1)$$

Determination of total soluble solids (TSS), titratable acidity (TA), and vitamin C

TA, TSS and vitamin C content were measured on DATs 4, 6, 8, and 10 following standard methods. On a particular date, a single fruit from each replication was destroyed to determine TA, TSS and vitamin C. Moreover, three mangoes (out of 10) were spent on the chemical analysis. A Hand Refractometer (REF 105) was used to measure the total soluble solids (˚Brix) when a drop of juice is placed on its prism using the AOAC technique (2012). The amount of TA was quantified by titrating 5.0 ml of aliquot (fresh juice was extracted from the fruits of each replication and diluted with distilled water, maintaining a ratio of 1:2 (e.g., 10 ml juice: 20 ml water), and 5 ml aliquot was used for titration) against NaOH (0.1 N) as stated by Nerdy (2018) as follows (2):

$$\text{Malic acid (\%)} = \frac{0.0067 \times \text{Vol. of NaOH} \times 30 \times 100}{5 \times 10} \qquad (2)$$

Where,

0.0067 =	Milli-equivalent weight of malic acid
30 =	Total volume (ml)
5 =	Extract juice sample (ml)
10 =	Volume of aliquot (ml)

The dye (2,6-dichlorophenol indophenol) titration method was followed to evaluate Vitamin C (3) (mg/100g) (Nerdy, 2018; Lekhon et al., 2024).

$$\text{Vitamin C (mg100g}^{-1}\text{)} = \frac{e \times d \times b}{c \times a} \qquad (3)$$

Where a= Weight of specimen, b= Volume produced with metaphosphoric acid, c= Volume of the aliquot, d= Dye factor, and e= reading of the Burette (mean).

Assessment of disease incidence (%) and disease severity (%)

Disease incidence is defined as the percentage of diseased mangos. Fruits with infections were identified by their visual symptoms. Using the following formula, the proportion of disease incidence in mangos was calculated (4) (Dhali et al., 2024; Sivakumar et al., 2002):

$$\text{\% Disease incidence} = \frac{\text{Number of infected mangos}}{\text{Total number of mangos under study}} \times 100 \qquad (4)$$

The percentage of the wounded area of infected fruit indicates the disease severity, and was calculated through eye estimation. Severity of Disease was scored according to Sivakumar et al. (2002) using the scale as follows: 1= 0% of fruit surface rotten; 2 = 1-25% of fruit surface rotten; 3 = 26-50% of fruit surface rotten; 4 = 51-75% of fruit surface rotten; and 5 = 76-100% of fruit surface rotten.

Shelf life (days)

The storage life of mangoes was determined by calculating the number of days needed for them to ripen completely while retaining their best eating quality and marketing potential. Moreover, it was determined based on physical parameters including weight loss, skin color, firmness, and disease severity.

Statistical analysis

A one-way Analysis of Variance (ANOVA) for single-factor CRD was performed on the data through IBM SPSS Statistics for Windows (Version 27.0.1.0) IBM Corp. (2020), Armonk, NY, USA]. Tukey's Honestly Significance Difference (HSD) Test was used to compare treatment means at a 5% level using the same software.

RESULTS AND DISCUSSION

Microenvironment of the experimental room

The weather was hot and humid during the experimental period (25 July to 3 August 2022). The temperature ranges in the storage room were 28.5 to 30.9 °C, with an average of 29.47 °C, while the relative humidity ranges from 85% to 90%, with a mean of 84.7% (Appendix I). Such warm and humid weather favors quick growth of the disease and fungal decay and deteriorates postharvest fruit qualities (do Nascimento Nunes, 2008), leading to significant postharvest loss.

Cumulative weight loss

Weight losses (%) of mango increased gradually throughout storage and varied significantly ($P < 0.05$) only at DATs 7 and 9 (Table 1). At DAT 9, the highest weight loss (31.4%) was reported for control and the lowest (19.34%) for 0.5% CNF. A similar trend was found at DAT 7, in which control showed maximum weight loss (21.53%) while the minimum (13.74%) was recorded for 0.5% CNF (Table 1). Chitosan coating inhibited mango weight loss (Zhu et al., 2008; Parvin et al., 2023), which agrees with our present evaluation. It may occur due to a reduction in transpiration rate due to CNF application. Moreover, higher weight was lost from the uncoated mango compared to the chitosan and cinnamon essential oil treated fruits (Yu et al., 2021).

Table 1. Effect of chitin nanofiber (CNF) on cumulative weight loss of mango at different days after treatment (DAT).

Treatments	Cumulative weight loss (%)								
	DAT 1	DAT 2	DAT 3	DAT 4	DAT 5	DAT 6	DAT 7	DAT 8	DAT 9
Control	0.863[a]	3.4600 [a]	6.0567[a]	8.073[a]	11.320[a]	16.233[a]	21.530[a]	24.807[a]	31.400[a]
CNF0.1%	1.0800[a]	3.2533[a]	5.4300[a]	7.520[a]	9.893[a]	12.840[a]	15.827[b]	18.687[a]	21.347[bc]
CNF 0.3%	1.0567[a]	3.1833[a]	5.0467[a]	7.163[a]	10.077[a]	13.260[a]	15.397[b]	20.327[a]	23.953[b]
CNF 0.5%	1.0800[a]	3.8033[a]	6.0233[a]	7.940[a]	10.660[a]	12.167[a]	13.740[b]	17.630[a]	19.340[c]
P	0.8734	0.7025	0.5555	0.7976	0.6265	0.2303	0.0218	0.0711	0.0006

The data represent the mean of three measurements. The mean values were separated by Tukey's HSD Test at 5%. The mean is not statistically different among the treatments if a column has a similar letter(s).

Fruit firmness

As time passed in storage, the firmness of mango changed gradually from hard to over-ripe, which an indicator of fruit is ripening. From DAT 1 to DAT 3, firmness remained unchanged irrespective of the treatments (Fig. 1). However, fruits started ripening from DAT 4. Significant variation was observed among coated and uncoated fruits at DATs 5, 6, 7, and 8. Additionally, the changes in firmness were faster for control (2.67, 3, 3.67, 4.33, 6, 6). The slower changes were recorded for CNF 0.1% (1.33, 1.67, 1.67, 2, 5, 6) on DATs 5, 6, 7, 8, 9, and 10, respectively, which means the rate of changes in firmness was slower in CNF treated fruits. Firmness changed slowly if mango fruits coated with chitosan (Hasan et al., 2020). These changes in firmness occur as a result of converting starch into sugar.

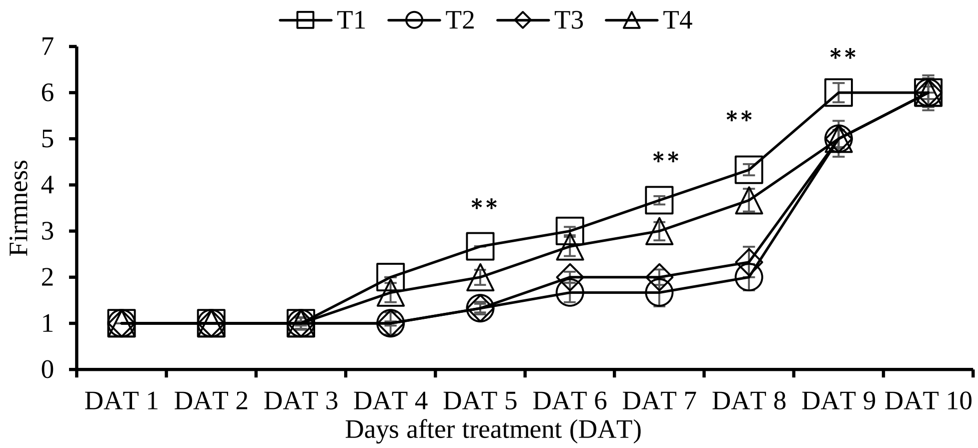


Fig. 1. Effects of chitin nanofiber on firmness of mango. T1= Control, T2= CNF 0.1%, T3= CNF 0.3%, T4= CNF 0.5%. Firmness was measured hedonically based on a scale of 1-6, in which 1 = Hard green, 2 = Sprung, 3 = Between sprung and eating ripe, 4 = Eating ripe, 5 = Over ripe, 6 = rotten. The error bar represents the mean ± SE (standard error) of three measurements. ** means significance at a 1% probability.

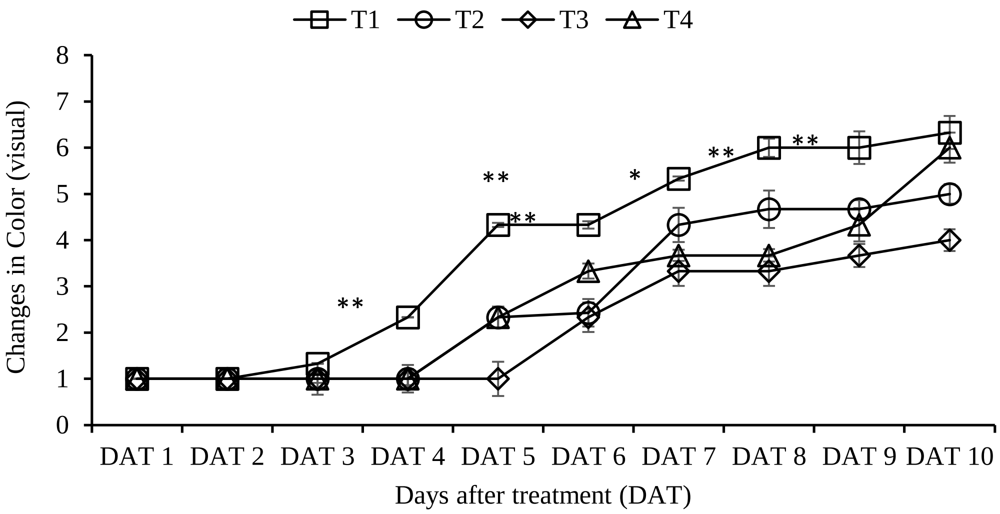


Fig. 2. Effect of chitin nanofiber on the visual color of mango. T1= Control, T2= CNF 0.1%, T3= CNF 0.3%, T4= CNF 0.5%. Color was measured visually using a numerical rating scale of 1-7, where 1 = green, 2 = breaker, 3 = up to 25% yellow, 4 = 25-<50% yellow, 5 = 50-<75% yellow, 6= 75-100% yellow, and 7= blackened. The error bar represents the mean ± SE (standard error) of the three measurements. *, ** mean significance at 5% and 1% probability, respectively.

Changes in fruit skin color

Visual color

A statistically significant difference (P < 0.01) was observed in the color change of mango among the treated and untreated mangoes (Fig. 2). Uncoated control fruits showed faster color change (4.33, 4.33, 5.33, 6, 6, 6.33) than CNF 0.3% treated fruits (1, 2.33, 3.33, 3.33, 3.67, 4) on DATs 5, 6, 7, 8, 9, and 10, respectively. The most noticeable changes during fruit storage are green to breaker color changes of mango peel. Therefore, color change indicates degradation of chlorophyll. As a result of color change, pulp becomes softer, and fruits produce a characteristic aroma. From our study, CNF 0.3% treated fruits required longer periods to reach different stages of ripening compared to the other treatments. Similarly, chitosan coating also delayed the color changes of mangoes (Hasan et al., 2020). The green color of mangoes turned yellow through chlorophyll breakdown as the process of ripening advances (Doreyappy-Gowda and Huddar, 2001).

Table 2. Color coordinates (L*, a*, b*, C*, h°) of mango as influenced by chitin nanofiber (CNF) at different days after treatment (DAT).

Treatments	DAT 1	DAT 3	DAT 5	DAT 7	DAT 9
	L*				
Control	42.67^{b}	43.00^{b}	44.77^{a}	47.57^{ab}	48.57^{a}
CNF 0.1%	46.33^{a}	43.73^{b}	46.27^{a}	50.00^{a}	48.57^{a}
CNF 0.3%	43.77^{b}	44.27^{ab}	44.27^{a}	46.20^{b}	48.63^{a}
CNF 0.5%	45.97^{a}	47.17^{a}	47.67^{a}	50.33^{a}	46.90^{a}
P	0.0067	0.0654	0.6784	0.0279	0.5564
	a*				
Control	-8.18^{a}	-7.13^{a}	-6.07^{a}	-3.79^{a}	-2.10^{a}
CNF 0.1%	-7.13^{a}	-6.60^{a}	-3.43^{a}	-2.67^{a}	-1.18^{a}
CNF 0.3%	-8.13^{a}	-6.36^{a}	-4.67^{a}	-3.41^{a}	-3.34^{a}
CNF 0.5%	-8.72^{a}	-7.23^{a}	-7.20^{a}	-5.75^{a}	-4.47^{a}
P	0.4562	0.9496	0.6227	0.9034	0.6851
	b*				
Control	17.61^{a}	18.10^{a}	20.76^{a}	26.14^{a}	28.53^{a}
CNF 0.1%	19.15^{a}	19.07^{a}	22.81^{a}	26.57^{a}	29.11^{a}
CNF 0.3%	18.25^{a}	19.81^{a}	20.76^{a}	24.53^{a}	27.28^{a}
CNF 0.5%	19.10^{a}	20.60^{a}	23.14^{a}	24.80^{a}	26.073^{a}
P	0.7790	0.2550	0.9109	0.7201	0.5351
	C*				
Control	19.42^{a}	19.52^{b}	21.80^{a}	26.77^{a}	28.99^{a}
CNF 0.1%	20.85^{a}	21.28^{ab}	23.85^{a}	27.32^{a}	29.69^{a}
CNF 0.3%	20.13^{a}	21.31^{ab}	22.47^{a}	25.69^{a}	28.32^{a}
CNF 0.5%	20.94^{a}	24.90^{b}	27.72^{a}	27.35^{a}	26.58^{a}
P	0.7450	0.2031	0.5730	0.9073	0.5471
	h°				
Control	114.93^{a}	111.40^{a}	106.63^{a}	99.03^{a}	90.13^{a}
CNF 0.1%	111.10^{a}	110.07^{a}	102.13^{a}	96.07^{a}	92.67^{a}
CNF 0.3%	114.13^{a}	109.07^{a}	103.87^{a}	99.23^{a}	98.70^{a}
CNF 0.5%	112.83^{a}	108.33^{a}	98.83^{a}	96.23^{a}	94.83^{a}
P	0.7942	0.9545	0.8186	0.9521	0.6727

Data presented as the mean of three measurements where an L* is lightness; a* is redness or greenness; and b* is blueness or yellowness. C* represents chroma mean color saturation, and h° represents hue angle. The mean values were separated by Tukey's HSD Test at 5%. The mean is not statistically different among the treatments if a column has similar letter(s).

Subjective color of mango

Lightness (L*) of mango gradually increased over time, irrespective of the treatments (Table 2). On DAT 7, significant variation *(P* < 0.05) was recorded where the maximum L* value was found in CNF-coated fruits, which means lightness of yellow color (most luminous). Nunes et al. (2007) also found an increased L* value of mango if coated with chitosan at postharvest. The value of a* indicates the amount of green or yellow color, and a* is more negative in CNF 0.5% coated mango than the control, indicating greener mango peel (Table 2). The shifting of a* value towards positive means more redness and ripening. Similarly, a more negative a* value was obtained if mangoes were treated with nano-chitosan (Ngo et al., 2021). Though there were no significant variations among the treatments, changes in b* values were slow at 0.5% CNF treatment than the control. However, a significant change in b* values were reported for nano-chitosan coated mangoes (Ngo et al., 2021). A progressive increase in the C* value was recorded, though nonsignificant (Table 2). However, the C* value of bananas increased initially, and declined later (Al-Dairi & Pathare, 2024). The C* might not reduce after a certain period due to varietal differences or environmental conditions. The hue angle (h°) of the mangoes decreased gradually, which is an indication of ripening (Table 2). The maximum h° (98.70) was obtained from CNF 0.3% treated mangoes, and the minimum was recorded for control (90.13). CNF coating showed the highest retention of h°, indicating a delay in the ripening process. Similarly, the ripening of mango is retarded by pectin/nano-chitosan treatment (Ngo et al., 2021).

Table 3. Effects of chitin nanofiber (CNF) on total soluble solid (TSS, °Brix), titratable acidity (TA %), and vitamin C content (mg 100g-1) of mango at different days after treatment (DAT).

Treatments	DAT 4	DAT 6	DAT 8	DAT 10
	TSS (°Brix)			
Control	8.2667^{c}	15.667^{b}	27.000^{a}	25.667^{a}
CNF 0.1%	10.167^{b}	8.0000^{c}	15.333^{b}	15.000^{c}
CNF 0.3%	13.567^{a}	16.333^{a}	27.667^{a}	21.000^{b}
CNF 0.5%	10.233^{b}	16.333^{a}	9.3333^{c}	11.667^{d}
P	0.0000	0.0000	0.0000	0.0000
	TA (%)			
Control	0.4233^{a}	0.3300^{a}	0.1800^{a}	0.3100^{a}
CNF 0.1%	0.4067^{a}	0.2633^{b}	0.1667^{a}	0.1933^{b}
CNF 0.3%	0.3633^{a}	0.2500^{b}	0.2533^{a}	0.1933^{b}
CNF 0.5%	0.4567^{a}	0.2400^{b}	0.1767^{a}	0.1800^{b}
P	0.8286	0.0708	0.4735	0.0001
	Vitamin C (mg $100g^{-1}$)			
Control	19.67^{b}	21.00^{b}	15.78^{b}	12.33^{b}
CNF 0.1%	34.33^{a}	32.00^{a}	29.33^{a}	24.33^{a}
CNF 0.3%	30.67^{a}	28.33^{ab}	24.00^{ab}	22.33^{ab}
CNF 0.5%	29.67^{ab}	25.67^{ab}	22.33^{ab}	20.00^{ab}
P	0.012	0.04	0.03	0.02

The data represent the mean of three measurements. The mean values were separated by Tukey's HSD Test at 5%. The mean is not statistically different among the treatments if a column has a similar letter(s).

Chemical attributes

The TSS content of mangoes increased gradually, and declined later (Table 3). Moreover, a rapid increase of TSS was observed in control and CNF 0.3% coated fruit. In contrast, the TSS value in 0.5% CNF steadily increased during storage. At DAT 10, the minimum TSS was recorded in 0.5% CNF (11.667 °Brix) followed by 0.1% CNF (15 °Brix), and the maximum for the control (25.67 °Brix). Similar TSS content was observed if mangoes coated with chitosan and cinnamon oil (Yu et al., 2021). TSS content fluctuated in the present study, and the similar fluctuation was reported for the nano-chitosan and pectin/nano-chitosan coated mangoes (Ngo et al., 2021).

Generally, TA follows a decreasing trend after harvest. CNF coating had no effect on TA content (Table 3). However, numerically a higher TA (0.25%) was recorded for 0.3% CNF, and lower (0.11%) from the control. Similarly, the 'Amropali' mango had the highest TA content if treated with chitosan oligosaccharides (Nitu et al., 2025). TA influences fruit flavor and storage quality because of organic acid content (Yu et al., 2021).

The CNF (0.1%) retained higher vitamin C (34.33, 32, 29.33, and 24.33 mg/100g) than the control on the 4th, 6th, 8th, and 10th DAT, respectively (Table 3). Similarly, the vitamin C content of mango was higher than the control if coated with pectin/nano chitosan, may be by forming a thin film, which delayed physiological and biochemical processes (Ngo et al., 2021). Similarly, the vitamin C content of uncoated fruit was the lowest while the maximum retention was observed for chitosan-treated mangoes (Parvin et al., 2023). However, vitamin C content decreased gradually for all treatments; as time elapsed, fruits began to deteriorate, and vitamin C declined.

Incidence and severity of disease

The onset of the disease started from DAT 5 for mangoes. On the 8th, 9th, and 10th days after storage, significant variation (P < 0.05) was recorded among the coated and uncoated mangoes. The highest disease incidence (100%) was recorded for untreated mangoes, and the lowest incidence (62.5%) was observed for 0.3% CNF (Fig. 3). CNF film creates a physical barrier to the oxygen and carbon dioxide, and serves as antimicrobial agent that may reduce disease incidence in mangoes (El Knidri et al., 2020; Jayaprakash et al., 2021). Mangoes treated with chitosan also exhibited low disease incidence (Munira et al., 2024; Parvin et al., 2023).

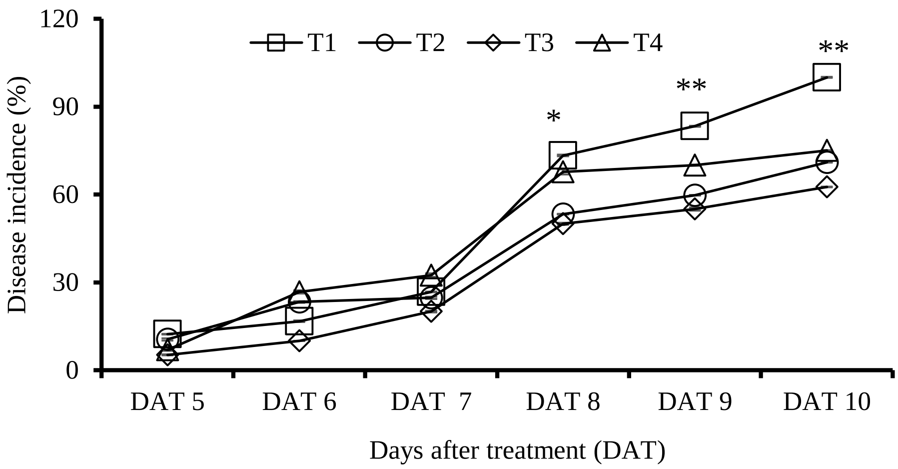


Fig. 3. Effects of chitin nanofiber on disease incidence of mango. T_1= Control, T_2= CNF 0.1%, T_3= CNF 0.3%, and T_4= CNF 0.5%. The error bar represents the mean ± SE (standard error) of three measurements. *, ** mean significance at 5% and 1% probability, respectively.

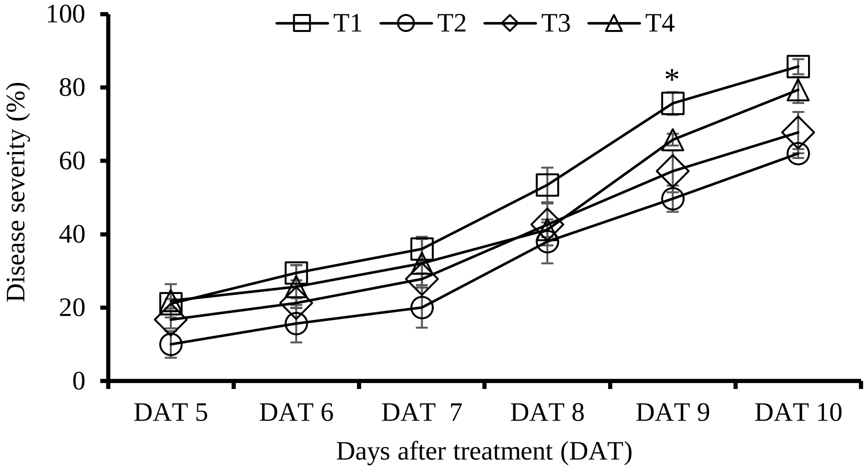


Fig. 4. Effects of chitin nanofiber on disease severity of mango. T_1= Control, T_2= CNF 0.1%, T_3= CNF 0.3%, and T_4= CNF 0.5%. The error bar represents the mean ± SE (standard error) of three measurements. *, ** mean significance at 5% and 1% probability, respectively.

The disease severity of mango gradually increased over the time (Fig. 4). The highest (85%) and the lowest (62%) disease severity were recorded from control and 0.1% CNF, respectively. Lower disease severity was reported if mangoes were treated with chitosan and 1-MCP (Wongmetha and Ke, 2012). Antimicrobial properties of CNF may reduce the severity of diseases in mangoes (El Knidri et al., 2020; Jayaprakash et al., 2021).

Shelf Life

Chitin nanofiber extended the keeping period of mangoes at storage. The highest shelf life (8.5 days) was obtained in mangoes coated with CNF 0.3%, followed by CNF 0.5% (8.33 days) at ambient conditions. The lowest shelf life was calculated from the untreated fruits (6.02 days) (Fig. 5). CNF (0.3%) prolonged the storage life of mangoes by 2.48 days than control. Similarly, irradiated chitosan also extended the shelf life of mangoes (Abbasi et al., 2009). Moreover, chitosan and chitosan with cinnamon oil prolonged the shelf life of mangoes (Yu et al., 2021; Parvin et al., 2023).

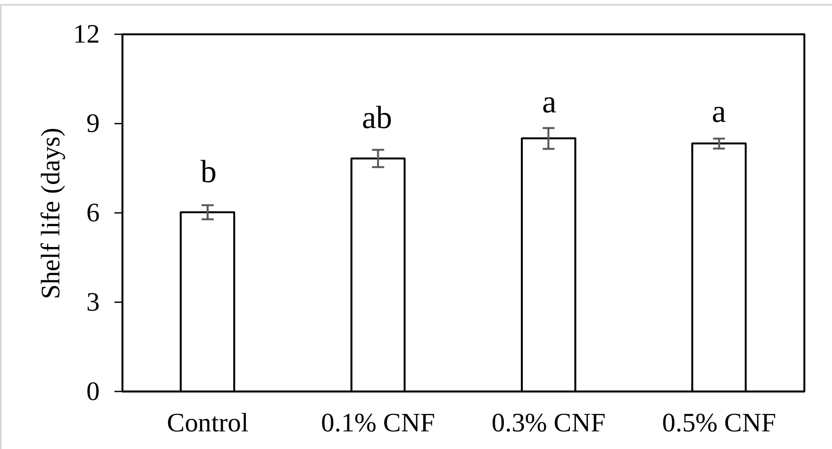


Fig. 5. Effects of chitin nanofiber on the shelf life of mango. The error bar represents mean ± SE (standard error). The treatment means are separated by Tukey's HSD Test at a 5% probability.

APPENDICES

Appendix I. Daily maximal and minimal room temperatures (°C) and relative humidity (%) at afternoon during the experimental period from 25 July to 03 August 2022.

Day	Room Temperature (°C)		Afternoon Relative Humidity (%)
	Maximum	Minimum	
7/25/2022	30.5	28.5	87
7/26/2022	30.1	28.8	85
7/27/2022	31	29.4	83
7/28/2022	30.3	29.3	85
7/29/2022	30.5	29.5	85
7/30/2022	31	30.5	80
7/31/2022	30.9	29.3	89
8/01/2022	30.1	29.2	88
8/02/2022	30.1	29.6	85
8/03/2022	29.7	29.1	85

CONCLUSION

Chitin nanofiber retains postharvest quality and prolongs the shelf life of mangoes. CNF (0.3%) coating decreases disease incidence by 37% and prolongs the shelf life of mangoes by 2.48 days compared to the control. CNF (0.1%) also retains 12 mg more vitamin C and decreases disease severity by 23% than untreated fruits. Therefore, CNF can be used for postharvest mango preservation. However, as the effectivity of the treatment varies due to varied concentrations, further research is required for other mangoes and different fruits. Moreover, the procedure of CNF extraction should be simple and economical.

Funding statement
This research was supported by the project from the Research and Innovation Centre of Khulna University, Khulna, Bangladesh; award # 06/2021 to the corresponding author.

Acknowledgement
The authors are thankful to the Research and Innovation Centre of Khulna University, Khulna, Bangladesh, for funding the research. The authors express their sincere gratitude to the Lab of Horticulture, Agrotechnology Discipline, Khulna University, Bangladesh, for their support during the experimental period.

Credit author statement
Shirazoom Munira: Methodology, Investigation, Analysis, Writing – preliminary draft; Md. Yamin Kabir: Supervision, Conceptualization, Analyzing, Writing – draft, review & editing, Fund acquisition.Shamim Ahmed Kamal Uddin Khan: Conceptualization, Co-supervision; Md. Iftekhar Shams: CNF preparation and supply.

Conflict of interest
The authors at this moment hereby declare that there is no conflict of interest.

REFERENCES

Abbasi, N. A., Iqbal, Z., Maqbool, M., & Hafiz, I. A. (2009). Postharvest quality of mango (*Mangifera indica* L.) fruit as affected by chitosan coating. *Pakistan Journal of Botany*, *41*(1), 343-357.

Al-Dairi, M., & Pathare, P. B. (2024). Evaluation of physio-chemical characteristics of ‘Fard’ banana using computer vision system. *Journal of Agriculture and Food Research*, *15*, 101057.

https://doi.org/10.1016/j.jafr.2024.101057

AOAC International (2012). Aromatic intermediates and derivatives. *In*: Official Methods of Analysis of AOAC International, 19th ed.; Latimar, G.W., Ed.; Association of Official Analytical Chemists: Washington, DC, USA; pp. A.IV.1–A.IV.17, ISBN1 0935584838; ISBN2 9780935584837.

BBS (2022). Yearbook of Agricultural Statistics of Bangladesh. Bangladesh Bureau of Statistics, Statistics and informatics Division, Ministry of Planning, Government People's Republic of Bangladesh, 28th series, 204.

Bini, K., Krishnan, A.G., Jacob, J., Supriya, R., & Thomas, K.S. (2024). Mango: The King of the Tropics. In: Uthup, T.K., Karumamkandathil, R. (eds) Economically Important Trees: Origin, Evolution, Genetic Diversity and Ecology. Sustainable Development and Biodiversity, vol 37. Springer, Singapore. https://doi.org/10.1007/978-981-97-5940-8_8.

Camatari, F. O. D. S., Santana, L. C. L. D. A., Carnelossi, M. A. G., Alexandre, A. P. S., Nunes, M. L., Goulart, M. O. F., & Silva, M. A. A. P. D. (2017). Impact of edible coatings based on cassava starch and chitosan on the post-harvest shelf life of mango (*Mangifera indica*) 'Tommy Atkins' fruits. *Food Science and Technology*, *38*, 86-95. Doi: https://doi.org/10.1590/1678-457X.16417

Dhali, S., Khan, S. A. K. U., Pérez, J. C. D., & Kabir, M. Y. (2024). Effects of calcium chloride on shelf life and quality of banana (*Musa sapientum* cv. Jin). *Journal of the Bangladesh Agricultural University*, *22*(4), 530-538. https://doi.org/10.3329/jbau.v22i4.78872

do Nascimento Nunes, M. C. (2008). Impact of environmental conditions on fruit and vegetable quality. *Stewart Postharvest Review*, *4*(4), 1-14.

Doreyappy-Gowda, I.N.D., & Huddar, A.G. (2001). Studies on ripening changes in mango (*Mangifera indica* L.) fruits. *Journal of Food Science and Technology*, *38*, 135-137.

El Knidri, H., Laajeb, A., & Lahsini, A. (2020). Chitin and chitosan: chemistry, solubility, fiber formation, and their potential applications. In *Handbook of Chitin and Chitosan* (pp. 35-57). Elsevier. https://doi.org/10.1016/B978-0-12-817970-3.00002-X

Fisk, C. L., Silver, A. M., Strik, B. C.,& Zhao, Y. (2008). Postharvest quality of hardy kiwifruit (*Actinidia arguta* 'Ananasnaya') associated with packaging and storage conditions. *Postharvest Biology and Technology*, *47*(3), 338-345. https://doi.org/10.1016/j.postharvbio.2007.07.015.

Giovannoni, J., Nguyen, C., Ampofo, B., Zhong, S., & Fei, Z. (2017). The epigenome and transcriptional dynamics of fruit ripening. *Annual Review of Plant Biology*, *68*(1), 61-84. https://doi.org/10.1146/annurev-arplant-042916-040906.

Hasan, M. Z., Morshed, M. N., Islam, M. A., Hera, M. H. R., & Hassan, M. K. (2020). Effects of different concentrations of chitosan on shelf life and quality of mango. *Sustainability in Food and Agriculture (SFNA) 1*(1), 21-26. http://doi.org/10.26480/sfna.01.2020.21.26

Hassan, M.K. (2006). Constitutive alkenyl resorcinol's and resistance to postharvest diseases in Mango. Ph.D. Thesis, school of agronomy and horticulture, The University of Quensland Australia, *286*. https://doi.org/10.14264/158131

Ifuku, S., Nogi, M., Abe, K., Yoshioka, M., Morimoto, M., Saimoto, H., & Yano, H. (2011). Simple preparation method of chitin nanofibers with a uniform width of 10–20 nm from prawn shell under neutral conditions. *Carbohydrate Polymers*, *84*(2), 762-764. https://doi.org/10.1016/j.carbpol.2010.04.039

Ifuku, S. (2014). Chitin and chitosan nanofibers: Preparation and chemical modifications. *Molecules*, *19*(11), 18367-18380. https://doi.org/10.3390/molecules191118367

Kabir, M. Y., & Hossain, S. K. (2024). Botanical extracts improve postharvest quality and extend the shelf life of papaya (*Carica papaya* L. cv. Shahi). *New Zealand Journal of Crop and Horticultural Science*, 1-17. https://doi.org/10.1080/01140671.2024.2348137

Lekhon, S. N. R., Khan, S. A. K. U., & Kabir, M. Y. (2024). Postharvest Quality of Calcium Chloride-Treated Strawberry (*Fragaria* x *ananassa* cv. Festival) in CoolBot Storage. *Journal of the Bangladesh Agricultural University*, *22*(4), 547-557. https://doi.org/10.3329/jbau.v22i4.78874

Li, D., & Xia, Y. (2004). Electrospinning of nanofibers: reinventing the wheel. *Advanced Materials*, *16*(14), 1151-1170. https://doi.org/10.1002/adma.200400719

Munira, S., Khan, S. A. K. U., & Kabir, M. Y. (2024). Chitosan Maintains Postharvest Quality and Improves the Shelf Life of Fruits. *Khulna University Studies*, 85-94. https://doi.org/10.53808/KUS.2024.21.02.1278-ls

Naleo, S., Sema, A., & Maiti, C. (2018). Effect of plant growth regulators and packaging on flowering, fruit quality and shelf life in mango cv. Amrapali. *Journal of Experimental Agriculture International*, *20*(6), 1-8. https://doi.org/10.9734/JEAI/2018/38059

Nerdy, N. (2018). Determination of vitamin C in various colors of bell pepper (*Capsicum annuum*) by Titration Method. *Alchemy Jurnal Penelitian Kimia*, *14*(1), 164-177. https://doi.org/10.20961/ALCHEMY.14.1.15738.164-178

Ngo, T. M. P., Nguyen, T. H., Dang, T. M. Q., Do, T. V. T., Reungsang, A., Chaiwong, N., & Rachtanapun, P. (2021). Effect of pectin/nanochitosan-based coatings and storage temperature on shelf life extension of "Elephant" Mango (*Mangifera indica* L.) Fruit. *Polymers*, *13*(19), 3430. https://doi.org/10.3390/polym13193430

Nitu, N. J., Ullah, M. S., Howlader, P., Mehedi, M. N. H., Meem, H. Z., & Bose, S. K. (2025). Chitosan oligosaccharides maintained postharvest quality and increased shelf life of mango. *Journal of Horticulture and Postharvest Research*, *8*(1), 43-66. 10.22077/jhpr.2024.7888.1395.

Nunes, M. C. N., Emond, J. P., Brecht, J. K., Dea, S., & Proulx, E. (2007). Quality curves for mango fruit (cv. Tommy Atkins and Palmer) stored at chilling and nonchilling temperatures. *Journal of Food Quality*, *30*(1), 104-120. https://doi.org/10.1111/j.1745-4557.2007.00109.x

Parvin, N., Rahman, A., Roy, J., Rashid, M. H., Paul, N. C., Mahamud, M. A., & Kader, M. A. (2023). Chitosan coating improves postharvest shelf-life of mango (*Mangifera indica* L.). *Horticulturae*, *9*(1), 64. https://doi.org/10.3390/horticulturae9010064

Rahman, H., & Akter, A. (2019). Performance of Mango (*Mangifera indica* L.) Genotypes at Jamalpur Region of Bangladesh. *International Journal of Genetic Engineering and Recombination*, *5*(1), 19-37.

Sampa, A. Y., Alam, M. A., Latif, M. A., & Islam, M. M. (2019). Socio-economic status and rationale of mango cultivation based on some selected areas in Rajshahi district of Bangladesh. *Research in Agriculture Livestock and Fisheries*, *6*(1), 79-90.

Shams, M. I., Kabir, M. Y., Ali, M. Y., Billah, M., Bristi, M. J. S., Kaminaka, H., ... & Ifuku, S. (2025). Exploring shrimp-derived chitin nanofiber as a sustainable alternative to urea for rice (Oryza sativa cv. BRRI dhan67) Cultivation. *Applied Nano*, *6*(2), 6. https://doi.org/10.3390/applnano6020006

Sellitto, V. M., Zara, S., Fracchetti, F., Capozzi, V., & Nardi, T. (2021). Microbial biocontrol as an alternative to synthetic fungicides: Boundaries between pre-and postharvest applications on vegetables and fruits. *Fermentation*, *7*(2), 60. https://doi.org/10.3390/fermentation7020060

Shamshina, J. L., Oldham, T., & Rogers, R. D. (2019). Applications of chitin in agriculture. In *Sustainable agriculture reviews 36: chitin and chitosan: applications in food, agriculture, pharmacy, medicine and wastewater treatment* (pp. 125-146). Cham: Springer International Publishing. https://link.springer.com/chapter/10.1007/978-3-030-16581-9_4.

Shao, Y. Z., Zeng, J. K., Hong, T. A. N. G., Yi, Z. H. O. U., & Wen, L. I. (2019). The chemical treatments combined with antagonistic yeast control anthracnose and maintain the quality of postharvest mango fruit. *Journal of Integrative Agriculture*, *18*(5), 1159-1169. https://doi.org/10.1016/S2095-3119(18)62128-8

Sivakumar, D., Hewarathgamagae, N. K., Wilson Wijeratnam, R. S., & Wijesundera, R. L. C. (2002). Effect of ammonium carbonate and sodium bicarbonate on anthracnose of papaya. *Phytoparasitica*, *30*, 486-492. https://doi.org/10.1007/BF02979753.

Thakur, R. R., Mangaraj, S., Tripathi, M. K., Singh, K. P., & Jadhav, M. L. (2022). Design of a modified atmosphere storage system for shelf life enhancement of mango (*Mangifera indica*, cv. Amrapali). *Journal of Food Processing and Preservation*, *46*(9), e16600. https://doi.org/10.1111/jfpp.16600.

Wongmetha, O., & Ke, L. S. (2012). The quality maintenance and extending storage life of mango fruit after postharvest treatments. *International Journal of Agricultural and Biosystems Engineering*, *6*(9), 798-803. https://doi.org/10.1999/1307-6892/8065

Xia, Y., Yang, P., Sun, Y., Wu, Y., Mayers, B., Gates, B., & Yan, H. (2003). One-dimensional nanostructures: synthesis, characterization, and applications. *Advanced Materials*, *15*(5), 353-389. https://doi.org/10.1002/adma.200390087.

Yu, K., Xu, J., Zhou, L., Zou, L., & Liu, W. (2021). Effect of chitosan coatings with cinnamon essential oil on postharvest quality of mangoes. *Foods*, *10*(12), 3003.

Zhang, H., Li, R., & Liu, W. (2011). Effects of chitin and its derivative chitosan on postharvest decay of fruits: A review. *International Journal of Molecular Sciences*, *12*(2), 917-934.

Zhu, X., Wang, Q., Cao, J., & Jiang, W. (2008). Effects of chitosan coating on postharvest quality of mango (*Mangifera indica* L. cv. Tainong) fruits. *Journal of Food Processing and Preservation*, *32*(5), 770-784. https://doi.org/10.1111/j.1745-4549.2008.00213.x

JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH
2025, VOL. 8(4), 553-560

Journal of Horticulture and Postharvest Research

Journal homepage: www.jhpr.birjand.ac.ir

University of Birjand

Comparative analysis of tocopherol content in pulp and peel of eight apple cultivars

Elhadi M. Yahia[1,*], José de Jesús Ornelas-Paz[2] and Claudia Inés Victoria-Campos[3]

1, Faculty of Natural Sciences, Autonomous University of Querétaro, Querétaro, Mexico.
2, Research Center for Food and Development, Cuauhtémoc, Chihuahua, Mexico.
3, Faculty of Nursing and Nutrition, Autonomous University of San Luis Potosí, San Luis Potosí, Mexico.

ARTICLE INFO

Original Article

Article history:
Received 5 March 2025
Revised 15 May 2025
Accepted 27 May 2025

Keywords:
Antioxidants
Malus domestica
Nutrition
Postharvest
Vitamin E

DOI: 10.22077/jhpr.2025.9049.1483
P-ISSN: 2588-4883
E-ISSN: 2588-6169

***Corresponding author:**
Faculty of Natural Sciences, Autonomous University of Querétaro, Querétaro, Mexico.

Email: yahia@uaq.mx

ABSTRACT

Purpose: Tocopherols (vitamin E) are important bioactive components in some fruits, possessing potent antioxidant activity and exerting a significant influence on metabolic pathways, human nutrition, and health. However, the content of tocopherols in apple fruit peels and pulp has scarcely been investigated. **Research method:** Fruits of eight apple cultivars were evaluated for color, total soluble solids (TSS), and tocopherols from both the pulp and peels were extracted and analyzed. **Findings**: The content of α-tocopherol (0.073–0.656 and 0.01–0.02 mg/100 g fresh weight (FW) in the peel and pulp, respectively) was higher than that δ-tocopherol (0.002–0.01 and 0.0001–0.0014 mg 100 g FW in the peel and pulp, respectively), and both tocopherols were higher in the peels than in the pulp. The content of α-tocopherol followed the order: 'Braeburn' > 'Golden Delicious' > 'Rome' > 'Red Delicious' and 'Royal Gala' > 'Jonagold' > 'Fuji' > 'Granny Smith'. Low levels of δ-tocopherol were detected in the peels. 'Granny Smith' apples had the highest δ-tocopherol content in the peel (0.01 mg 100 g FW), whereas 'Rome Beauty', 'Royal Gala' and 'Fuji' apples exhibited the lowest levels (0.002 mg 100 g FW). **Research limitations:** There were no limitations identified. **Originality/Value:** Our results indicate that tocopherols content in apple peels and pulp is relatively low compared to other types of fruits rich in vitamin E. However, regular consumption of whole apples may contribute to daily vitamin E intake and help prevent the oxidation of lipophilic biomolecules.

INTRODUCTION

Apples (*Malus domestica* Borkh) are among the most consumed fruit in the world and, therefore, they are an important source of bioactive compounds for human nutrition and health. Epidemiological studies have related the daily consumption of an apple (100 g/d) with a decrease in all mortality causes (Hodgson et al., 2016). Several bioactive compounds have widely been identified and quantified in apple fruit, including vitamin C, phenolic compounds, phytosterols, chlorophyll, and carotenoids (Bohn & Bouayed, 2020). However, other bioactive compounds in apple fruit such as tocopherols have received scarce attention (Akšic et al., 2021; Bohn & Bouayed, 2020; Górnaś et al., 2024; Fernández-Cancelo et al., 2022). In the past, the term Vitamin E included eight toco-chromanols naturally existing in plant materials (α-, β-,γ- and σ- tocopherol and the respective tocotrienols), which were highly recognized as quenchers of free radicals in lipid environments (Falk & Munné-Bosch, 2010). However, in recent years international health boards agreed that α-tocopherol is the only human bioactive form of vitamin E (Institute of Medicine, 2000; EFSA NDA Panel, 2024). The Daily Recommended Intake (DRI) of vitamin E is 15 mg/d of α-tocopherol, but its consumption usually is below this amount (IOM, 2001). In European dietary surveys, the consumption of vitamin E in adults varied from 9.7 to 16 mg/ day (EFSA, 2024). Vitamin E deficiency is associated with serious health problems related to neurological function, energy-sulfured amino acids, and single-carbon metabolism (Blaner et al., 2021). The bioavailability of vitamin E from enriched apples has been estimated to be nearly 10%, with this efficiency increased up to 20% and 33%, with 6% and 21% of dietary fat in meals, respectively (Bruno et al., 2006).

The intrinsic, but not relative, antioxidant activity of flavonoids as chain-breaking antioxidants was measured for few flavonoids and was found to be smaller than that of α-tocopherol (Pedrielli & Skribsted, 2002). Furthermore, there is an intimate relationship between polyphenolic compounds and vitamin E, which increase the importance of tocopherols in the apple matrix. For example, higher antioxidant effects of quercetin, epicatechin, and ferulic acid have been observed when they are in combination with α-tocopherol, due to a significant synergistic effect (Pedrielli & Skribsted, 2002; Trombino et al., 2004). In humans, a diet rich in vitamin E is correlated with the reduction of free radicals, and consequently protection against oxidation of some molecules like phospholipids in cellular membranes and low-density lipoproteins (Blaner et al., 2021). In addition, this vitamin seems to reduce the risk to suffer some types of cancer (prostate, liver, bladder, and pancreatic cancers) and a decrease in Alzheimer’s disease incidence (Blaner et al., 2021; Yang et al., 2019).

In apples, like in some other fruits, the endogenous levels of tocopherols appear to have a great technological importance due to their protection of cellular membranes during low night temperatures in the pre-harvest stage and during cold storage in the post-harvest stage, which affects the commercial quality of the fruit (Fernández-Cancelo et al., 2022). Tocopherols also play important roles as antioxidants in chloroplasts and thylakoids, however, they are also involved in germination, phytohormonal balance, carbohydrate metabolism, growth, flowering, leaf senescence, and stress resistance in plants, fruits, and seeds (Falk & Munné-Bosch, 2010; Fernández-Cancelo et al., 2022; Sadiq et al., 2019). In Mexico, apples are extensively cultivated, where more than 800,000 tons being produced in 2022, placing the country within the top 20 producer countries in the world (FAOSTAT, 2022; SADER, 2023). However, several tons of apple pomace with significant quantities of bioactive compounds including tocopherols, are produced and wasted annually, and represent a significant source of environmental pollution. Although the content of tocopherols has been reported in the seeds of several apple cultivars, their content in apple skins and pulp has been scarcely reported (Fernández-Cancelo et al., 2022; Bianchi et al., 2020). Thus, the objective of this work was to investigate the tocopherol content in the peel and pulp of eight important apple cultivars.

MATERIALS AND METHODS

Chemicals and solvents

High-performance liquid chromatography (HPLC) grade methanol, reactive grade anhydrous granular sodium sulphate (Na_2SO_4), anhydrous ethanol, and petroleum ether were purchased from J.T. Baker (Baker Mallinckrodt, México). α- and σ-tocopherol standard compounds were purchased from Sigma (Sigma-Aldrich, St. Louis, MO). HPLC-grade water was prepared by a Milli-Qplus purification system (Millipore Corp., Bedford, MA, USA).

Plant material

Fruits from eight apple cultivars were obtained from local market in Querétaro, Mexico. Six cultivars (‘Fuji’, ‘Rome Beauty’, ‘Jonagold’, ‘Red Delicious’, ‘Royal Gala’ and ‘Braeburn’) were imported, while ‘Granny Smith’ and ‘Golden Delicious’ apples were produced in Mexico. Twenty apples of each cultivar were selected for uniform size and color, and freedom from defects.

Total soluble solids and color measurement

All apples were ripe and evaluated for total soluble solids content (% TSS) and color. TSS was measured in juice obtained from a representative portion of each fruit using a hand refractometer (ATAGO, Co. Ltd., Japan). The external color was evaluated by taking 2 readings in two opposite regions around of equatorial zone of each fruit, using a CM-2002 Minolta spectrophotometer (Minolta Co. Ltd., Japan), calibrated with a white pattern and zeroed on each occasion just before sampling.

Extraction and analysis of tocopherols

The apple peel was separated from the pulp using a potato peeler. Samples of one gram of peel or pulp tissue from each of five randomly selected fruit were ground in a mortar and Na_2SO4 was added until complete dehydration. Samples were extracted with 25 ml of ethanol for 15 min using an ultrasonic bath and then centrifuged at 5000 x *g* for 5 min at 2 °C. The pellet was eliminated, and 3.5 mL of petroleum ether was added to the apple extract, samples were then shaken, and 5 mL of water was added. The mixture was centrifuged again under the same conditions described above and the upper layer was separated and evaporated. The obtained residue was dissolved in 1.5 mL of methanol and filtered through a polyethylene membrane with a pore size of 0.45 μm (Millipore Corp., Bedford, MA) before injection of 50 μL into the High Performance Liquid Chromatographic (HPLC) system.

The HP 1100 series HPLC system (Hewlett-Packard GmbH, Waldbronn, Germany) was equipped with a fluorescence detector (FLD). The mobile phase was methanol/water (95:5 % v/v) and flow rate was at 1 mL/min through a Symmetry C18 analytical column (4.6 x 150 mm, 3.5 mm; Waters Co., Milford, MA) which was kept at 25°C. The tocopherols were monitored at λ_{ex}= 294 nm, λ_{em}= 326 nm. The identification and quantification of α- and σ-tocopherol were achieved by standard compounds.

Statistical analysis

All analyses were performed in triplicate, the collected data were analyzed by a one-way ANOVA and comparison of the means by the test of Tukey-Kramer, all analyses considered a $p < 0.05$ as statistical significance using JMP software (SAS Institute Inc., Cary, NC).

RESULTS

Color and total soluble solids content

The luminosity was higher in 'Granny Smith', 'Golden Delicious' and 'Fuji' apples, representing the lighter aspect of these fruits, whereas lower values were observed in 'Rome' and 'Red Delicious' apples, indicating their darker aspects (Table 1). The a* values representing the redder (positive values) to greener (negative values) coloration, were higher in 'Red Gala' apples which represent their intense coloration, followed by 'Red Delicious', 'Jonagold' and 'Rome' apples. The 'Granny Smith' apples had negative a* values, indicating the green color of the fruit of this cultivar. 'Golden Delicious' apples had the highest value of b* variable, indicating their yellow color. 'Fuji' and 'Braeburn' apples had a combination of lower redness and intermediate b* values, which describe their bicolor peels.

The TSS measured for the eight apple cultivars are shown in Table 1, and all cultivars were within the range of 11.7 to 15.4 °Brix. Color and TSS are important commercial indicators of fruit quality and ripeness. Apple color is an important fruit quality attribute that influences the consumer's preference. The ranges of the skin color are related to both the absolute and relative concentrations of at least three pigments. Chlorophylls are the most abundant in the epidermal and hypodermal cell layers of unripe green apples, while the color of ripe red apples is due to the content of anthocyanins, and carotenoids are the most abundant pigment in ripe yellow cultivars (Fernández-Cancelo et al., 2022). The purpose of the color evaluation in this study was to characterize and indicate the physiological stage of the fruit at the moment of evaluation. The color variables were similar to fruits of the same cultivars cultivated in Greece ('Granny Smith', 'Golden Delicious' and 'Fuji' apples), Belgium ('Jonagold' apples), Argentina ('Granny Smith' and 'Red Delicious'), and India ('Golden Delicious', 'Granny Smith', 'Red Gala', and 'Red Delicious') (Drogoudi et al., 2008; Gwanpua et al., 2014; Kumar et al., 2018; Piagentini & Pirovani, 2017).

TSS (°Brix) is a good indicator of fruit sugar content and sweetness. For many apple cultivars, like in 'Golden Delicious', the TSS needs to be around 12%, while others, like 'Royal Gala', it needs to be above 12% (Hoehn et al., 2003). In others cultivars such as 'Granny Smith', a proper TSS is around 11%. All eight cultivars in our study had adequate values of color and TSS for consumption and good quality (Table 1).

Table 1. Fruit maturity parameters of the eight apple cultivars analyzed.

Cultivar	TSS	Color variables L*	a*	b*
'Granny Smith'	11.7 ± 0.2 c	69.4 ± 0.3 a	-5.6 ± 0.2 g	37.1 ± 0.3 b
'Red Gala'	13.4 ± 0.3 b	53.6 ± 0.8 c	27.7 ± 0.7 a	19.0 ± 0.6 e
'Golden Delicious'	15.4 ± 0.2 a	74.1 ± 0.4 a	4.2 ± 0.4 f	42.0 ± 0.3 a
'Red Delicious'	13.5 ± 0.3 b	40.9 ± 0.3 d	20.9 ± 0.4 c	5.2 ± 0.3 f
'Jonagold'	14.6 ± 0.3 a	52.1 ± 1.0 c	23.5 ± 0.9 b	21.0 ± 0.9 de
'Rome'	12.9 ± 0.2 b	38.0 ± 0.3 e	23.1 ± 0.4 b	5.3 ± 0.3 f
'Fuji'	13.1 ± 0.3 b	61.8 ± 1.1 b	7.5 ± 1.2 e	25.3 ± 0.9 c
'Braeburn'	12.7 ± 0.2 b	55.6 ± 0.9 c	14.8 ± 1.0 d	23.1 ± 1.0 cd

TSS: Total soluble solids, the color variables include: Lightness (L*), green to red (a*), and blue to yellow (b*). Different letters within the same column indicate significant differences between apple varieties.

Content of tocopherols

The content of α-tocopherol (0.073 – 0.656 and 0.01 – 0.02 mg 100 g^{-1} FW in peel and pulp, respectively) was higher than δ-tocopherol (0.002 – 0.01 and 0.0001 – 0.0014 mg 100 g^{-1} FW in peel and pulp, respectively) in fruit of all apple cultivars (Fig. 1 and 2). The α-tocopherol content (Fig. 1) was considerably lower in the pulp than in the peel, and was 0.01-0.02 mg 100 g^{-1} of fresh pulp of 'Braeburn', which had the highest content. In both pulp and peel tissues, the levels of δ-tocopherol were low (Fig. 2). The peel of 'Granny Smith' and 'Braeburn' apples had the highest amount of δ-tocopherol (0.01 and 0.007 mg 100 g^{-1} FW, respectively). In the pulp, the content of δ-tocopherol was lower than 0.0015 mg 100 g^{-1} of fresh pulp in all of the cultivars, being 'Granny Smith' apples with the highest content. This vitamer was not detected in 'Red Delicious' apples. In the chromatograms (Fig. 3), it was possible to observe several peaks (at least 3 of them) between the δ- and α-tocopherol peaks that exhibited similar fluorescent characteristics to tocopherols. Therefore, it is suspected that these apples contain at least another tocopherol, β or γ, tocotrienols or both.

The content of α-tocopherol in the tested apple cultivars was higher than that reported in yellow peels of 'Golden Reinders' apples cultivated in the mountain and valley regions of Spain (0.0105 – 0.012 mg 100 g^{-1}) (Fernández-Cancelo et al., 2022). The peel of 'Granny Smith' and 'Fuji' apples had the lowest amount of α-tocopherol (Fig. 1) possibly because these cultivars do not show the process of the chlorophyll degradation during ripening. Chlorophyll biodegradation produces phytol, which is a limiting factor in the synthesis of tocopherols (Fernández-Cancelo et al., 2022; Sadiq et al., 2019). The rest of the cultivars, especially 'Golden Delicious' and 'Braeburn', had a high amount of α-tocopherol in the peel (Fig. 1), and exhibited chlorophyll degradation and biosynthesis of carotenoids. Interestingly, the content of α-tocopherol was significantly lower in the apple pulp than reported previously for 24 different cultivars from Italy (0.13 – 0.33 mg 100 g^{-1} FW) (Bianchi et al., 2020).

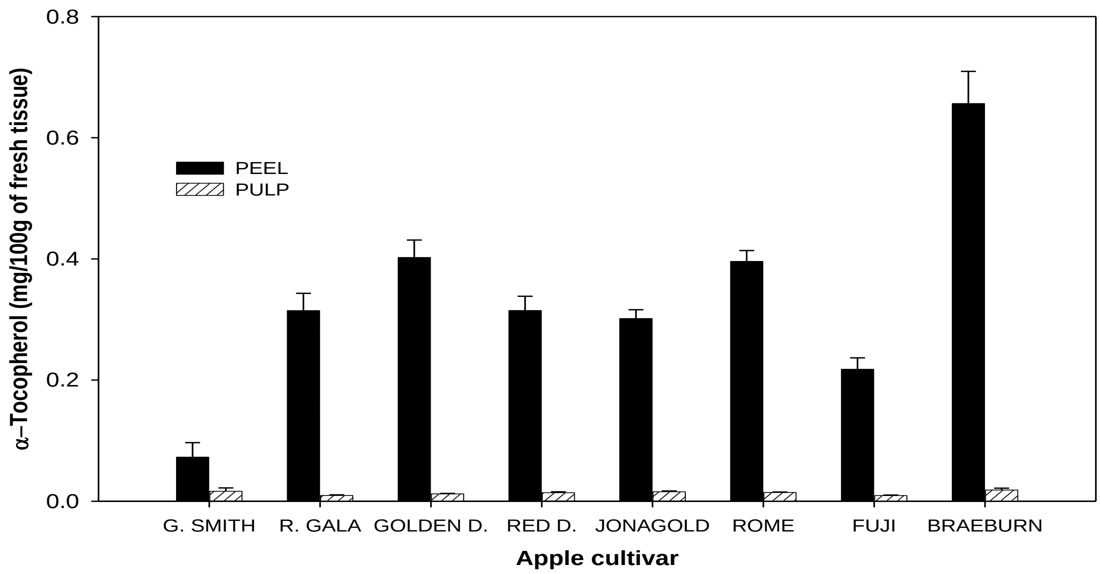


Fig. 1. α-Tocopherol content in the peel and pulp of eight apple cultivars of apples. Upper bars are the standard error of the mean. Different letters in peel and pulp bars indicate significant differences between apple cultivars. The asterisk indicates significant differences between the peel and the pulp (p<0.05).

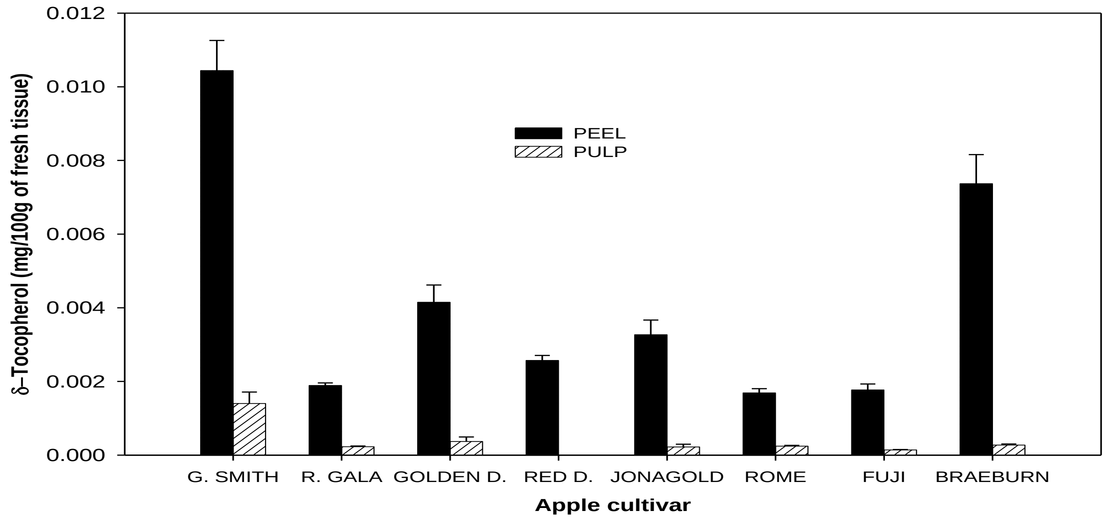


Fig. 2. δ-Tocopherol content in the peel and pulp of eight apple cultivars. Upper bars are the standard error of the mean. Different letters in peel and pulp bars indicate significant differences between apple cultivars. The asterisk indicates significant differences between the peel and the pulp ($p<0.05$).

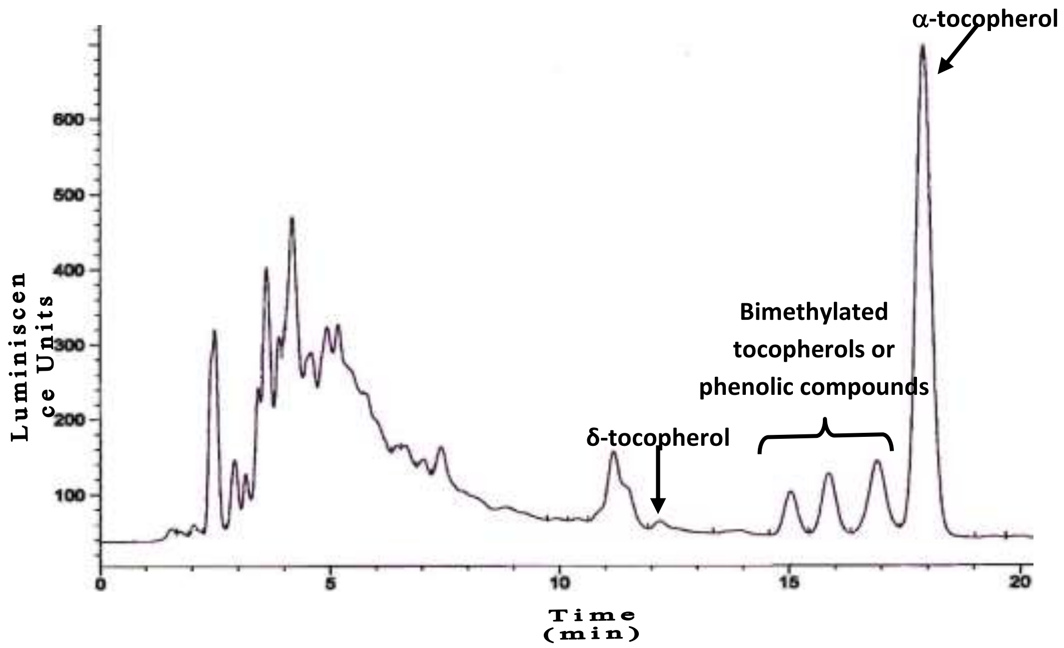


Fig. 3. Typical High Performance Liquid Chromatography (HPLC) chromatogram of tocopherols contained in extracts of apple peel.

Fernández-Cancelo et al. (2022) found γ-tocopherol in yellow peels of 'Golden Reinders' apples, however, at lower values than that of δ-tocopherol in our study (0.001 – 0.004 mg 100 g^{-1}). The variation in the content of tocopherols among the different cultivars might be explained by genetic determinants (Akšic et al., 2021), but no significant relationship was found between the apple origin and the content of tocopherols (Górnaś et al., 2023). Fernández-Cancelo et al. (2022) found up to 10-fold higher content of γ-tocopherols in peels of apples from mountain regions than in fruits from valleys in Spain, however, α-tocopherol was not affected.

The tentative identification of other tocopherols must be taken with discretion since most of the authors point out that it is not possible to separate β or γ-tocopherol using reversed phase (Górnaś et al., 2024), despite the fact that there are reports that indicate the opposite (Akšic et al., 2021), and some peaks could be a mixture of bimethylated tocopherols. On the other hand, these peaks could originate from phenolic compounds such as catechin or epicatechin, which are excited at similarλ, and also fluoresce.

CONCLUSION

Our results indicate that fruits of the apple cultivars analyzed contain low levels of α-tocopherol (vitamin E). However, these low levels represent an important loss of phytochemicals and antioxidant compounds when the peel of the fruit is removed, as is the custom of some consumers due to safety or other reasons, not only because of the loss of tocopherols, but also because of the synergistic and regenerative effects of these with other antioxidant compounds.

Conflict of interest
The authors declare no conflict of interest.

Author statement
EMY was responsible of the investigation, conceptualization, and funding acquisition, reviewing, editing supervision, writing the original draft. JJOP was responsible for methodology, data analysis, data curation, writing the original draft, reviewing and editing. CIVC was responsible for data curation, writing the original draft, reviewing and editing.

REFERENCES

Akšić, M. F, Lazarević, K., Šegan, S., Natić, M., Tosti, T., Ćirić, I., & Meland, M. (2021). Assessing the fatty acid, carotenoid, and tocopherol compositions of seeds from apple cultivars (*Malus domestica* Borkh.) grown in Norway. *Foods*, *10*(8), 1956. https://doi.org/10.3390/foods10081956

Bianchi, F., Soini, E., Ciesa, F., Bortolotti, L., Guerra, W., Robatscher, P., & Oberhuber, M. (2020). L-ascorbic acid and α-tocopherol content in apple pulp: A comparison between 24 cultivars and annual variations during three harvest seasons. *International Journal of Food Properties*, *23*(1),1624-1638. https://doi.org/10.1080/10942912.2020.1820515

Blaner, W. S., Shmarakov, I. O., & Traber, M. G. (2021). Vitamin A and vitamin E: will the real antioxidant please stand up?. *Annual Review of Nutrition*, *41*(1),105-131. https://doi.org/10.1146/annurev-nutr-082018-124228

Bohn, T., & Bouayed, J. (2020). Apples: an apple a day, still keeping the doctor away?'. In A. K. Jaiswal (Ed.), *Nutritional Composition and Antioxidant Properties of Fruits and Vegetables*, (pp. 595-612). Academic Press. https://doi.org/10.1016/B978-0-12-812780-3.00037-4

Bruno, R. S., Leonard, S. W., Park, S. I., Zhao, Y., & Traber, M.G. (2006). Human vitamin E requirements assessed with the use of apples fortified with deuterium-labeled α-tocopheryl acetate. *The American Journal of Clinical Nutrition*, *83*(2), 299-304. https://doi.org/10.1093/ajcn/83.2.299

Drogoudi, P. D., Michailidis, Z., & Pantelidis, G. (2008) Peel and flesh antioxidant content and harvest quality characteristics of seven apple cultivars. *Scientia Horticulturae*, *115*(2),149-153. https://doi.org/10.1016/j.scienta.2007.08.010

EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens. (2024). Scientific opinion on the tolerable upper intake level for vitamin E. *EFSA Journal*, *22*, e8953. https://doi.org/10.2903/j.efsa.2024.8953

Falk, J., & Munné-Bosch, S. (2010). Tocochromanol functions in plants: antioxidation and beyond. *Journal of Experimental Botany*, *61*(6), 1549-1566. https://doi.org/10.1093/jxb/erq030

FAOSTAT. (2022). Data on Crops and livestock products. Apple production. https://www.fao.org/faostat/en/#data/QCL. Accessed, August 15, 2024.

Fernández-Cancelo, P., Iglesias-Sanchez, A., Torres-Montilla, S., Ribas-Agustí, A., Teixidó, N., Rodriguez-Concepcion, M., & Giné-Bordonaba, J. (2022). Environmentally driven transcriptomic and metabolic changes leading to color differences in "Golden Reinders" apples. *Frontiers in Plant Science*, *13*, 913433. https://doi.org/10.3389/fpls.2022.913433

Górnaś, P., Lācis, G., Mišina, I., & Ikase, L. (2023). Tocopherols in cultivated apple *Malus* sp. seeds: composition, variability and specificity. *Plants*, 12(5), 1169. https://doi.org/10.3390/plants12051169

Górnaś, P., Symoniuk, E., & Soliven, A. (2024). Reversed phase HPLC with UHPLC benefits for the determination of tocochromanols in the seeds of edible fruits in the Rosaceae family. *Food Chemistry*, *460*, 140789. https://doi.org/10.1016/j.foodchem.2024.140789

Gwanpua, S. G., Vicent, V., Verlinden, B. E., Hertog, M. L. A. T. M., Nicolai, B. M., & Geeraerd A. H. (2014). Managing biological variation in skin background colour along the postharvest chain of 'Jonagold' apples. *Postharvest Biology and Technology*, *93*, 61-71. https://doi.org/10.1016/j.postharvbio.2014.02.008

Hodgson, J. M., Prince, R. L., Woodman, R. J., Bondonno, C. P., Ivey, K. L., Bondono, N., Rimm, E. B., Ward, N. C., Croft, K. D., & Lewis, J. R. (2016). Apple intake is inversely associated with all-cause and disease-specific mortality in elderly women. *British Journal of Nutrition*, 115(5), 860-867. https://doi.org/10.1017/S0007114515005231

Hoehn, E., Gasser, F., Guggenbühl, B., & Künsch, U. (2003). Efficacy of instrumental measurements for determination of minimum requirements of firmness, soluble solids, and acidity of several apple varieties in comparison to consumer expectations. *Postharvest Biology and Technology*, *27*(1), 27-37. https://doi.org/10.1016/S0925-5214(02)00190-4

Institute of Medicine (US) Panel on Dietary Antioxidants and Related Compounds. (2000). Dietary reference intakes for vitamin C, vitamin E, selenium, and carotenoids. US: National Academy Press. pp. 531.

Kumar, P., Sethi, S., Sharma, R. R., Singh, S., Saha, S., Sharma, V. K., Verma, M. K., & Sharma, S. K. (2018). Nutritional characterization of apple as a function of genotype. *Journal of Food Science and Technology*, *55*, 2729-2738. https://doi.org/10.1007/s13197-018-3195-x

Pedrielli, P., & Skibsted, L. H. (2002) Antioxidant synergy and regeneration effect of quercetin, (−)-epicatechin, and (+)-catechin on α-tocopherol in homogeneous solutions of peroxidating methyl linoleate. *Journal of Agricultural and Food Chemistry*, *50*(24), 7138-7144. https://doi.org/10.1021/jf020437l

Piagentini, A.M., & Pirovani, M. E. (2017) Total phenolics content, antioxidant capacity, physicochemical attributes, and browning susceptibility of different apple cultivars for minimal processing. *International Journal of Fruit Science*, *17*(1), 102-116. https://doi.org/10.1080/15538362.2016.1262304

SADER, (2023), Secretaría de Agricultura y Desarrollo Rural. Todo sobre la manzana. https://www.gob.mx/agricultura/es/articulos/todo-sobre-la-manzana. Accessed, August 24.

Sadiq, M., Akram, N. A., Ashraf, M., Al-Qurainy, F., & Ahmad, P. (2019). Alpha-tocopherol-induced regulation of growth and metabolism in plants under non-stress and stress conditions. *Journal of Plant Growth Regulation*, *38*, 1325-1340. https://doi.org/10.1007/s00344-019-09936

Trombino, S., Serini, S., Di Nicuolo, F., Celleno, L., Andò, S., Picci, N., Calviello, G., & Palozza, P. (2004). Antioxidant effect of ferulic acid in isolated membranes and intact cells: synergistic interactions with α-tocopherol, β-carotene, and ascorbic acid. *Journal of Agricultural and Food Chemistry*, *52*(8), 2411-2420. https://doi.org/10.1021/jf0303924

Yang, C. S., Luo, P., Zeng, Z., Wang, H., Malafa, M., & Suh, N. (2020). Vitamin E and cancer prevention: Studies with different forms of tocopherols and tocotrienols. *Molecular Carcinogenesis*, *59*(4), 365-389. https://doi.org/10.1002/mc.23160

JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH
2025, VOL. 8(4), 561-580

Journal of Horticulture and Postharvest Research

Journal homepage: www.jhpr.birjand.ac.ir

University of Birjand

Environmental and physiological determinants of growth and phytochemical variation in Kratom (*Mitragyna speciosa*): A review

Bujaningrum Ega Agustina[1], Berna Elya[1,*] and Roshamur Cahyan Forestrani[1]

1, Faculty of Pharmacy Universitas Indonesia; Depok 16424 West Java, Indonesia

ARTICLE INFO

Original Article

Article history:
Received 2 August 2025
Revised 30 September 2025
Accepted 3 October 2025

Keywords:
Environmental factors
Growth optimization
Kratom *(Mitragyna speciosa)*
Physiological determinants
Phytochemical variation

DOI: 10.22077/jhpr.2025.9870.1557
P-ISSN: 2588-4883
E-ISSN: 2588-6169

***Corresponding author:**
Faculty of Pharmacy Universitas Indonesia; Depok 16424 West Java, Indonesia.

Email: berna.elya@farmasi.ui.ac.id

ABSTRACT

Purpose: Kratom (*Mitragyna speciosa*) is a tropical plant native to Southeast Asia, widely used for traditional and emerging therapeutic purposes. This review investigates the environmental and physiological determinants of phytochemical variation in *Mitragyna speciosa* (kratom), with a focus on optimizing alkaloid yield and raw material quality. As kratom gains commercial traction, particularly in Western markets, the need for standardizing cultivation practices becomes increasingly urgent. **Findings:** Geographic origin significantly influences mitragynine content, with native Southeast Asian samples displaying higher and more consistent levels than those cultivated elsewhere. Environmental factors such as light exposure, temperature, soil acidity, and nutrient composition play crucial roles in modulating both plant morphology and secondary metabolite biosynthesis. Internally, leaf maturity, organ specificity, and vein color are linked to variable alkaloid profiles, reflecting genetic and developmental influences. Alkaloid biosynthesis is regulated not only by climate and geography but also at the molecular level through gene–environment interactions. Studies across continents and related species further underscore the diversity and adaptability of the genus. These findings suggest that integrated, site-specific agronomic strategies are essential to support kratom's evolution as a standardized phytopharmaceutical. **Limitations:** Most existing studies are regionally constrained, lack standardized methodology, and rarely incorporate multi-site or molecular validation, limiting their broader applicability. **Directions for future research:** Future work should prioritize field-based, multi-environment trials to validate the effects of environmental variables on alkaloid biosynthesis and biomass traits. In addition, controlled environment experiments, genetic profiling, and enzyme-specific biosynthetic studies are essential to refine cultivation protocols and support regulatory frameworks. Integrative agronomic and phytochemical modeling is also needed to guide the development of standardized and high-quality kratom production systems.

INTRODUCTION

Kratom (*Mitragyna speciosa* Korth.), a member of the Rubiaceae family, is a tropical plant traditionally used across Southeast Asia, including Indonesia, Thailand, Malaysia, the Philippines, Vietnam, and Papua New Guinea (Löfstrand et al., 2014). It is known by various local names, such as "Kratom" in Thailand, "kadamba" in Indonesia, "ketum" and "biak" in Malaysia, "giam" in Vietnam, and "mambog" in the Philippines (Chua, 2001). *Mitragyna speciosa* thrives in humid environments, particularly along rivers, swamps, and alluvial lowlands. Its natural habitat includes freshwater swamp forests, riverbanks, and peat soils (Nilus et al., 2011).

Historically, Kratom has been used by local communities to treat various ailments. In Kalimantan, Indonesia, for example, Kratom leaves are brewed like tea to relieve fatigue, enhance stamina, and alleviate pain and fever (Utomo et al., 2022). Over the past decade, global interest in this plant has grown due to its diverse pharmacological properties, including analgesic, anxiolytic, and anti-inflammatory effects in treating chronic conditions (Sitthiphon & Prakasit, 2025). Some studies have even reported its potential as an alternative therapy in opioid cessation programs (Singh et al., 2023; Tabanelli et al., 2023; Indriyanti et al., 2024). Despite its medicinal and ethnobotanical significance (Arief et al., 2025; Begum et al., 2025), Kratom remains controversial and often mischaracterized due to its association with substance misuse (Cinosi et al., 2015; Predescu et al., 2025), leading to legal restrictions in several countries (Diversion Control Division, 2024). Nevertheless, Kratom retains high popularity and increasing international market demand (Grundmann et al., 2023), with commercial products such as powders, capsules, tablets, and concentrated extracts fetching prices as high as USD 3.57 per gram of concentrated extract (Prevete et al., 2024). With Kratom's rapid expansion into international markets, especially in Western countries, commercial interest has intensified and diversified product demand. However, this accelerated growth highlights the absence of standardized cultivation protocols, which are urgently needed to ensure consistency in alkaloid yield, product quality, and regulatory compliance across different production regions.

Chemically, Kratom's pharmacological effects are primarily attributed to its alkaloid content, particularly mitragynine, which can constitute up to 66% of the total alkaloid profile (Veeramohan et al., 2018). Other alkaloids, including speciogynine, speciociliatine, and paynantheine, contribute between 1% and 9% (Ameline et al., 2024). In concentrated preparations such as resins and tinctures, mitragynine content can reach up to 40%. These alkaloids not only influence pharmacological potency but also play a critical role in determining product quality and market value (Cinosi et al., 2015; Grundmann et al., 2023).

Given that alkaloid biosynthesis and accumulation are heavily influenced by environmental conditions, a comprehensive understanding of ecological determinants such as climate, light, soil, seasonal variation, and geographic location is essential for sustainable and high-quality Kratom cultivation. Despite growing demand and commercial exploitation, there remains a lack of consolidated knowledge on how environmental variables interact with plant physiology to shape both biomass and bioactive compound profiles. To date, no integrative review has systematically examined the environmental and physiological factors affecting Kratom's growth and chemical quality in diverse cultivation contexts.

This review aims to bridge that critical gap by systematically examining the relationship between environmental factors and Kratom's phytochemical quality. It represents the first scientific effort to integrate both ecological and physiological perspectives into a unified framework, providing a foundation for developing sustainable cultivation strategies, improving raw material quality, and advancing evidence-based Kratom product development on a global scale. Given Kratom's increasing global prominence in herbal markets and its complex regulatory landscape, such insights are urgently needed to guide scientific research and industry practices.

MATERIALS AND METHODS

This study was conducted as a narrative literature review aimed at identifying and synthesizing scientific evidence regarding the environmental factors influencing the growth and phytochemical composition of *Mitragyna speciosa*. Literature searches were performed using major peer-reviewed academic databases, including PubMed, Scopus, Web of Science, Google Scholar, and the Directory of Open Access Journals (DOAJ).

Search terms were constructed using Boolean operators (AND/OR) and included combinations of relevant keywords such as: ("*Mitragyna speciosa*" OR "Kratom" OR "mitragynine") AND ("environment" OR "climate" OR "growing media" OR "light" OR "season" OR "soil") AND ("morphology" OR "plant organ" OR "phytochemistry" OR "alkaloid"). Additional keywords such as "plant nutrition," "Kratom strain," "leaf maturity"and "vegetative growth" were also used to expand the search coverage.

The literature search covered publications ranging from the oldest study published in 1983 to the most recent articles published in 2025 to ensure the inclusion of both foundational and the most recent advances in the field. Inclusion criteria were limited to original research articles and scientific reviews published in English or Indonesian, with full-text availability and direct relevance to the research topic. Editorials, commentaries, and non-scientific sources were excluded. The selection process involved screening titles, abstracts, and full texts to ensure alignment with the scope of the review. Eligible literature was then analyzed qualitatively to identify thematic patterns, trends, and correlations among environmental variables and both physiological and phytochemical responses of *Mitragyna speciosa*. The final body of evidence corresponds to the 96 publications cited in the reference list of this manuscript. Findings were synthesized narratively to establish a conceptual framework for optimizing ecophysiological determinants in sustainable Kratom cultivation.

RESULTS AND DISCUSSION

Phytochemical composition of *Mitragyna speciosa*

Wide arrays of natural compounds have been identified in *Mitragyna speciosa* as a result of extensive phytochemical investigations. These studies consistently revealed that the major classes of constituents included terpenoids, flavonoids, and most notably, alkaloids. Among these, indole-based alkaloids represent the predominant bioactive group. Some compounds are also present as oxindole derivatives with four- or five-membered ring structures, reflecting the plant's considerable chemical complexity (Takayama, 2004). A visual overview of these phytochemical classes and representative compounds is illustrated in Figure 1.

Mitragynine and its derivative, 7-hydroxymitragynine, exhibit strong affinity for opioid receptors and have demonstrated potent analgesic properties. Notably, 7-hydroxymitragynine has been reported to be up to 46 times more potent than mitragynine and 13 times stronger than morphine (Takayama, 2004). In addition to mitragynine, *M. speciosa* contains a range of structurally diverse and pharmacologically promising indole alkaloids such as ajmalicine, paynantheine, speciogynine, isopaynantheine, speciociliatine, and mitraciliatine. It also comprises oxindole-type alkaloids including isomitraphylline, isospeciofoline, speciofoline, corynoxine A, corynoxeine, and rhynchophylline. At least 19 alkaloids have been characterized in this plant (Flores-Bocanegra et al., 2020; Manwill et al., 2022). Alkaloid concentrations have been shown to vary significantly based on environmental factors. For example, in plants cultivated in the United States (University of Mississippi), mitraphylline was identified as the dominant alkaloid (León et al., 2009). In another study, mitragynine levels in Thai specimens were approximately 66% higher than those in Malaysian counterparts (Takayama et al., 1998). Numerous factors have been associated with this variation, including macronutrients (Phromchan et al., 2024), light exposure (Zhang et al., 2022), plant maturity (Veeramohan et al., 2023), seasonal and geographical conditions (Leksungnoen et al., 2022; Sengnon et al., 2023), soil characteristics (Rossalinda et al., 2024), and the other factors.

Beyond its rich alkaloid content, recent studies confirm that leaves of *Mitragyna speciosa* also contain various non-alkaloid bioactive compounds. These include at least 10 flavonoids, six terpenoids, and several phenylpropanoids, along with carboxylic acids, glycosides, phenols, and phenolic aldehydes (Veeramohan et al., 2023). Methanolic extracts of Kratom leaves contain epicatechin, and its polymeric form (condensed procyanidin tannins) exhibited notable biological activity against SARS-CoV-2, with an EC_{50} of 8.38 µg/mL. Other isolated non-alkaloid constituents include chlorogenic acid, saccharic acid 1,4-lactone, and inositol; each with distinct biological activities (Sureram et al., 2024). Ethanol extracts from red, white, and green Kratom variants have demonstrated the presence of phenolic and flavonoid compounds with antioxidant activity (Masriani et al., 2024). Furthermore, saponins such as daucosterol and triterpenoid saponins (quinovic acid 3-O-β-D-quinovopyranoside and quinovic acid 3-O-β-D-glucopyranoside) have been isolated from Kratom cultivated in the U.S. (León et al., 2009), while common phytosterols such as β-sitosterol and stigmasterol have been reported across *Mitragyna* species, including *M. speciosa* (Phongprueksapattana et al., 2008).

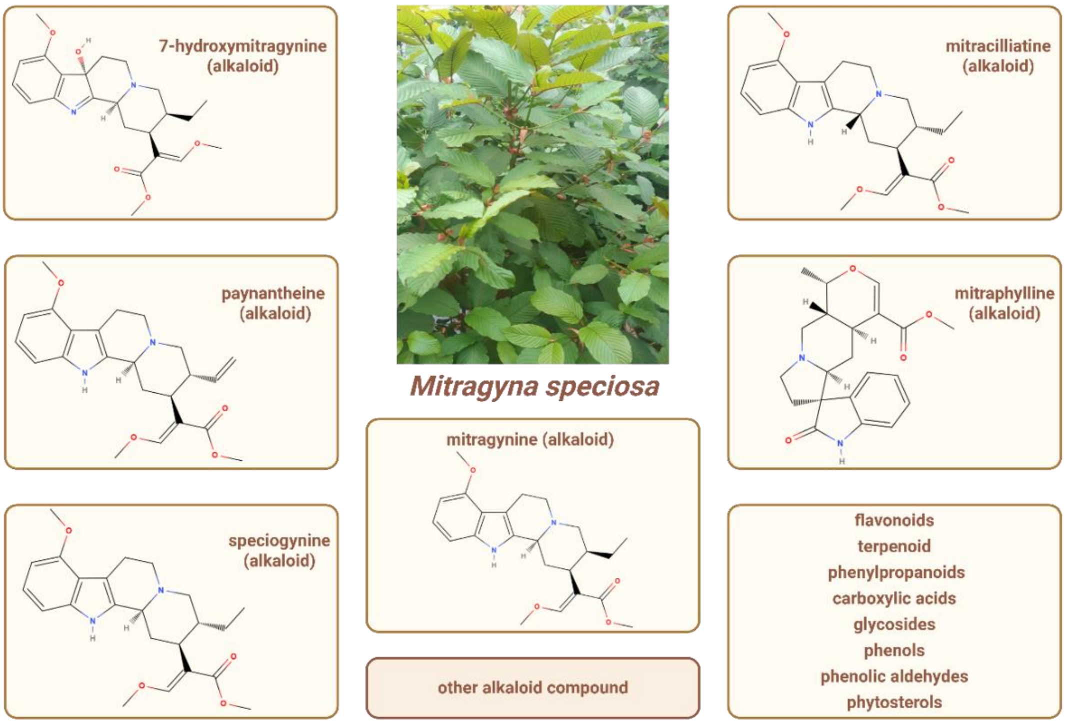


Fig. 1. Phytochemical constituents in *Mitragyna speciosa* leaves.

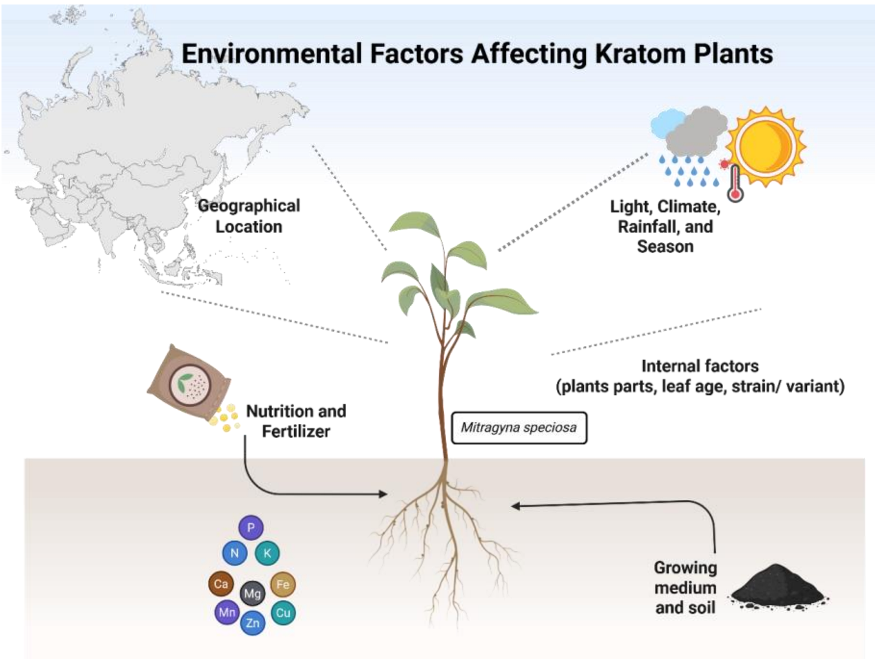


Fig. 2. Key environmental and physiological factors influencing the growth and phytochemical composition of *Mitragyna speciose.*

This rich and diverse phytochemical profile underscores *M. speciosa*'s wide-ranging pharmacological potential and the need for further exploration. It also raises important considerations regarding how environmental variables such as geography, seasonality, light intensity, and soil nutrient status shape the composition and concentration of alkaloids and other secondary metabolites in this plant (Fig. 2).

Pharmacological effects and uses of Mitragyna speciosa

Mitragyna speciosa is widely recognized for its complex pharmacological profile, primarily attributed to its key alkaloids, mitragynine and 7-hydroxymitragynine. These compounds exhibit notable affinity for opioid receptors, particularly the μ-opioid receptor, which accounts for the plants reported analgesic and mild euphoric effects (Takayama, 2004; Chear et al., 2021). Notably, 7-hydroxymitragynine has demonstrated significantly higher agonist potency than morphine, positioning it as a promising candidate for the development of plant-derived analgesics (Kruegel et al., 2019).

Historically, *Mitragyna speciosa* has been widely utilized in ethnomedicinal practices across Southeast Asia, particularly in Thailand, Malaysia, and Indonesia. In Thailand, Kratom leaves are traditionally chewed by laborers to enhance energy and stamina during fieldwork. In Malaysia and West Kalimantan, Indonesia, the leaves are brewed as tea to relieve pain, fatigue, and symptoms of diarrhea. Additionally, Kratom has been used as a traditional remedy for opioid withdrawal and as a natural sedative alternative (Cinosi et al., 2015). Thai folk medicine also employs *M. speciosa* leaves to treat cough, diarrhea, hyperglycemia, and pain (Nakaphan et al., 2016).

Table 1. Geographical variation in Kratom phytochemistry.

No	Location/ Country	Sample Type	Cultivation	Mitragynine Content	Notes	Reference
1	Kedah & Penang Malaysia	Fresh leaves	Cultivated	9.38-18.85 mg g^{-1}	Other alkaloids: corynoxine; corynoxine B; isospeciofoline; mitragynine oxindole B; corynantheidine; speciogynine; paynantheine; speciociliatine; speciociliatine N(4)-oxide; 7-hydroxymitragynine; isocorynantheidine; ajmalicine; mitraphylline	(Chear et al., 2021)
2	Malaysia	Mature leaves	Not specified	318.3 mg (± 12%) of total alkaloid	other alkaloids: 7α-hydroxy-7h-mitragynine; speciogynine; (+)-pinoresinol; mitralactonal; speciociliatine; paynantheine; mitragynaline; 3,4, 5,6-dehydromitragynine; mitrasulgynine	(Takayama et al., 1998)
3	United States	Young plants (<5 years)	Cultivated	8 mg (0.044%)	Dominant alkaloid: mitraphylline (24 mg) Other alkaloids: ajmalicine; corynantheidine; 7-hydroxymitragynine; isomitraphylline; paynantheine; isocorynantheidine	(León et al., 2009)
4	Kalimantan, Indonesia	Mature leaves	Wild	13.94-17.69 mg g^{-1} (leaf powder), 15.87-48.52 mg g^{-1} (ethanolic extract)	High mitragynine content observed in wild plants	(Novianry et al., 2024)
5	Kalimantan, Indonesia	Mature leaves	Not specified	1.4% (leaf powder) 4.5%-5.03% (crude extract) 38.56%-45.86% (alkaloid extract)	Includes paynantheine, speciogynine, speciociliatine	(Bayu et al., 2024)
6	Indonesia	Not specified	Not specified	0.37%-1.70% (leaf powder)	-	(Tanti et al., 2021)
7	Thailand	Mature leaves Plant age 5 to 25	Cultivated	7.5-26.6 mg g^{-1}	-	(Leksungnoen et al., 2022)

		years				
8	Thailand	Plant age 3 to 60 years	Cultivated	0.39%-3.46%	Includes paynantheine, speciogynine	(Sengnon et al., 2023)

Numerous studies have explored the pharmacological potential of Kratom leaves through in vitro, in vivo, and ethnographic approaches. While these studies confirm diverse activities such as anti-inflammatory and analgesic most evidence derives from preclinical models (Kafo et al., 2024; Rahmawati et al., 2024; Tuntiyasawasdikul et al., 2024; Alford et al., 2025; Mahaprom et al., 2025; Sornsenee et al., 2025). Kratom has also demonstrated sedative properties, as evidenced by experimental studies that reported central nervous system, depressant effects and traditional uses supporting its calming potential (Prasetya & Sudarwati, 2023; Obeng et al., 2024). In addition, several investigations have highlighted its anti-obesity activity, showing that Kratom extracts may influence metabolic regulation and body weight control in preclinical models (Janthongkaw et al., 2023; Pansai et al., 2024; Tampanna et al., 2025). Evidence of anti-cancer potential has also emerged, with in vitro and in silico studies demonstrating cytotoxic and antiproliferative effects against selected cancer cell lines, including those derived from lung cancer and brain cancer (Viwatpinyo et al., 2023; Bayu et al., 2024; Priatna et al., 2025) . Finally, Kratom has been increasingly studied in the context of opioid cessation, where observational and early clinical studies suggest its possible role as a harm-reduction tool, though methodological limitations and regulatory concerns still constrain firm conclusions (Romeu et al., 2020; Singh et al., 2020, 2023). This wide-ranging pharmacological activity reflects the plant's intricate phytochemical composition. Consequently, understanding the environmental factors influencing the synthesis and concentration of bioactive compounds is essential for elucidating the variability in *M. speciosa*'s biological effects and ensuring consistency in its quality and efficacy as a medicinal plant.

Phytochemical variation of *Mitragyna speciosa* across geographical regions

Comparative analysis across various geographical regions reveals substantial variation in the mitragynine content of *Mitragyna speciosa* leaves. As summarized in Table 1, samples originating from Southeast Asia such as Thailand and Malaysia generally exhibited higher and more consistent levels of mitragynine. In contrast, cultivated specimens from the United States showed markedly lower concentrations, averaging approximately 0.044%. In these samples, mitraphylline was reported as the dominant alkaloid, which differs significantly from the mitragynine, dominant profiles typically found in Asian specimens. Notably, wild-harvested plants from Kalimantan, Indonesia, demonstrated relatively high mitragynine content, suggesting that natural tropical conditions may play a pivotal role in promoting the biosynthesis of mitragynine, as opposed to cultivated environments outside the species' native habitat.

Beyond Asia, the genus *Mitragyna* is also found across the African continent, although represented by distinct species. For instance, *M. stipulosa*, *M. ciliata*, and *M. inermis* are native to West Africa, and their alkaloid profiles include compounds such as rotundifoline, isorotundifoline, rhynchophylline, isorhynchophylline, mitraphylline, ciliaphylline, and speciophyllin. In East Africa, *M. robustipulata* has also been identified, with its leaves containing rotundifoline, isorotundifoline, rhynchophylline, isorhynchophylline, mitraphylline, and isomitraphylline (Shellard, 1983). This species diversity emphasizes that while the *Mitragyna* genus is pantropical in distribution, its phytochemical profile is highly species-specific and environmentally dependent.

Table 2. Effects of light exposure, climate, and seasonal factors on the alkaloid content and growth of *Mitragyna speciose*.

No	Environmental Factor	Condition / Treatment	Impact	Location	Reference
1	Season	Comparison between rainy and dry seasons	Highest mitragynine (MG) content (up to 4.94% w/w) at end of dry season; broader and greener leaves; rainy season reduces MG content (to 0.74%)	Thailand	(Sengnon et al., 2023)
2	Rainfall and precipitation	Seasonal variation	Ideal rainfall <800 mm; mitragynine content decreases with high precipitation; June identified as optimal period	Thailand	(Sengnon et al., 2023)
3	Sunlight exposure	Medium to full sunlight	Cool and dry season leads to lower alkaloid content	Thailand	(Sengnon et al., 2023)
4	Light intensity	18 MJ $m2^{-1}$ per jam	Larger stem circumference and plant height; broader leaves; mitragynine content 18–21 mg g^{-1}	Thailand	(Leksungnoen et al., 2022)
		Greenhouse shaded (~40% light, ~25% full sunlight) vs. non-shaded vs. direct sun	Mitragynine content 31-40% higher under GH-shaded conditions; highest total chlorophyll, plant height, leaf size under GH-shaded; 35% higher paynantheine; 2x corynoxine concentration vs. other conditions	United States	(Zhang et al., 2022)
5	High light intensity	1200 μmol m^{-2} s^{-1}	Highest total dry mass (incl. stems: 23.83 ± 6.92 g); higher leaf thickness (0.20 ± 0.03 mm)	Thailand	(Leksungnoen et al., 2025)
6	Average temperature	25°C to 28°C	Highest mitragynine content recorded within this temperature range	-	(Leksungnoen et al., 2022; Zhang et al., 2022)

Light exposure, climate, and seasonal factors

Referring to Table 2, the analysis of various environmental conditions highlights the central role of climatic dynamics in influencing the variability of alkaloid content in *Mitragyna speciosa*. The plant's differential responses to seasonal shifts, temperature fluctuations, and variations in light intensity have been consistently observed across multiple study locations. These significant environmental effects are reflected not only in the accumulation of mitragynine but also in key morphological traits such as leaf area, stem height, and total biomass. The interplay among these environmental parameters appears to modulate the physiological efficiency of the plant in synthesizing secondary metabolites. Notably, variations in light treatment (whether through natural intensity or artificial shading) produce measurable effects on both the primary and secondary alkaloid concentrations. Taken together, these findings underscore the critical importance of controlled environmental conditions in optimizing alkaloid production in Kratom cultivation.

Table 3. Effects of nutritional and growing media factors on growth and alkaloid content in *Mitragyna speciose.*

No	Nutritional / Growing Media Factor	Condition / Treatment	Impact on Plant and Alkaloids	Reference
1	Soil type	Mineral soil (alluvial, red-yellow podzolic soil) vs peat soil	Peat soil has high porosity and elevated N and P content, supporting superior vegetative growth. Addition of dolomite lime and chicken manure improves plant height, leaf number, and dry weight.	(Edi et al., 2024; Rossalinda et al., 2024)
2	Soil element content	High Ca and Mg content	Increases mitragynine content	(Leksungnoen et al., 2022; Sengnon et al., 2023)
3	Soil pH	pH 4-6 (acidic)	Optimal zone for growth and alkaloid accumulation	(Edi et al., 2024; Leksungnoen et al., 2022; Sengnon et al., 2023)
4	Soil water content (volumetric & retention)	High	Positively associated with increased mitragynine levels	(Leksungnoen et al., 2022; Sengnon et al., 2023)
5	Soil water potential	−0.03 MPa vs −0.7 MPa	High water potential (−0.03 MPa) improves plant height and stem biomass. Medium potential (−0.4 MPa) yields highest MG content (0.63% w/w)	(Leksungnoen et al., 2025)
6	NPK fertilizer dosage	Application of Osmocote Plus (N, P, K) 15-9-12 (doses: 0, 46, 74, and 96 g per container)	High doses increase mitragynine content (up to 2.58% per plant); enhance plant height, leaf number, leaf area, SPAD value*, and photosynthetic efficiency; minor alkaloids highest at low to moderate doses	(Zhang et al., 2020)
7	Nutrient composition	Macronutrients (N, P, K, Ca, Mg) and micronutrients (Fe, Mn, Zn, Cu)	Increases mitragynine, paynantheine, and total alkaloid content	(Sengnon et al., 2023)
8	Liquid organic fertilizer	150 ml L^{-1}	Enhances leaf number, root and shoot dry weight; no significant effect on plant height, leaf area, and chlorophyll content	(Antoni et al., 2023)

*SPAD value = an index of relative chlorophyll concentration using SPAD device meter.

Nutritional and growing media factors

Table 3 demonstrates that both growing media and nutrient availability significantly influence vegetative growth and alkaloid accumulation in *Mitragyna speciosa*. Plants cultivated in peat-based media exhibit superior vegetative performance, characterized by increased plant height, leaf number, root volume, and total dry biomass. This advantage is attributed to the physical and chemical properties of peat soils, such as high porosity and abundant nitrogen and phosphorus content. Soil pH within the mildly to moderately acidic range (pH 4–6) was shown to support both optimal growth and alkaloid accumulation. Additionally, soil water potential and its moisture retention capacity contributed to higher mitragynine content. Fertilization (both organic and inorganic) enhanced biomass parameters and physiological efficiency. Although alkaloid concentration per unit leaf weight did not always increase proportionally, total alkaloid content per plant improved as biomass increased under moderate to high fertilization regimes. Furthermore, minor alkaloids such as paynantheine and corynantheidine also increased depending on nutrient composition and dosage. These findings underscore the importance of nutrient and substrate management as key strategies in optimizing high-quality Kratom raw material production.

Table 4. Internal factors affecting *Mitragyna speciosa.*

No	Internal Factor	Treatment	Part / Condition Analyzed	Results	Reference
1	Plant part	Leaves, stems, and twigs	Phytochemical content by plant part	Leaves and stems contain alkaloids, steroids, quinones, saponins, tannins; twigs contain alkaloids, quinones, saponins	(Sofia et al., 2022)
		Roots and stems	Phytochemical content by plant part	Roots contain alkaloids, phenols, terpenoids, flavonoids, tannins, saponins; stems contain alkaloids, phenols, steroids, terpenoids, flavonoids, tannins	(Putri et al., 2023)
		Leaves, stems, bark, and roots	Alkaloid profile by plant organ (via LC-MS)	Mitragynine, speciogynine, paynantheine, speciociliatine dominant in leaves; lower in stems and bark; not detected in roots	(Schotte et al., 2023)
2	Leaf age	Young vs. mature leaves	Top 2 leaves vs. 7-10th leaves from shoot tip	Mature leaves had 1.2x higher mitragynine and 3.3x higher 7-hydroxymitragynine; 86 metabolites shared, 5 unique to each leaf type	(Veeramohan et al., 2023)
		Five developmental stages (S1–S5)	S1 (7-15 days) S2 (15-30 days) S3 (30-45 days) S4 (45-60 days) S5 (60-75 days)	Highest mitragynine in S1-S2; highest chlorophyll, carotenoids, stomatal density in S5; antioxidant activity increased with age	(Phromchan et al., 2024)
3	Variety/strain	Strain comparison/ vein leaf colors	Red Malay, Red Bali, Red Thai, Green Malay, White Borneo	Green Malay had highest mitragynine (59.7%) and total alkaloids (94.9%); White Borneo had highest extract yield (12.2 mg g^{-1})	(Boffa et al., 2018)
			Red, green, white Indonesia	White strain had highest ethanol extract yield (18.74%); all contained alkaloids, flavonoids, tannins, saponins, phenols, triterpenoids	(Masriani et al., 2023)
			Red dan green Thai	Green Thai had highest mitragynine (63.60 mg g^{-1}); both contained rutin and quercetin	(Janthongkaw et al., 2023)
			Red vein vs White vein (Pho Thong strain)	White strain had higher mitragynine (39.79 mg g^{-1} ± 1.81); both had rutin and quercetin	(Nawaka et al., 2025)
			Green vein Bali, Maeng Da, Red Indo, Red Borneo	Green strains had higher mitragynine (42.29-52.81 mg g^{-1}) than red strains (26.68%-33.84 mg g^{-1})	(Ng & Ha, 2024)

In addition to external environmental influences, alkaloid content in *Mitragyna speciosa* is also determined by internal factors, including anatomical structure, organ maturity, and genetic variation. Different plant organs (roots, stems, and leaves) exhibit uneven distribution of alkaloids. Leaf maturity further influences the concentration of active compounds, with specific developmental stages linked to peak alkaloid levels. Genetic factors, such as strain or variety, also shapen the alkaloid profile; plants with different leaf vein colors (e.g., red, green, or white) have been reported to contain varying ratios of mitragynine and other alkaloids. These phenotypic differences reflect the complex interplay between genetic background and environmental conditions, which ultimately influence the plant's phytochemical composition and pharmacological potential. Therefore, precise selection of plant parts and varietal identification are essential steps for standardizing and developing evidence-based Kratom products. A summary of these internal determinants and their phytochemical implications is presented in Table 4.

As a member of the Rubiaceae family, *Mitragyna speciosa* (Kratom) exhibits significant potential as a high-value agricultural commodity. Over the past decade, global demand for Kratom products has increased substantially, particularly in markets such as the United States and Europe, positioning this plant as an emerging botanical commodity. Its derivatives are now widely available in various forms, including powders, capsules, teas, and liquid concentrates, reflecting diversified usage driven by its unique physiological effects, acting as a stimulant at low doses and as a sedative-analgesic at higher doses (Singh et al., 2019). This trend presents strategic opportunities for tropical Kratom-producing countries, such as Indonesia, Thailand, and Malaysia, to advance cultivation systems and product development. Nevertheless, the industry continues to face challenges, notably the variability in active compound content caused by environmental factors, genetic diversity, and the lack of established quality standards and regulatory frameworks.

The chemical composition of *Mitragyna speciosa* reflects the complexity of tropical secondary metabolism, which generates not only a dominant bioactive compound but a synergistic phytochemical profile (Hassan et al., 2013). Mitragynine, the primary alkaloid, is recognized for its major pharmacological activities (Utar et al., 2011; Suhaimi et al., 2016; Yunusa et al., 2023; Obeng et al., 2024). However, the presence of other alkaloids such as 7-hydroxymitragynine, speciogynine, paynantheine, and speciociliatine contributes complementary biological effects. For instance, speciogynine has been shown to significantly reduce mechanical allodynia in cancer-induced bone pain models (Ortiz et al., 2024), while mitraphylline and rhynchophylline exhibit mild modulatory activity on the opioid system (Sudmoon et al., 2025). In vivo studies have further revealed that 7-hydroxymitragynine possesses strong analgesic potential at doses of 17.8 mg/kg, acting as a partial μ-opioid receptor agonist with 41% efficacy in [^{35}S]GTPγS binding assays (Calvache et al., 2021). In addition to alkaloids, the presence of flavonoids, phenols, tannins, and saponins also supports antioxidant and immunomodulatory functions. These synergistic interactions align with the polycomponent pharmacodynamics principle in phytopharmaceutical development (Saleh et al., 2012; Zailan et al., 2022; Masriani et al., 2024; Phromchan et al., 2024; Sornsenee et al., 2025).

The distribution of bioactive compounds in Kratom is spatially regulated, with leaves serving as the principal site of accumulation. This pattern is supported by studies on other plant species. In *Pterocarpus erinaceus*, phytochemical analysis revealed that leaf tissues contain significantly higher levels of bioactive compounds such as proteins, flavonoids, phenols, and saponins, compared to stems and roots (Alagbe et al., 2024). Separately, methanolic leaf extracts of *Moringa oleifera* have demonstrated the strongest antipyretic activity relative to extracts from stems and roots, with efficacy that approached or even exceeded that of the positive control (Jamil et al., 2025). In Kratom, metabolomic profiling confirms the presence of mitragynine, speciogynine, paynantheine, and speciociliatine in leaf tissue, while roots and stems predominantly contain biosynthetic precursors like strictosidine. The biosynthesis of mitragynine follows a monoterpenoid indole alkaloid pathway originating from strictosidine aglycone, catalyzed by stereospecific enzymes such as MsDCS1, MsDCS2, and MsEnolMT. Rational enzyme modifications have been shown to alter biosynthetic outputs, although mitragynine remains the predominant product due to its structural stability and efficient conversion (Schotte et al., 2023).

Leaf maturity also plays a crucial role in alkaloid accumulation. Studies have demonstrated that fully developed leaves, particularly in developmental stages S2 to S4 (15–60 days), exhibit the highest mitragynine concentrations, with stage S3 (30–45 days) identified as the optimal harvest window (Phromchan et al., 2024). Similar trends are observed in *Mangifera indica*, where bioactive content (mangiferin) declines with leaf aging (Nguyen et al., 2024). Additionally, vein color, a common phenotypic trait used to classify Kratom strains into red, green, and white has been linked to distinct alkaloid profiles. This variation is likely associated with inherited pigmentation traits and underlying differences in metabolic activity and biosynthetic capacity (Deng & Harbaugh, 2006; Kan et al., 2021). Differences in *Mitragyna speciosa* strains or varieties are also utilized by users to achieve specific pharmacological effects. Red vein Kratom is commonly used for its relaxing properties, green vein for pain relief, and white vein for its stimulant effects. However, analyses of several commercial Kratom products have reported no significant differences in mitragynine content across these strains (Huisman et al., 2023). This observation aligns with findings from previous studies, which also showed that the variation in mitragynine levels among red, green, and white strains was relatively minor (Boffa et al., 2018; Smith et al., 2024).

Beyond internal factors, phytochemical accumulation is profoundly influenced by geography and environmental conditions. Variables such as temperature, rainfall, light intensity, and photoperiod collectively shapen ecophysiological dynamics and gene expression (Kumar et al., 2017; Georgescu et al., 2022; Saravanakumar et al., 2022). In Southeast Asia, stable tropical climates with average temperatures of 25–28°C, high humidity, and filtered sunlight are optimal for alkaloid biosynthesis. In contrast, plants cultivated in subtropical regions such as the United States exhibit lower mitragynine levels and higher concentrations of alternative alkaloids like mitraphylline (León et al., 2009). A comparative study of *Euphorbia hirta* from Ethiopia and India revealed that, although both samples contained similar phytochemical constituents, the ethanolic extract from the Indian specimen exhibited greater compound diversity, with GC-MS identifying 15 compounds in the Indian sample compared to 12 in the Ethiopian one (Saravanakumar et al., 2022). Another comparative study on eight types of berries showed that specimens from Romania consistently exhibited higher total phenolic content (TPC) and radical scavenging activity (RSA) than those from Russia (Georgescu et al., 2022). These findings underscore the critical role of geographic factors, including agroclimatic conditions and local growing environments, in influencing the synthesis and accumulation of secondary metabolites, the overall phytochemical composition, and the biological activity of plants. Nevertheless, controlled greenhouse environments can offset ecological limitations, enabling competitive mitragynine yields even outside the native habitat (Zhang et al., 2022).

Climatic variables not only serve as environmental inputs but also as molecular signals regulating biosynthetic gene expression. For instance, light influences both photosynthesis and secondary metabolite synthesis through photoreceptors and redox signaling pathways (Thoma et al., 2020; Borbély et al., 2022). In *Aloe vera*, plants grown in highland semi-arid agroclimates exhibit antioxidant activity 10–12% higher than those from humid tropical zones, supporting the impact of light and temperature on metabolite production (Kumar et al., 2017). Precision agronomic strategies, including light and temperature management, thus become essential for maximizing alkaloid biosynthesis in Kratom.

The availability of nutrients and the characteristics of the growing medium are key agronomic determinants that influence the efficiency of secondary metabolism in *Mitragyna speciosa*. Nutritional imbalance not only affects morphological parameters but also disrupts the biosynthetic pathways of alkaloids involving specific enzymes such as strictosidine synthase and cytochrome P450 monooxygenase (Nguyen & Dang, 2021; Kim et al., 2023). Both macro- and micronutrients including nitrogen, phosphorus, magnesium, and calcium support alkaloid biosynthesis by serving as precursors and essential enzymatic cofactors (Hank et al., 2003; Qi et al., 2024). Soil pH further influences nutrient availability, with acidic soils impeding the uptake of key elements like phosphorus and iron (Huang et al., 2023; Yang et al., 2024). Peat-based soils, characterized by high porosity, elevated organic matter, and strong water-holding capacity, provide ideal microecological conditions for Kratom growth. Studies in Kapuas Hulu, Indonesia, showed that Kratom grown near peatlands (580–650 meters from riverbanks) exhibited higher mitragynine content (15.49%) and improved morphological traits compared to plants near water sources (Pramulya et al., 2024). These outcomes are attributed to low pH, high C-organic content, and superior cation exchange capacity (Hikmatullah & Sukarman, 2014; Cao, 2019).

Considering the diverse endogenous and exogenous factors influencing the alkaloid biosynthetic pathways, standardizing Kratom quality requires a systemic approach that integrates plant genetics, physiology, and environmental management. Genetic diversity has been shown to contribute to chemotypic variation and differential alkaloid accumulation across populations of *Mitragyna speciosa* (Leksungnoen et al., 2022; Manwill et al., 2022). Moreover, leaf developmental stage and harvesting phase significantly influence the phytochemical profile, with mature leaves generally containing higher levels of mitragynine and related alkaloids compared to younger tissues (Veeramohan et al., 2023; Phromchan et al., 2024). Findings from multiple studies suggest that a combination of superior cultivars, optimal harvesting phases, and favorable edaphoclimatic conditions such as stable temperatures, moderate light intensity, and growing media with high cation exchange capacity (e.g., managed peat) can maximize the accumulation of mitragynine and other supporting bioactive compounds (Hikmatullah & Sukarman, 2014; Zhang et al., 2020; Edi et al., 2024; Chongdi et al., 2025). Therefore, an adaptive agroecological cultivation strategy serves as a critical foundation for developing *Mitragyna speciosa* into a sustainable and evidence-based global phytopharmaceutical commodity.

CONCLUSION

The phytochemical profile of *Mitragyna speciosa* is determined by the interplay of intrinsic factors such as organ type, leaf maturity, and genetic variability, together with extrinsic variables including light exposure, temperature, soil characteristics, and nutrient availability. Mitragynine remains the dominant alkaloid underpinning its pharmacological activity, while synergistic contributions from minor alkaloids and non-alkaloid metabolites reinforce the plant's multicomponent bioactivity. These findings emphasize the need for precision cultivation strategies guided by ecophysiological data to achieve consistent quality in Kratom leaf biomass as raw material for phytopharmaceutical development. Nevertheless, challenges such as high genetic variability and regulatory heterogeneity may hinder large-scale consistency. Future studies should therefore focus on controlled cultivation systems that integrate optimized environmental parameters, multi-location and cross-country cultivation trials to assess genotype, environment interactions, and standardized post-harvest protocols to ensure reproducible phytochemical outcomes. Collectively, these efforts could strengthen the scientific basis for the sustainable cultivation and global utilization of *M. speciosa* as a strategic herbal commodity.

Conflict of interest

The authors declare that there are no conflicts of interest related to this article. This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Acknowledgments

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

REFERENCES

Alagbe, J. O., Shittu, M. D., Adesina, A. Y., Grace, C. J., Cincinsoko, K. M., Oluwafemi, B. S., & Erikanobong, E. (2024). The approximate mineral and phytochemical content of the leaves, stem bark, and roots of *Pterocarpus erinaceus* in India. *Cerrado: Agricultural and Biological Research, 1*(1), 32–41. https://doi.org/10.14295/cerrado.v1i1.562

Alford, A. S., Moreno, H. L., Benjamin, M. M., Dickinson, C. F., & Hamann, M. T. (2025). Exploring the therapeutic potential of mitragynine and corynoxeine: Kratom-derived indole and oxindole alkaloids for pain management. *Pharmaceuticals*, *18*(2), 222. https://doi.org/10.3390/ph18020222

Ameline, A., Gheddar, L., Arbouche, N., Blanchot, A., Raul, J. S., & Kintz, P. (2024). Testing for Kratom alkaloids in fingernail clippings – not only mitragynine. *Journal of Pharmaceutical and Biomedical Analysis*, *243*. https://doi.org/10.1016/j.jpba.2024.116078

Antoni, Zakiah, Z., & Mukarlina. (2023). Pertumbuhan bibit Kratom merah (*Mitragyna speciosa* Korth.) dengan pemberian pupuk organik cair kotoran ayam potong [Growth of red Kratom seedlings (*Mitragyna speciosa* Korth.) with the provision of liquid organic fertilizer from broiler chicken manure]. *Journal Buana Sains*, *23*(2), 1412–1638. https://doi.org/doi.org/10.33366/bs.v23i2.4453

Arief, I., Sunnardianto, G. K., Khairi, S., & Saputri, W. D. (2025). The potential of *Mitragyna speciosa* leaves as a natural source of antioxidants for disease prevention. *Journal of Integrative Bioinformatics*, *21*(4). https://doi.org/10.1515/jib-2023-0030

Bayu, A., Rahmawati, S. I., Karim, F., Panggabean, J. A., Nuswantari, D. P., Indriani, D. W., Ahmadi, P., Witular, R., Dharmayanti, N. L. P. I., & Putra, M. Y. (2024). An in vitro examination of whether Kratom extracts enhance the cytotoxicity of low-dose doxorubicin against A549 human lung cancer cells. *Molecules*, *29*(6). https://doi.org/10.3390/molecules29061404

Begum, T., Arzmi, M. H., Khatib, A., Uddin, A. B. M. H., Aisyah Abdullah, M., Rullah, K., Mat So'ad, S. Z., Zulaikha Haspi, N. F., Nazira Sarian, M., Parveen, H., Mukhtar, S., & Ahmed, Q. U. (2025). A review on *Mitragyna speciosa* (Rubiaceae) as a prominent medicinal plant based on ethnobotany, phytochemistry and pharmacological activities. *Natural Product Research*, *39*(6), 1636–1652. https://doi.org/10.1080/14786419.2024.2371564

Boffa, L., Ghè, C., Barge, A., Muccioli, G., & Cravotto, G. (2018). Alkaloid profiles and activity in different *Mitragyna speciosa* strains. *Natural Product Communications*, *13*(9), 1111–1116. https://doi.org/10.1177/1934578X1801300904

Borbély, P., Gasperl, A., Pálmai, T., Ahres, M., Asghar, M. A., Galiba, G., Müller, M., & Kocsy, G. (2022). Light intensity- and spectrum-dependent redox regulation of plant metabolism. *Antioxidants*, *11*(7), 1311. https://doi.org/10.3390/antiox11071311

Calvache, M. P. G., Obeng, S., Leon, F., Gamez-Jimenez, L. R., Patel, A., Ho, N. P., Crowley, M. L., Pallares, V., Mottinelli, M., McCurdy, C. R., McMahon, L. R., & Hiranita, T. (2021). In vitro and in vivo pharmacological comparison of Mu-Opioid receptor activity of the Kratom (*Mitragyna speciosa*) alkaloid mitragynine and its metabolite 7-hydroxymitragynine. *Alzheimer's & Dementia*, *17*(S9). https://doi.org/10.1002/alz.058605

Cao, S. (2019). Research and application of peat in agriculture. *IOP Conference Series: Earth and Environmental Science*, *384*(1), 012174. https://doi.org/10.1088/1755-1315/384/1/012174

Chear, N. J.-Y., León, F., Sharma, A., Kanumuri, S. R. R., Zwolinski, G., Abboud, K. A., Singh, D., Restrepo, L. F., Patel, A., Hiranita, T., Ramanathan, S., Hampson, A. J., McMahon, L. R., & McCurdy, C. R. (2021). Exploring the chemistry of alkaloids from Malaysian *Mitragyna speciosa* (Kratom) and the role of oxindoles on human opioid receptors. *Journal of Natural Products*, *84*(4), 1034–1043. https://doi.org/10.1021/acs.jnatprod.0c01055

Chongdi, S., Uthairatsamee, S., Ngernsaengsaruay, C., Andriyas, T., & Leksungnoen, N. (2025). Regional variability in growth and leaf functional traits of *Mitragyna speciosa* in Thailand. *International Journal of Plant Biology*, *16*(1), 24. https://doi.org/10.3390/ijpb16010024

Chua, L. S. L. , S. G. H. (2001). *Mitragyna speciosa* (Korth.) Havil. PROSEA Foundation, Bogor, Indonesia. prota4u.org/prosea

Cinosi, E., Martinotti, G., Simonato, P., Singh, D., Demetrovics, Z., Roman-Urrestarazu, A., Bersani, F. S., Vicknasingam, B., Piazzon, G., Li, J. H., Yu, W. J., Kapitány-Fövény, M., Farkas, J., Di Giannantonio, M., & Corazza, O. (2015). Following "the Roots" of Kratom (*Mitragyna speciosa*): the evolution of an enhancer from a traditional use to increase work and productivity in Southeast Asia to a recreational psychoactive drug in Western countries. *BioMed Research International*, *2015*. https://doi.org/10.1155/2015/968786

Deng, Z., & Harbaugh, B. K. (2006). Independent inheritance of leaf shape and main vein color in Caladium. *Journal of the American Society for Horticultural Science*, *131*(1), 53–58. https://doi.org/10.21273/JASHS.131.1.53

Diversion Control Division, D. (2024). Kratom *(Mitragyna speciosa* Korth*).*

Edi, S. U., Indrawati, U. S. Y. V., & . J. (2024). Characterization of physicochemical properties and and heavy metals content of soils under Kratom (*Mitragyna speciosa*) Cultivation, Kapuas Hulu District, Indonesia. *Indian Journal of Agricultural Research*, 58, 1109-1114. https://doi.org/10.18805/IJARe.AF-845

Flores-Bocanegra, L., Raja, H. A., Graf, T. N., Augustinović, M., Wallace, E. D., Hematian, S., Kellogg, J. J., Todd, D. A., Cech, N. B., & Oberlies, N. H. (2020). The chemistry of Kratom *Mitragyna speciosa*: updated characterization data and methods to elucidate indole and oxindole alkaloids. *Journal of Natural Products*, *83*(7), 2165–2177. https://doi.org/10.1021/acs.jnatprod.0c00257

Georgescu, C., Frum, A., Virchea, L.-I., Sumacheva, A., Shamtsyan, M., Gligor, F.-G., Olah, N. K., Mathe, E., & Mironescu, M. (2022). Geographic variability of berry phytochemicals with antioxidant and antimicrobial properties. *Molecules*, *27*(15), 4986. https://doi.org/10.3390/molecules27154986

Grundmann, O., Hendrickson, R. G., & Greenberg, M. I. (2023). Kratom: History, pharmacology, current user trends, adverse health effects and potential benefits. *Disease-a-Month*, *69*(6), 101442. https://doi.org/10.1016/J.DISAMONTH.2022.101442

Hank, H., Lásló, I., Bálványos, I., Kursinszki, I., Kovács, Gy., Szöke, E., & Töth, E. (2003). Effect of magnesium on the growth and alkaloid production of hairy root cultures. *Acta Horticulturae*, *597*, 271–274. https://doi.org/10.17660/ActaHortic.2003.597.39

Hassan, Z., Muzaimi, M., Navaratnam, V., Yusoff, N. H. M., Suhaimi, F. W., Vadivelu, R., Vicknasingam, B. K., Amato, D., von Hörsten, S., Ismail, N. I. W., Jayabalan, N., Hazim, A. I., Mansor, S. M., & Müller, C. P. (2013). From Kratom to mitragynine and its derivatives: Physiological and behavioural effects related to use, abuse, and addiction. *Neuroscience & Biobehavioral Reviews*, *37*(2), 138–151. https://doi.org/10.1016/j.neubiorev.2012.11.012

Hikmatullah, & Sukarman. (2014). Physical and chemical properties of cultivated peat soils in four trial sites of ICCTF in Kalimantan and Sumatra, Indonesia. *Journal of Tropical Soils*, *19*(3), 131–141.

https://doi.org/DOI:10.5400/jts.2014.19.3.131

Huang, K., Li, M., Li, R., Rasul, F., Shahzad, S., Wu, C., Shao, J., Huang, G., Li, R., Almari, S., Hashem, M., & Aamer, M. (2023). Soil acidification and salinity: the importance of biochar application to agricultural soils. *Frontiers in Plant Science*, *14*, 1206820. https://doi.org/10.3389/fpls.2023.1206820

Huisman, G., Menke, M., Grundmann, O., Schreiber, R., & Mason, N. (2023). Examining the psychoactive differences between Kratom strains. *International Journal of Environmental Research and Public Health*, *20*(14), 6425. https://doi.org/10.3390/ijerph20146425

Indriyanti, N., Hajrah, H., Priastomo, M., Samsul, E., Prabowo, W. C., & Prasetya, F. (2024). Efektivitas ekstrak kratom (*Mitragyna speciosa*) pada inhibisi withdrawal syndrome model adiksi morfin [Effectiveness of kratom (*Mitragyna speciosa*) extract on the inhibition of withdrawal syndrome in a morphine addiction model]. *Jurnal Mandala Pharmacon Indonesia*, *10*(1), 145–150. https://doi.org/10.35311/jmpi.v10i1.535

Jamil, S., Turabi, T. H., Ahmad, S., Riaz, M., Wariss, H. M., & Akter, Q. S. (2025). Comparative investigation of the nutritional profiling and antipyretic activity of *Moringa oleifera* leaves, bark, and root from different sites of Punjab, Pakistan. *Food Science & Nutrition*, *13*(1), e4706. https://doi.org/10.1002/fsn3.4706

Janthongkaw, A., Klaophimai, S., Khampaya, T., Yimthiang, S., Yang, Y., Ma, R., Bumyut, A., & Pouyfung, P. (2023). Effect of green and red Thai Kratom (*Mitragyna speciosa*) on pancreatic digestive enzymes (alpha-glucosidase and lipase) and acetyl-carboxylase 1 activity: A possible therapeutic target for obesity prevention. *PLOS ONE*, *18*(9), e0291738. https://doi.org/10.1371/journal.pone.0291738

Kafo, A., Elsalami, R., & Hassan, M. (2024). *Mitragyna speciosa* Korth. downregulates macrophage inflammatory responses by inhibiting TLR-4 and increasing IL-10 production. *İstanbul Journal of Pharmacy*, *54*(3), 350–358. https://doi.org/10.26650/IstanbulJPharm.2024.1424150

Kan, P.-W., Cheng, Y.-C., & Yeh, D.-M. (2021). Mechanism of leaf vein coloration and inheritance of leaf vein color, flower form, and floral symmetry in Gloxinia. *Journal of the American Society for Horticultural Science*, *146*(3), 178–183. https://doi.org/10.21273/JASHS05034-20

Kim, K., Shahsavarani, M., Garza-García, J. J. O., Carlisle, J. E., Guo, J., De Luca, V., & Qu, Y. (2023). Biosynthesis of Kratom opioids. *New Phytologist*, *240*(2), 757–769. https://doi.org/10.1111/nph.19162

Kruegel, A. C., Uprety, R., Grinnell, S. G., Langreck, C., Pekarskaya, E. A., Le Rouzic, V., Ansonoff, M., Gassaway, M. M., Pintar, J. E., Pasternak, G. W., Javitch, J. A., Majumdar, S., & Sames, D. (2019). 7-Hydroxymitragynine is an active metabolite of mitragynine and a key mediator of its analgesic effects. *ACS Central Science*, *5*(6), 992–1001. https://doi.org/10.1021/acscentsci.9b00141

Kumar, S., Yadav, M., Yadav, A., & Yadav, J. P. (2017). Impact of spatial and climatic conditions on phytochemical diversity and in vitro antioxidant activity of Indian *Aloe vera* (L.) Burm. f. *South African Journal of Botany*, *111*, 50–59. https://doi.org/10.1016/j.sajb.2017.03.012

Leksungnoen, N., Andriyas, T., Ngernsaengsaruay, C., Uthairatsamee, S., Racharak, P., Sonjaroon, W., Kjelgren, R., Pearson, B. J., McCurdy, C. R., & Sharma, A. (2022). Variations in mitragynine content in the naturally growing Kratom (*Mitragyna speciosa*) population of Thailand. *Frontiers in Plant Science*, *13*, 1028547. https://doi.org/10.3389/FPLS.2022.1028547

Leksungnoen, N., Andriyas, T., Ku-Or, Y., Chongdi, S., Tansawat, R., Aramrak, A., Ngernsaengsaruay, C., Uthairatsamee, S., Sonjaroon, W., Thongchot, P., Ardsiri, S., & Pongchaidacha, P. (2025). The effect of light intensity and polyethylene-glycol-induced water stress on the growth, mitragynine accumulation, and total alkaloid content of Kratom (*Mitragyna speciosa*). *Horticulturae*, *11*(3), 272. https://doi.org/10.3390/horticulturae11030272

León, F., Habib, E., Adkins, J. E., Furr, E. B., McCurdy, C. R., & Cutler, S. J. (2009). Phytochemical characterization of the leaves of *Mitragyna speciosa* grown in USA. *Natural Product Communications*, *4*(7), 907–910. https://doi.org/10.1177/1934578X0900400705

Löfstrand, S. D., Krüger, Å., Razafimandimbison, S. G., & Bremer, B. (2014). Phylogeny and generic delimitations in the sister tribes Hymenodictyeae and Naucleeae (Rubiaceae). *Systematic Botany*, *39*(1), 304–315. https://doi.org/10.1600/036364414X678116

Mahaprom, K., Chokpaisarn, J., Kunworarath, N., Paduka, W., Phoopha, S., Limsuwan, S., & Neamsuvan, O. (2025). In vivo analgesic, anti-inflammatory activities, and phytochemical profile of Thai herbal Kratom recipe, a traditional Thai herbal medicine for muscle pain relief. *Journal of Ethnopharmacology*, *343*, 119442. https://doi.org/10.1016/j.jep.2025.119442

Manwill, P. K., Flores-Bocanegra, L., Khin, M., Raja, H. A., Cech, N. B., Oberlies, N. H., & Todd, D. A. (2022). Kratom (*Mitragyna speciosa*) validation: quantitative analysis of indole and oxindole alkaloids reveals chemotypes of plants and products. *Planta Medica*, *88*(9–10), 838–852. https://doi.org/10.1055/a-1795-5876

Masriani, M., Melania, P., Muharini, R., Alimuddin, A. H., & Sartika, R. P. (2024). Total phenolic and flavonoids content, and antioxidant activity of Kratom (*Mitragyna speciosa* Korth.) leaf ethanol extract. *Jurnal Natural*, *24*(1), 16–21. https://doi.org/10.24815/jn.v24i1.33125

Masriani, M., Muharini, R., Wijayanti, D. K., Melanie, P., & Widiansari, M. L. (2023). Phytochemical Screening of Ethanol Extracts from Three Variants of Kratom Leaves (*Mitragyna speciosa* Korth.). *Hydrogen: Jurnal Kependidikan Kimia*, *11*(2), 192. https://doi.org/10.33394/hjkk.v11i2.7122

Nakaphan, T., Teerachaisakul, M., Puttum, S., Sompimai, K., & Nootim, P. (2016). Traditional use of Kratom (*Mitragyna speciosa* Korth) among folk healers in southern Thailand. *Journal of Thai Traditional and Alternative Medicine*, *14*(3), 274-285.

Nawaka, N., Lertcanawanichakul, M., Porntadavity, S., Pussadhamma, B., & Jeenduang, N. (2025). Kratom leaf extracts exert hypolipidaemic effects via the modulation of PCSK9 and LDLR pathways in HepG2 cells. *Scientific Reports*, *15*(1), 15696. https://doi.org/10.1038/s41598-025-00711-1

Ng, K., & Ha, T. (2024). Extraction and detection of mitragynine in Kratom leaves by high-performance liquid chromatography. *Natural Product Research*, *39*(14), 4074-4079. https://doi.org/10.1080/14786419.2024.2331602

Nguyen, T., & Dang, T. T. (2021). Cytochrome P450 enzymes as key drivers of alkaloid chemical diversification in plants. *Frontiers in Plant Science*, *12*. https://doi.org/10.3389/fpls.2021.682181

Nguyen, H. T., Miyamoto, A., Hoang, H. T., Vu, T. T. T., Pothinuch, P., & Nguyen, H. T. T. (2024). Effects of maturation on antibacterial properties of Vietnamese mango (*Mangifera indica*) Leaves. *Molecules*, *29*(7), 1443. https://doi.org/10.3390/molecules29071443

Nilus, R., Lee Ying Fah, & Alexander Hastie. (2011). Species selection trial in burnt peat swamp vegetation in southwest coast of Sabah, Malaysia. In *Proceedings of the International Symposium Rehabilitation of Tropical Rainforest Ecosystems, Serdang, Kuala Lumpur, Malaysia*.

Novianry, V., Astuti, P., & Andriani. (2024). Comparative analysis of mitragynine content in Kratom leaves (*Mitragyna speciosa* Korth) from Kabupaten Kapuas Hulu Using HPLC Method. *SCISCITATIO*, *5*(2), 87–93. https://doi.org/10.21460/sciscitatio.2024.52.183

Obeng, S., Crowley, M. L., Mottinelli, M., León, F., Zuarth Gonzalez, J. D., Chen, Y., Gamez-Jimenez, L. R., Restrepo, L. F., Ho, N. P., Patel, A., Martins Rocha, J., Alvarez, M. A., Thadisetti, A. M., Park, C. R., Pallares, V. L. C., Milner, M. J., Canal, C. E., Hampson, A. J., McCurdy, C. R., Hiranita, T. (2024). The *Mitragyna speciosa* (Kratom) alkaloid mitragynine: Analysis of adrenergic α2 receptor activity in vitro and in vivo. *European Journal of Pharmacology*, *980*, 176863. https://doi.org/10.1016/j.ejphar.2024.176863

Ortiz, Y. T., Mukhopadhyay, S., McCurdy, C. R., McMahon, L. R., & Wilkerson, J. L. (2024). Efficacy and receptor activity of mitragynine and speciogynine in attenuating cancer-induced bone pain associated mechanical allodynia. *The Journal of Pain*, *25*(4), 12. (Abstract). https://doi.org/10.1016/j.jpain.2024.01.060

Pansai, N., Wungsintaweekul, J., & Wichienchot, S. (2024). The effects of *Mitragyna speciosa* extracts on intestinal microbiota and their metabolites in vitro fecal fermentation. *Journal of the Science of Food and Agriculture*, *104*(14), 8500–8510. https://doi.org/10.1002/jsfa.13677

Phongprueksapattana, S., Putalun, W., Keawpradub, N., & Wungsintaweekul, J. (2008). *Mitragyna speciosa*: hairy root culture for triterpenoid production and high yield of mitragynine by regenerated plants. *Zeitschrift Für Naturforschung C*, *63*(9–10), 691–698. https://doi.org/10.1515/znc-2008-9-1014

Phromchan, W., Defri, I., Saensano, C., Chookaew, A., Chiarawipa, R., & Sriwiriyajan, S. (2024). Morphological and physiological properties of Kratom (*Mitragyna speciosa*) leaves: Macronutrients, phytochemicals, antioxidants, and mitragynine content. *Plant Science Today*, *11*(2), 762-770. https://doi.org/10.14719/pst.2991

Pramulya, M., Salsabila, U., & Ramadhan, T. H. (2024). Examining the impact of cultivated land distance from riparian areas on the growth and quality of red Kratom alkaloids. *Journal of Global Innovations in Agricultural Sciences*, 605–612. https://doi.org/10.22194/JGIAS/24.1351

Prasetya, R., & Sudarwati, T. (2023). Kratom (*Mitragyna speciosa*) leaf ethanol extract showed in vivo analgesic activity. *Pharmacology and Clinical Pharmacy Research*, *8*, 102–107. https://doi.org/doi:10.15416/pcpr.v8i2.40727

Predescu, I.-A., Jîjie, A.-R., Pătraşcu, D., Pasc, A.-L.-V., Piroş, E.-L., Trandafirescu, C., Oancea, C., Dehelean, C. A., & Moacă, E.-A. (2025). Unveiling the complexities of medications, substance abuse, and plants for recreational and narcotic purposes: An in-depth analysis. *Pharmacy*, *13*(1), 7. https://doi.org/10.3390/pharmacy13010007

Prevete, E., Catalani, V., Singh, D., Kuypers, K. P. C., Theunissen, E. L., Townshend, H. D., Banayoti, H., Ramaekers, J. G., Pasquini, M., & Corazza, O. (2024). A preliminary inventory of Kratom (*Mitragyna Speciosa*) products and vendors on the darknet and cryptomarkets. *Journal of Psychoactive Drugs*, *56*(4), 485–495. https://doi.org/10.1080/02791072.2023.2242361

Priatna, P. A., Rahmah, S., Widyowati, R., & Sukadirman. (2025). Identification of LC-MS/MS and docking analysis of topoisomerase iiα inhibition from Kratom leaves (*Mitragyna speciosa*) as potential anticancer agents. *International Journal of Applied Pharmaceutics*, 119–125. https://doi.org/10.22159/ijap.2025.v17s1.18

Putri, M., Wardoyo, E. R. P., & Kurniatuhadi, R. (2023). Potensi ekstrak metanol akar dan batang kratom (*Mitragyna speciosa* Korth.) sebagai antibakteri *Propionibacterium acnes* ATCC 6919 penyebab jerawat [Potential of methanol extract of kratom (*Mitragyna speciosa* Korth.) roots and stems as antibacterial against *Propionibacterium acnes* ATCC 6919, the causative agent of acne]. *Protobiont*, *12*(2), 43–49.

Qi, Y., Gao, P., Yang, S., Li, L., Ke, Y., Zhao, Y., Huang, F., & Yu, L. (2024). Unveiling the impact of nitrogen deficiency on alkaloid synthesis in konjac corms (*Amorphophallus muelleri* Blume). *BMC Plant Biology*, *24*(1), 923. https://doi.org/10.1186/s12870-024-05642-z

Rahmawati, S. I., Indriani, D. W., Ningsih, F. N., Hardhiyuna, M., Firdayani, F., Ahmadi, P., Rosyidah, A., Septiana, E., Dharmayanti, N. L. P. I., Bayu, A., & Putra, M. Y. (2024). Dual anti-inflammatory activities of COX-2/5-LOX driven by Kratom alkaloid extracts in lipopolysaccharide-induced RAW 264.7 cells. *Scientific Reports*, *14*(1), 28993. https://doi.org/10.1038/s41598-024-79229-x

Romeu, A. G., Cox, D. J., Smith, K. E., Dunn, K. E., & Griffiths, R. R. (2020). Kratom (*Mitragyna speciosa*): User demographics, use patterns, and implications for the opioid epidemic. *Drug and Alcohol Dependence*, *208*, 107849. https://doi.org/10.1016/J.DRUGALCDEP.2020.107849

Rossalinda, B., Astina, A., & Palupi, T. (2024). Respon pertumbuhan bibit kratom terhadap jenis tanah yang berbeda [Response of kratom seedlings growth to different soil types]. *Journal Sains Pertanian Equator*, *13*(2), 576. https://doi.org/10.26418/jspe.v13i2.77021

Saleh, S. R., Hasan, M. H., Said, Md. I. M., Adenan, M. I., & Adam, A. (2012). Antioxidant, anti-inflammatory and antinociceptive activities of *Mitragyna speciosa* and *Erythroxylum cuneatum*. *2012 IEEE Symposium on Humanities, Science and Engineering Research*, 1087–1091. https://doi.org/10.1109/SHUSER.2012.6268787

Saravanakumar, V., Masi, C., Neme, I., Arjun, K., & Dinakarkumar, Y. (2022). Geographical comparison of phytoconstituents in *Euphorbia hirta*: A pilot study in Ethiopia and India. *Bulletin of Pioneering Researches of Medical and Clinical Science*, *1*(2), 34–41. https://doi.org/10.51847/ErNYBrhrFF

Schotte, C., Jiang, Y., Grzech, D., Dang, T.-T. T., Laforest, L. C., León, F., Mottinelli, M., Nadakuduti, S. S., McCurdy, C. R., & O'Connor, S. E. (2023). Directed biosynthesis of mitragynine stereoisomers. *Journal of the American Chemical Society*, *145*(9), 4957–4963. https://doi.org/10.1021/jacs.2c13644

Sengnon, N., Vonghirundecha, P., Chaichan, W., Juengwatanatrakul, T., Onthong, J., Kitprasong, P., Sriwiriyajan, S., Chittrakarn, S., Limsuwanchote, S., & Wungsintaweekul, J. (2023). Seasonal and geographic variation in alkaloid content of Kratom (*Mitragyna speciosa* (Korth.) Havil.) from Thailand. *Plants*, *12*(4), 949. https://doi.org/10.3390/PLANTS12040949/S1
Shellard, E. J. (1983). Mitragyna: A note on the alkaloids of African species. *Journal of Ethnopharmacology*, *8*(3), 345–347. https://doi.org/10.1016/0378-8741(83)90073-9
Singh, D., Narayanan, S., Grundmann, O., Dzulkapli, E. Bin, & Vicknasingam, B. (2019). Effects of Kratom (*Mitragyna Speciosa* Korth.) use in regular users. *Substance Use & Misuse*, *54*(14), 2284–2289. https://doi.org/10.1080/10826084.2019.1645178
Singh, D., Yeou, N. J. C., Narayanan, S., Leon, F., Sharma, A., McCurdy, C. R., Avery, B. A., & Balasingam, V. (2020). Patterns and reasons for Kratom (*Mitragyna speciosa*) use among current and former opioid poly-drug users. *Journal of Ethnopharmacology*, *249*, 112462. https://doi.org/10.1016/j.jep.2019.112462
Singh, D., Azuan, M. A., & Narayanan, S. (2023). Kratom (*Mitragyna speciosa*) use in a sample of drug-dependent adolescents in rehabilitation for drug use in Malaysia. *Journal of Ethnicity in Substance Abuse*, 1–16. https://doi.org/10.1080/15332640.2023.2293941
Sitthiphon, B., & Prakasit, W. (2025). Understanding the effects and clinical potential of Kratom (*Mitragyna speciosa*): A narrative review. *The Bangkok Medical Journal*, *21*(1), 73–77. https://doi.org/10.31524/bkkmedj.2025.13.002
Smith, K. E., Panlilio, L. V., Sharma, A., McCurdy, C. R., Feldman, J. D., Mukhopadhyay, S., Kanumuri, S. R. R., Kuntz, M. A., Hill, K., & Epstein, D. H. (2024). Time course of Kratom effects via ecological momentary assessment, by product type, dose amount, and assayed alkaloid content. *Drug and Alcohol Dependence*, *264*, 112460. https://doi.org/10.1016/j.drugalcdep.2024.112460
Sofia, N., Yuniarti, Y., & Rosidah, R. (2022). Uji fitokimia terhadap tanaman obat kratom (*Mitragyna speciosa*) di KHDTK ULM [Phytochemical test of kratom (*Mitragyna speciosa*) medicinal plant in KHDTK ULM]. *Jurnal Sylva Scienteae*, *5*(2), 218-224. https://doi.org/10.20527/jss.v5i2.5356
Sornsenee, P., Chimplee, S., & Romyasamit, C. (2025). Evaluation of antibacterial, antibiofilm, antioxidant, and anti-inflammatory activities of Kratom leaves (*Mitragyna speciosa*) fermentation supernatant containing *Lactobacillus rhamnosus* GG. *Probiotics and Antimicrobial Proteins*, *17*(1), 328–340. https://doi.org/10.1007/s12602-023-10142-x
Sudmoon, R., Tanee, T., Wonok, W., Ameamsri, U., Liehr, T., Daduang, S., Siripiyasing, P., & Chaveerach, A. (2025). Discovery of rhynchophylline and mitraphylline in two Thai Mitragyna species and the investigation of their biological activity via opioid gene expression analysis. *Scientific Reports*, *15*(1), 5865. https://doi.org/10.1038/s41598-025-89715-5
Suhaimi, F. W., Yusoff, N. H. M., Hassan, R., Mansor, S. M., Navaratnam, V., Müller, C. P., & Hassan, Z. (2016). Neurobiology of Kratom and its main alkaloid mitragynine. *Brain Research Bulletin*, *126*, 29–40. https://doi.org/10.1016/J.BRAINRESBULL.2016.03.015
Sureram, S., Chutiwitoonchai, N., Pooprasert, T., Sangsopha, W., Limjiasahapong, S., Jariyasopit, N., Sirivatanauksorn, Y., Khoomrung, S., Mahidol, C., Ruchirawat, S., & Kittakoop, P. (2024). Discovery of procyanidin condensed tannins of (−)-epicatechin from Kratom, *Mitragyna speciosa*, as virucidal agents against SARS-CoV-21. *International Journal of Biological Macromolecules*, *273*. https://doi.org/10.1016/j.ijbiomac.2024.133059
Tabanelli, R., Brogi, S., & Calderone, V. (2023). Targeting opioid receptors in addiction and drug withdrawal: where are we going? *International Journal of Molecular Sciences*, *24*(13), 10888. https://doi.org/10.3390/ijms241310888
Takayama, H., Kurihara, M., Kitajima, M., Said, I. M., & Aimi, N. (1998). New indole alkaloids from the leaves of Malaysian *Mitragyna speciosa*. *Tetrahedron*, *54*(29), 8433–8440. https://doi.org/10.1016/S0040-4020(98)00464-5
Takayama, H. (2004). Chemistry and pharmacology of analgesic indole alkaloids from the Rubiaceous plant, *Mitragyna speciosa*. *Chemical and Pharmaceutical Bulletin*, *52*(8), 916–928. https://doi.org/10.1248/CPB.52.916
Tampanna, N., Wanitsuwan, W., Chewatanakornkul, S., Wangkulangkul, P., Theapparat, Y., Detarun, P., & Wichienchot, S. (2025). The role of Kratom (*Mitragyna speciosa* Korth.) extract in medical

foods for obese patients: Effects on gut microbiota in a colon model. *Food Research International*, *204*, 115935. https://doi.org/10.1016/j.foodres.2025.115935

Tanti, Lalangi, C. A., Arfiyani, E., Ningtias, W., & Maulida, E. N. (2021). Mitragynine percentages of various Kratom variants seized in Indonesia: A quantitative analysis using liquid chromatography-photo diode array detector. *International Journal of Applied Pharmaceutics*, *13*(5), 252–256. https://doi.org/10.22159/ijap.2021v13i5.41910

Thoma, F., Somborn-Schulz, A., Schlehuber, D., Keuter, V., & Deerberg, G. (2020). Effects of light on secondary metabolites in selected leafy greens: A review. *Frontiers in Plant Science*, *11*, *497*. https://doi.org/10.3389/fpls.2020.00497

Tuntiyasawasdikul, S., Junlatat, J., Tabboon, P., Limpongsa, E., & Jaipakdee, N. (2024). *Mitragyna speciosa* ethanolic extract: Extraction, anti-inflammatory, cytotoxicity, and transdermal delivery assessments. *Industrial Crops and Products*, *208*, 117909. https://doi.org/10.1016/j.indcrop.2023.117909

Utar, Z., Majid, M. I. A., Adenan, M. I., Jamil, M. F. A., & Lan, T. M. (2011). Mitragynine inhibits the COX-2 mRNA expression and prostaglandin E 2 production induced by lipopolysaccharide in RAW264.7 macrophage cells. *Journal of Ethnopharmacology*, *136*(1), 75–82. https://doi.org/10.1016/j.jep.2011.04.011

Utomo, R. S., Wibowo, M. A., Nurmainah, N., & Burhansyah, R. (2022). Local culture of Kratom (*Mitragyna speciosa*) consumption in Kapuas Hulu district. *AIP Conference Proceedings*, *2563*(1), 050026. https://doi.org/10.1063/5.0104736

Veeramohan, R., Azizan, K. A., Aizat, W. M., Goh, H. H., Mansor, S. M., Yusof, N. S. M., Baharum, S. N., & Ng, C. L. (2018). Metabolomics data of *Mitragyna speciosa* leaf using LC-ESI-TOF-MS. *Data in Brief*, *18*, 1212–1216. https://doi.org/10.1016/j.dib.2018.04.001

Veeramohan, R., Zamani, A. I., Azizan, K. A., Goh, H. H., Aizat, W. M., Razak, M. F. A., Yusof, N. S. M., Mansor, S. M., Baharum, S. N., & Ng, C. L. (2023). Comparative metabolomics analysis reveals alkaloid repertoires in young and mature *Mitragyna speciosa* (Korth.) Havil. Leaves. *PLoS ONE*, *18*(3), e0283147. https://doi.org/10.1371/JOURNAL.PONE.0283147

Viwatpinyo, K., Mukda, S., & Warinhomhoun, S. (2023). Effects of mitragynine on viability, proliferation, and migration of C6 rat glioma, SH-SY5Y human neuroblastoma, and HT22 immortalized mouse hippocampal neuron cell lines. *Biomedicine & Pharmacotherapy*, *166*, 115364. https://doi.org/10.1016/j.biopha.2023.115364

Yang, S., Xu, Y., Tang, Z., Jin, S., & Yang, S. (2024). the impact of alkaline stress on plant growth and its alkaline resistance mechanisms. *International Journal of Molecular Sciences*, *25*(24), 13719. https://doi.org/10.3390/ijms252413719

Yunusa, S., Hassan, Z., & Müller, C. P. (2023). Mitragynine inhibits hippocampus neuroplasticity and its molecular mechanism. *Pharmacological Reports*, *75*(6), 1488–1501. https://doi.org/10.1007/s43440-023-00541-w

Zailan, N. F. Z., Sarchio, S. N. E., & Hassan, M. (2022). Evaluation of phytochemical composition, antioxidant and anti-diabetic activities of *Mitragyna speciosa* methanolic extract (MSME). *Malaysian Journal of Medicine and Health Sciences*, *18*(s21), 93–100. https://doi.org/10.47836/mjmhs.18.s21.15

Zhang, M., Sharma, A., León, F., Avery, B., Kjelgren, R., McCurdy, C. R., & Pearson, B. J. (2020). Effects of nutrient fertility on growth and alkaloidal content in *Mitragyna speciosa* (Kratom). *Frontiers in Plant Science*, *11*. https://doi.org/10.3389/fpls.2020.597696

Zhang, M., Sharma, A., Leó, F., Avery, B., Kjelgren, R., Mccurdyid, C. R., & Pearsonid, B. J. (2022). Plant growth and phytoactive alkaloid synthesis in Kratom [*Mitragyna speciosa* (Korth.)] in response to varying radiance. https://doi.org/10.1371/journal.pone.0259326

JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH
2025, VOL. 8(4), 581-594

Journal of Horticulture and Postharvest Research

Journal homepage: www.jhpr.birjand.ac.ir

University of Birjand

Extending vase life and reducing ethylene production in rose cut flowers using Calamondin *(Citrus macrocarpa)* extract

Lorelyn Joy Noble Turnos-Milagrosa[1,*]

1, Horticulture Division, Department of Crop Science, College of Agriculture, University of Southern Mindanao, Philippines

ARTICLE INFO

Original Article

Article history:
Received 30 April 2025
Revised 30 September 2025
Accepted 4 October 2025

Keywords:
Cut flowers
Ethylene
Flower longevity
Hydrating solutions
Postharvest

DOI: 10.22077/jhpr.2025.9319.1511
P-ISSN: 2588-4883
E-ISSN: 2588-6169

***Corresponding author:**
Horticulture Division, Department of Crop Science, College of Agriculture, University of Southern Mindanao, Philippines.

Email: lturnos@usm.edu.ph

ABSTRACT

Purpose: Floral preservative vase solutions commonly use citric acid to reduce the pH of water, thereby extending the vase life of cut flowers. This study was conducted to explore the potential of hydrating solutions in extending the vase life of roses and reducing the production of ethylene. **Research Method:** Cut roses were used to evaluate the efficacy of hydrating solutions (calamondin juice, cinnamon and garlic powder extracts and bamboo wood vinegar) and stem cutting every two days to increase the vase life and slow down changes in the visual quality parameters when added in vase solution. **Findings:** Cut roses subjected to calamondin extract (20 ml/L) had a statistically longer vase life of 11.67 days (37.29% higher than the control), followed by bamboo wood vinegar (9.67 days or 13.76%), while untreated samples had 8.50 days (*p value = 0.000*). Roses treated with cinnamon powder and ginger extract attained limit of fitness for decoration at Day 8. During the peak of ripening at day 7, calamondin treatments reduced the ethylene concentration by 10.60% and resulted in the highest carbon dioxide (0.87% vol), which was statistically different from the other treatments. **Research limitations:** No limitations were identified in the study. **Originality/Value:** The study demonstrated that the addition of calamondin extract significantly slows down deteriorative changes in cut flowers. Given the economic feasibility and widespread availability of calamondin fruits in local markets, this postharvest treatment presents a practical and cost-effective option for flower vendors.

INTRODUCTION

Flowers or flower buds cut from the plant are referred to as cut flowers, and each species of cut flower has a unique vase life and freshness longevity, which are significantly influenced by preharvest factors, harvesting activities and postharvest handling conditions. Vase longevity, which is usually determined by a flower's sensitivity to ethylene, is a major factor influencing consumers' decision to purchase and repurchase cut flowers (Rihn et al., 2015). The vase life of cut flowers is limited due to microbial infection, depletion of organic reserve through respiration, withering, poor water quality, storage temperature, ethylene sensitivity and mechanical damage (Vehniwal & Abbey, 2019). Lower storage temperatures can reduce carbon dioxide production, as flowers undergo high respiratory activity that depletes the already limited organic substrates in the petals.

Tinebra et al. (2021) emphasized that the postharvest storage potential is inversely related to respiration, especially in flowers where organic respiratory reserves are minimal. The quality of a cut flower depends on its morphological traits and longevity, which are measured by the duration of these characteristics. Shelf life is a critical factor in determining the market value of cut flowers (Cavalcante et al., 2021).

Among cut flowers, cultivated roses have the highest production area and market share worldwide, and their ornamental and economic value is significantly influenced by flower opening and senescence (Chen et al., 2023). In the Philippines, cut flower production is limited to a small number of growers. There has been increasing demand for cut flowers in recent years, which triggered greater production; however, despite the expansion of cultivation areas, there is still a shortfall in supply.

Rose (*Rosa* spp.), belonging to the Rosaceae family, is one of the most in-demand ornamental plants in the world for its high economic and cultural importance. Cut rose flowers play a significant role in the rose cultivation industry (Mortazavi et al., 2007). Energy transformation and metabolic activity continue in roses after detachment from the parent plant due to their high content of moisture and richness in endogenous enzymes. Cut roses have a comparatively short vase life, which affects flower longevity, petal abscission, functional petal life, neck bending, wilting and overall deterioration and senescence.

Vase life is strongly influenced by temperature, sunlight exposure during vase storage, hydrating solutions, and the stem's ability to absorb and uptake of water. Previous studies (Darandeh & Hadavi, 2012; Kandel et al., 2021) revealed that adding citric acid in the vase solutions improved flower longevity and extended the postharvest life of cut flowers by controlling microbial activity and lowering the solution's pH. This study evaluated the potential of citric acid from calamondin, cinnamon powder, ginger extracts, and bamboo wood vinegar to extend the postharvest life and reduce ethylene production in the cut rose's samples

Floral preservative solutions are among the most widely used strategies of the flower vendors to enhance quality of cut flowers and other ornamentals (Nguyen et al., 2020). Holding solutions may contain a mixture of plant growth regulators, carbohydrates, mineral salts, organic acids and ethylene inhibitors. Among the many citrus fruit sources of citric acid, calamondin (also known as calamansi) was selected as the key ingredients of the vase solution due to its higher citric acid content and other beneficial properties, making it potentially more valuable, accessible, and economically feasible than other citrus commodities.

Essential oil made from ginger rhizome extracts contains linalool and borneol, and also possesses antifungal and antibacterial properties (Ma et al., 2021), which could enhance plant development. In addition, cinnamon essential oil, which is mostly derived from aldehydes, is popular for its potent antioxidant and antimicrobial properties (Shu et al., 2024), and was found to reduce pathogen infections and rate of deterioration in many vegetables and fruits. Moreover, bamboo wood vinegar or pyrolysis oil, produced from natural carbonization (Sudaria et al., 2016) has acetic acid as the main component and has exhibited potential in enhancing the harvest and postharvest qualities of fruits and vegetables.

MATERIALS AND METHODS

Materials

The materials used in this study included 'Hybrid tea' cut roses (purchased from a rose farm in Valencia, Bukidnon, Philippines), vases, sterile distilled water, hydrating solutions prepared from calamondin fruits, cinnamon powder, ginger, bamboo wood vinegar, BH-4S Portable multi-gas detector, and digital weighing scale.

Methods

Experimental design and treatments

Experimental site: The experiment was conducted in the Postharvest Laboratory Room of the College of Agriculture, University of Southern Mindanao, Kabacan, Cotabato, Philippines. The room conditions during the study were maintained at a temperature range of 25–31 °C, relative humidity of approximately 60%, and light intensity between 700 and 750 lux.

The study was carried out a factorial Completely Randomized Design with two factors and three replications per treatment combination. Factor A refers to the stem cutting (without cutting and with cutting), while factor B refers to the hydrating solutions (untreated/water only, calamondin extract (20 ml/L), cinnamon powder (20 g/L), ginger extract (20 g/L) and bamboo wood vinegar (1 ml/L)). There were 10 cut roses per experimental unit as the test materials. Stems (approximately 60 cm in length) of rose cut flowers were put in vase containers (5 cut flowers per vase, 2 vases per experimental unit) with hydrating solution treatments added to 500 ml of water. Rose stem cutting was done above the nodes, with *45^o* angle, at 2 cm stem length. For Factor A, cut roses assigned to the stem cutting treatment were trimmed using sharp, clean cutters at 2-day intervals. The vases used to store the roses were sanitized with a dilute bleach solution prior to addition of vase solution.

Preparation of hydrating solutions

Preparation of calamondin extract: Calamondin juice was manually extracted and homogenized using a mixture of methanol and water. The resulting solution was thoroughly mixed and then diluted with distilled water to achieve a concentration of 20 mL per liter for use as a vase solution. The final pH of the prepared vase solution was 2.80.

Preparation of cinnamon powder: A commercially available cinnamon powder was dissolved in distilled water at a concentration of 20 g per liter. The final pH of the prepared vase solution was 5.0.

Preparation of ginger extract: Ginger extract was prepared from fresh ginger rhizomes, which were washed and dried at 40 °C for 48 hours. The dried rhizomes were ground and stored at 15 °C prior to extraction. Soxhlet extraction was performed using 85% ethanol, followed by concentration and drying of the extract. The final product was stored at 5 °C and subsequently mixed with distilled water at a concentration of 20 g/L for use as a vase solution. The final pH of the prepared solution was 3.6.

Preparation of bamboo wood vinegar: A basic wood vinegar extraction system was established, consisting of key components such as a burning chamber, smoke/gas generator, smoke pipe, smoke chamber, and wood vinegar collector. The process began by stacking bamboo materials inside the smoke/gas generator, which were then left to sit for 90 days to undergo essential purification. The resulting bamboo wood vinegar was mixed with distilled water at a concentration of 1 mL/L for use as a vase solution. The final pH of the prepared solution was 3.4.

Postharvest quality parameters

Data parameters include: A) visual quality rating: rating of 9 – excellent, field fresh, no defect; 7 – good condition, minor defects; 5 – fair condition, moderate defects; 3 – limit of marketability; 2 - poor, serious defects, limit of fitness for decoration; 1 – unfit for decoration; B) bending, discoloration and wilting: 0 – No bending/discoloration/wilting; 1-1-25% bending/discoloration/wilting; 2–26-50% bending/discoloration/wilting; 3 – above 50% bending/discoloration/wilting. Data parameters such as visual quality, neck bending, and petal discoloration and wilting were monitored at days 1, 2,4, 6, 8, 10, 11, 12, while weight loss at days 6, 9 and 11.

Vase life in cut roses refers to the duration (in days) during which the flowers maintain their decorative value, specifically until they reach the threshold of fitness for decoration (VQR-2), following treatment with hydrating vase solutions. It is measured from the time the rose is cut and placed in water until it loses its ornamental appeal due to wilting, petal discoloration, or other signs of senescence.

Gases monitoring: The BH-4S Portable Multi-Gas Detector was used to monitor ethylene, carbon dioxide, and oxygen levels through natural diffusion of ambient air into the device. Gas measurements were conducted every other day, beginning from the initial setup of the experiment.

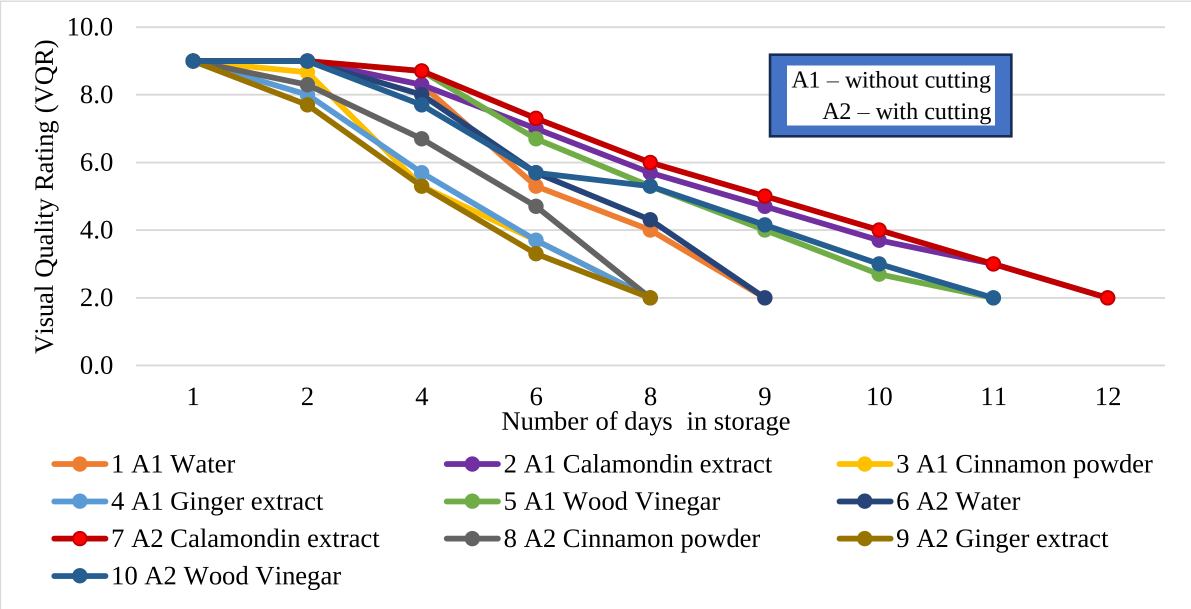
Statistical analysis

All data parameters were subjected to appropriate statistical data analysis using the Statistical Tool for Agricultural Research (STAR), software version 2.0.1. Analysis of Variance (ANOVA) was employed to determine significant differences among treatment means, with the F-test used to assess overall significance. The level of significance was set at 5%, and treatments showing significant differences were further analysed using the Least Significant Difference (LSD) test.

RESULTS

Visual quality rating and vase life

The visual quality rating (VQR) of the treated rose cut flowers is presented in Figure 1. Roses treated with cinnamon powder and ginger extract reached the VQR-2 threshold (the limit of fitness for decoration) earliest, on day 8. In contrast, those treated with calamondin extract and bamboo wood vinegar remained in good condition with minor defects on Day 8, and only reached VQR-2 on days 11 and 9, respectively. For samples subjected to stem recutting every two days, VQR ratings were generally similar to those of samples without stem cutting, provided they received the same hydrating solution treatments. Figures 2 and 3 provide photo documentation of the treated cut flowers at 6 and 11 days after treatment application, showing that the calamondin treatment consistently maintained superior physical appearance compared to the other treatments.



VQR Rating: 9 – excellent, field fresh, no defect; 7 – good, minor defects; 5 – fair, moderate defects; 3 – limit of marketability; 2 - poor, serious defects, limit of fitness for decoration; 1 – unfit for decoration

Fig. 1. Visual quality rating (VQR) of rose cut flowers treated with various hydrating solutions and stem cutting up to 12 days in storage. USM, Kabacan, Cotabato, Philippines, 2025.

Fig. 2. Cut roses treated with various hydrating solutions and stem cutting at day 6. USM, Kabacan, Cotabato, Philippines, 2025.

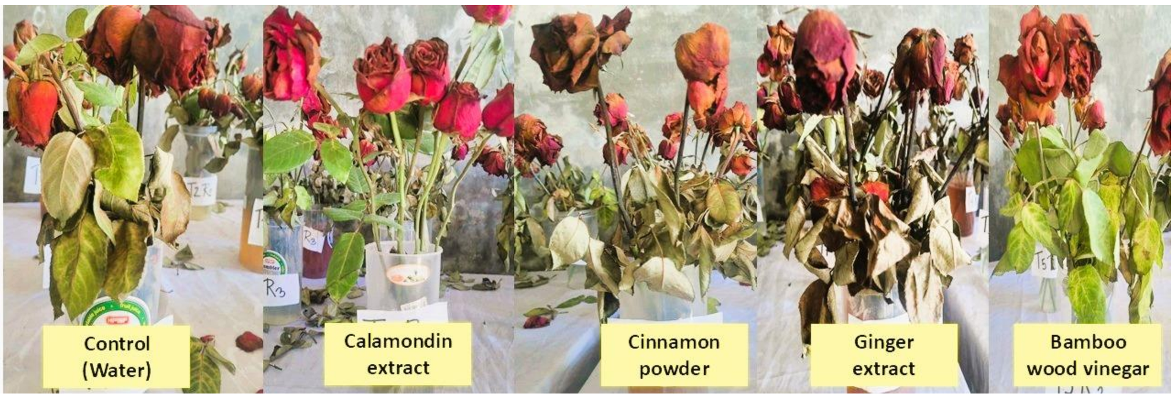


Fig. 3. Cut roses treated with various hydrating solutions and stem cutting at day 11. USM, Kabacan, Cotabato, Philippines, 2025.

Table 1. Vase life or number of days to which the roses attain VQR-2 (limit of fitness for decoration) after treating the cut flowers to four hydrating solutions and to stem recutting for every 2 days. University of Southern Mindanao, Kabacan, Cotabato, Philippines, 2025.

Treatments	Without cutting	With cutting	Factor B**
Water	8.33	8.67	8.50[c]
Calamondin extract	11.67	11.67	11.67[a]
Cinnamon	6.67	7.00	6.83[d]
Ginger	6.67	6.67	6.67[d]
Bamboo wood vinegar	9.00	10.33	9.67[b]
Factor A	8.47	8.87	

	F value	Pr (>F)
Factor A	3.27	0.0855
Factor B	71.14	0.0000
Factor A:Factor B	0.45	0.3308

CV (%) - 6.99; Mean – 8.67 days. **highly significant, Least Significant Difference (LSD) Test. Means followed by different letters are significantly different at p<0.05.

Table 2. Neck bending, petal discoloration and wilting of rose cut flowers treated with various hydrating solutions and stem cutting at days 8 and 11 after treatment application. USM, Kabacan, Cotabato, Philippines, 2025.

	Day 8		Day 11	
Treatments	Without Cutting	With Cutting	Without Cutting	With Cutting
	Neck Bending			
Water	3.0	3.0	3.0	3.0
Calamondin extract	1.7	1.7	2.0	2.3
Cinnamon powder	3.0	3.0	3.0	3.0
Ginger extract	3.0	3.0	3.0	3.0
Bamboo wood vinegar	3.0	2.7	3.0	3.0
	Petal Discoloration			
Water	3.0	3.0	3.0	3.0
Calamondin extract	2.0	2.0	2.0	2.0
Cinnamon powder	3.0	3.0	3.0	3.0
Ginger extract	3.0	3.0	3.0	3.0
Bamboo wood vinegar	2.3	2.0	3.0	3.0
	Wilting			
Water	3.0	3.0	3.0	3.0
Calamondin extract	1.3	1.7	2.3	2.3
Cinnamon powder	3.0	3.0	3.0	3.0
Ginger extract	3.0	3.0	3.0	3.0
Bamboo wood vinegar	2.3	2.7	3.0	3.0

Rating Scale: 0 – No bending/discoloration/wilting; 1 - 1-25% bending/discoloration/wilting; 2 – 26-50% bending/discoloration/wilting; 3 – above 50% bending/discoloration/wilting.

Table 1 revealed highly significant differences in the vase life of cut roses based on the type of hydrating solution used (Factor B). However, no significant differences were observed with respect to stem recutting every other two days (Factor A), nor in the interaction between the two factors. Among the treatments, calamondin extract significantly extended the vase life of cut roses to 11.67 days, followed by bamboo wood vinegar at 9.67 days, compared to 8.50 days in the untreated control. No significant interaction was found between hydrating solution type and stem recutting in terms of their effect on vase life.

Additional data parameters reflecting the physical attribute of cut rose flowers on days 8 and 11 after treatment application are presented in Table 2, including neck bending, petal discoloration, and wilting. Among the treatments, calamondin extract demonstrated superior performance. On day 8, cut flowers in all other treatments exhibited notable deterioration in physical appearance, whereas those treated with calamondin showed minimal signs of decline, with neck bending rated at 1.7, petal discoloration at 2.0, and wilting ranging from 1.3 to 1.7—corresponding to a verbal description of 1–25% bending, discoloration, and wilting. Furthermore, no visible differences were observed in the physical characteristics of rose samples regardless of whether stems were recut every other two days.

Table 3. Weight loss percentage (%) of rose cut flowers treated with various hydrating solutions and stem cutting at days 3, 6, 9 and 11 of storage. USM, Kabacan, Cotabato, Philippines. 2025.

Treatments	Without cutting	With cutting	Factor B**
Water	8.33	8.67	8.50^{c}
	Factor A		
	Day6**	Day9*	Day11**
Without cutting	54.72	65.47^{b}	67.47^{b}
With cutting	46.51	79.09^{a}	85.31^{a}
	Factor B		
Treatments	Day6*	Day9^{ns}	Day11 ^{ns}
Water	51.66^{a}	68.71	76.00
Calamondin extract	43.60^{b}	65.13	75.42
Cinnamon powder	52.48^{a}	68.37	76.70
Ginger extract	53.73^{a}	64.93	76.22
Bamboo wood vinegar	51.60^{a}	71.74	77.59
	Factor A × B		
	Day6 ^{ns}	Day9^{ns}	Day11^{ns}
Without Stem Cutting			
Water	55.08^{a}	65.44	68.94
Calamondin extract	52.22^{a}	65.56	66.94
Cinnamon powder	54.62^{a}	63.66	65.33
Ginger extract	55.81^{a}	63.28	66.14
Bamboo wood vinegar	55.86^{a}	69.42	69.98
With Stem Cutting			
Water	48.24^{b}	71.97	83.06
Calamondin extract	34.98^{b}	64.75	83.90
Cinnamon powder	50.33^{a}	73.08	88.07
Ginger extract	51.64^{a}	66.58	86.31
Bamboo wood vinegar	47.35^{b}	74.06	85.20
CV (a) %	11.10	8.60	7.49
CV (b) %	6.41	7.83	4.55
Mean	50.61	67.78	76.39

	Day 6		Day 9		Day 11	
	F-value	Pr (>F)	F-value	Pr (>F)	F-value	Pr (>F)
Factor A	24.01	0.0001	5.14	0.0346	106.66	0.0000
Factor B	4.59	0.0086	1.54	0.2288	0.18	0.9468
FactorA:FactorB	2.05	0.1258	0.70	0.6013	0.85	0.5092

** - highly significant, Least Significant Difference (LSD) Test, * - significant, ns – not significant
Means followed by different letters are significantly different at $p<0.05$.

Weight loss

The percentage weight loss of cut rose flowers as influenced by hydrating solutions and stem recutting, is presented in Table 3. A highly significant interaction was observed between stem recutting and hydrating solution treatents on day 6. Roses with stems recut every two days exhibited significantly lower weight loss when treated with calamondin extract, bamboo wood vinegar, or water, ranging from 34.98 to 48.24% However, on days 9 and 11, roses subjected to stem recutting showed consistently higher weight reduction compared to those without stem cutting. No significant differences were found among the hydrating solution treatments alone, or in the interaction between the two factors, with respect to weight reduction at these later stages.

Table 4. Ethylene (ppm) concentrations of rose cut flowers treated with various hydrating solutions and stem cutting at days 1,3, 5, 7 and 9 of storage. USM, Kabacan, Cotabato, Philippines, 2025.

	Ethylene (ppm)				
	Day1[ns]	Day3**	Day5**	Day7*	Day9**
Water	20.10	35.50[ab]	53.33[b]	88.00[a]	25.67[b]
Calamondin extract	20.06	30.50[c]	49.17[c]	78.67[b]	31.33[a]
Cinnamon powder	20.06	30.33[c]	49.17[c]	83.50[ab]	32.50[a]
Ginger extract	20.03	37.67[a]	54.33[b]	85.50[a]	25.17[b]
Bamboo wood vinegar	20.06	34.67[b]	60.33[a]	84.83[a]	25.67[b]
Ethylene (CV, %)	5.65	6.82	4.14	5.91	5.71
Ethylene (Mean)	20.06	33.73	53.27	84.10	28.07

	Day 3		Day 5		Day 7		Day 9	
	F-value	Pr (>F)	F-value	Pr (>F)	F-value	Pr (>F)	F-value	Pr (>F)
Factor A	0.23	0.6394	1.75	0.2004	0.49	0.4929	0.83	0.3728
Factor B	11.74	0.0000	26.11	0.0000	2.89	0.0485	29.37	0.0000
FactorA:FactorB	0.27	0.8915	1.22	0.3326	0.32	0.8597	0.67	0.6211

**highly significant, LSD Test, * - significant; ns – not significant
Means followed by different letters are significantly different at $p<0.05$.

Table 5. Carbon dioxide (% vol) concentrations of rose cut flowers treated with various hydrating solutions and stem cutting at Days 1,3, 5, 7 and 9 of storage. USM, Kabacan, Cotabato, Philippines, 2025.

	Carbon dioxide (% Vol)				
	Day 1**	Day3**	Day5[ns]	Day7*	Day9**
Water	0.75[a]	3.08[bc]	3.62	0.73[bc]	0.44[a]
Calamondin extract	0.74[ab]	3.23[a]	3.69	0.87[a]	0.31[c]
Cinnamon powder	0.73[bc]	3.18[ab]	3.71	0.79[b]	0.27[c]
Ginger extract	0.72[c]	3.00[c]	3.55	0.70[c]	0.41[b]
Bamboo wood vinegar	0.72[c]	3.04[c]	3.55	0.60[d]	0.39[b]
Carbon dioxide (CV, %)	2.18	3.06	4.27	8.58	8.80
Carbon dioxide (Mean)	0.73	3.11	3.62	0.74	0.36

	Day 1		Day 3		Day 5		Day 7		Day 9	
	F-value	Pr (>F)	F-value	Pr (>F)	F-value	Pr (>F)	F-value	Pr (>F)	F-value	Pr (>F)
Factor A	0.12	0.7343	4.06	0.0575	1.03	0.3218	0.70	0.4134	0.64	0.4337
Factor B	7.22	0.0009	6.10	0.0022	1.49	0.2422	15.29	0.0000	30.55	0.0000
FactorA:FactorB	0.68	0.6154	0.79	0.5464	0.20	0.9377	2.78	0.0550	0.61	0.6575

**highly significant, LSD Test, * - significant; ns – not significant
Means followed by different letters are significantly different at $p<0.05$.

Effects on ethylene, carbon dioxide and oxygen

Table 4 presents the ethylene production in cut rose flowers as influenced by four hydrating solutions and stem recutting every two days. Initial ethylene concentrations ranged from 20.03 to 20.10 ppm across all treatments. The peak in ethylene production was observed on day 7 of storage, with the highest concentration recorded in roses treated with water only (88.00 ppm), which was statistically comparable to other treatments except for calamondin extract, which showed a significantly lower concentration of 78.67 ppm. By day 9, ethylene levels in roses treated with calamondin extract and cinnamon powder were statistically comparable.

Tables 5 and 6 present the effects of hydrating solutions and stem recutting on carbon dioxide (CO_2) and oxygen (O_2) concentrations, respectively. During the peak of ethylene production on day 7, roses treated with calamondin extract exhibited the highest CO_2 concentration at 0.87%, which was statistically different from the other treatments. No significant differences were observed in O_2 concentrations among treatment means on day 7, with values ranging from 18.18% to 18.56%. A highly significant interaction between hydrating solution and stem cutting was recorded for oxygen levels on day 3. On this day, roses treated with any hydrating solution but without stem cutting showed statistically comparable oxygen concentrations to those treated with cinnamon powder and stem cutting, with values ranging from 19.95% to 20.50%.

Table 6. Oxygen (% vol) concentration of rose cut flowers treated with various hydrating solutions and stem cutting at days 1,3, 5, 7 and 9 of storage. USM, Kabacan, Cotabato, Philippines, 2025.

	Oxygen (% Vol)			
	Day 1**	Day5**	Day7ns	Day9**
Water	20.70a	19.62a	18.18	15.59b
Calamondin extract	20.28b	19.15c	18.56	16.33a
Cinnamon powder	20.77a	19.50ab	18.48	16.48a
Ginger extract	20.25b	19.28bc	18.28	14.22c
Bamboo wood vinegar	20.82a	19.29bc	18.52	15.65b

Oxygen at day 3: Factor A×B**	Without cutting	With cutting
Water	19.95a	20.03bc
Calamondin extract	20.00a	20.10b
Cinnamon powder	20.07a	20.50a
Ginger extract	20.03a	19.91c
Bamboo wood vinegar	20.10a	20.13b

	Day1	Day3	Day5	Day7	Day9
Oxygen (CV, %)	0.53	0.49	1.11	3.30	2.72
Oxygen (Mean)	20.56	20.08	19.37	18.41	15.65

	Day 1		Day 3		Day 5		Day 7		Day 9	
	F-value	Pr (>F)	F-value	Pr (>F)	F-value	Pr (>F)	F-value	Pr (>F)	F-value	Pr (>F)
Factor A	8.26	0.0094	8.38	0.0090	0.23	0.6339	0.03	0.8727	0.00	0.9763
Factor B	36.67	0.0000	9.52	0.0002	4.59	0.0086	0.45	0.7710	26.35	0.0000
FactorA:FactorB	1.19	0.3473	6.20	0.0021	2.00	0.1335	0.59	0.6769	1.42	0.2631

**highly significant, LSD Test, ns – not significant
Means followed by different letters are significantly different at p<0.05.

DISCUSSION

Vase life of cut flowers

Vase life refers to the duration which a cut flower maintains its aesthetic appeal when placed in a vase. This period is assessed through various physical attributes including flower opening, petal coloration, florets, pedicel and stem measurments, fresh weight, senescence patterns, foliage folding and overall longevity (De et al., 2015). Water uptake and water loss of cut flowers are some of the major indicators of the vase life (Frew et al., 2018). The quality and longevity of flowers are influenced by a combination of factors including genetical composition, pre-harvest conditions, techniques used in harvesting, postharvest practices such as handling, packaging, storage and transportation.

The key strategy used by many to extend the postharvest life of cut flowers is through natural products which have anti-microbial properties (lemon juice, fruit extracts and essential oils) which can be used as an alternative to synthetic chemical vase solutions. Postharvest treatments for cut flowers are very crucial in maintaining freshness, quality, and physical attributes, enhancing vase life (Hussen & Yassin, 2013) and reducing waste products of ornamentals by addressing concerns such as respiration, water uptake and microbial growth. Among these postharvest treatments are preservative solutions and stem recutting, in which cut stems are recut under water to prevent air bubbles from blocking water uptake.

Citric acid as postharvest treatments

Citric acid, primarily sourced from citrus fruits such as limes, lemons, oranges and tangerines, can also be found in vegetables and even animal tissues, though it is mostly concentrated in citrus. When citric acid is added to water, it lowers the pH, which is very beneficial for cut flowers. A reduction in pH helps improve water uptake, inhibits bacterial growth in the vase solution, and prevents petal wilting; therefore, extending the flower's vase life (Mehdikhah et al., 2016). Both the peel and the juice of calamondin contain various phytochemicals which can contribute to extending postharvest life of many commodities. They include flavonoids, phenolic compounds and essential oils with D-limonene as key components. These substances have high antioxidant and antimicrobial properties which aid in preventing the growth of microbes and other pathogenic microorganisms, delaying senescence, hence, prolonging the freshness of flowers.

Jadhav and Gurav (2018) reported maximum bud tightness in rose cut flowers, minimum curling of petals and head bending, and reduction of petal drop with the application of sucrose (7%) + citric acid (0.5%) + aluminum sulphate (0.5%). Meperanum et al. (2025) highlighted the potential of sugarcane vinegar and calamondin extract in extending the vase life of chrysanthemum cut flowers by up to 10 days (3 days longer than the untreated samples).

There are various mechanisms involved in the potential of calamondin fruit in delaying senescence and reducing ethylene production, such as the suppression of ethylene's effects, inhibition of ethylene synthesis as well as the influence of other plant hormones. There are reports that 1-methylcyclopropene and benzyladenine reduce ethylene production and delay the degreening in calamondin. There are natural antioxidants like flavonoids and ascorbic acid, and synthetic ethylene inhibitors which can affect development and fruit ripening and senescence, such as influencing ethylene signaling and production (Mwelase et al., 2024).

Ethylene on flower longevity and senescence

Ethylene significantly affects seed germination, root development, flower maturation and senescence, floral organ abscission (Scariot et al., 2014), fruit ripening, and response to biotic and abiotic stresses. Floral longevity, which is significantly influenced by petal wilting, withering and abscission, is controlled by ethylene through changes in endogenous levels (van Doorn & Woltering, 2008).

Ethylene has a significant impact in senescence regulation in ethylene sensitive flowers, but has little or no impact in ethylene insensitive flowers (Dar et al., 2021). For ethylene sensitive flowers, the senescence of the petal is accompanied by rapid ethylene production increase. The signaling pathway and mechanism of ethylene perception are based on the presence of its receptors, which are essential in carrying out the aging process (Ju et al., 2015). The postharvest life of cut flowers can be prolonged by substances that reduce the hormone production and that can block the ethylene action. Ethylene is synthesized as follows: L-methionine → S-adenosyl methionine (SAM) →ACC → ethylene.

Carbon dioxide and oxygen

Reducing respiration rate and effects of ethylene on sprouting, ripening, flower abscission and senescence can be attained by increasing carbon dioxide and decreasing oxygen concentrations. A study by Poonsri (2020) on *Dendrobium* orchid flowers revealed that low O_2 and high CO_2 significantly improved the storage life, helped retain the total content of anthocyanin, lowered fresh weight loss, respiration rate, ethylene production, protein degradation and protease activity. Low O_2 and high CO_2 restrict the exchange of gases; hence, slowing down the deterioration of produce (Singh & Kumar, 2008). CO_2 is known to have anti-microbial properties that are used in postharvest management. Higher concentrations of CO_2 make the surrounding environment not favorable for the majority of the pests, and can control the growth of microbes by limiting deterioration of tissues. Cut flower wilting can be delayed through high CO_2 and low O_2 packaging; while ethylene production can be reduced by altering internal atmosphere of low O_2 and high CO_2.

CONCLUSION

This study validated the potential of citric acid from calamondin fruit extract wherein the treated rose cut flowers had a vase life that was three days longer than the untreated cut roses. The slowing down of the flower deterioration with citric acid-treated cuttings was mainly due to lower ethylene and higher carbon dioxide concentrations which were noted in day 7 during the peak of ethylene production. The application of calamondin fruit extract is very feasible and economically viable due to the abundant supply of these calamondin/calamansi fruits in the local markets which are being sold at lower cost.

In addition, recutting the stems of cut flowers every two days revealed no significant advantage compared to the uncut roses stems. Therefore, with the application of calamondin extract in the vase hydrating solution, it is no longer recommended to do re-cutting because it will just be an additional work to do.

With these findings of the study, it is recommended that ornamental growers, flower vendors and buyers that for postharvest treatments of cut flowers, 20 ml/L calamondin extract may be used for pulsing. For future research trials on cut flowers, optimization of calamondin extract concentration could be explored, shelf life and stability could be assessed, and the addition of sucrose and other substances to calamondin could be evaluated for possible enhancement of postharvest attributes.

Conflict of interest

The author declares that there is no conflict of interest or competing interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

Cavalcante, L., de Araujo, F. F., Ribeiro, W. S., Santos, M. N. S., & Finger, F. (2021). Postharvest physiology of cut flowers. *Ornamental Horticulture, 27*(4), 374-387. https://doi.org/0.1590/2447-536x.v27i3.2372

Chen, Y. H., Miller, W. B., & Hay, A. (2023). Postharvest bacterial succession on cut flowers and vase water. *PLoS ONE, 18*(10), e0292537. https://doi.org/10.1371/journal.pone.0292537

Dar, R. A., Nisar, S., & Tahir, I. (2021). Ethylene: A key player in ethylene sensitive flower senescence: A review. *Scientia Horticulturae, 290*(7), *110491*. https://doi.org/10.1016/j.scienta.2021.110491

Darandeh, N., & Hadavi, E. (2012). Effect of pre-harvest foliar application of citric acid and malic acid on chlorophyll content and post-harvest vase life of Lilium cv. Brunello. *Frontiers in Plant Science, 2*, 1-6. https://doi.org/10.3389/fpls.2011.00106

De, L. C., De, P., & Chhetri, G. (2015). Pre- and postharvest physiology of *Cymbidium* orchids. *International Journal of Horticulture, 5*(6), 1-5. https://doi.org/10.5376/ijh.2015.05.0006 https://doi.org/10.1590/2447-536X.v27i4.2372

Frew, A., Weston, L. A., Reynolds, O. L., & Gurr, G. M. (2018). The role of silicon in plant biology: A paradigm shift in research approach. *Annals of Botany 121*(7), 1265-1273. https://doi.org/10.1093/aob/mcy009

Hussen, S., & Yassin, H. (2013). Review on the impact of different vase solutions on the postharvest life of rose flower. *International Journal of Agricultural Research and Review, 1*(2),13-17. https://doi.org/10.13140/RG.2.1.2133.3528

Jadhav, P. B., & Gurav, N. P. (2018). Extending the storage-life and shelf-life of a rose flowers cv. Dutch postharvest using chemical compounds and cold storage (Ecofrost). *International Journal of Research and Review, 5*(6),151-156.

Ju, C., Van de Poel, B., Cooper, E. D., Thierer, J. H., Gibbons, T. R., & Delwiche, C. F. (2015). Conservation of ethylene as a plant hormone over 450 million years of evolution. *Nature Plants, 1*(1), 14004. https://doi.org/10.1038/nplants.2014.4

Kandel, D., Atreya, P. N., & Poudel, S. (2021). Postharvest vase life of gerbera under different chemical treatments. *Nepalese Journal of Agricultural Sciences, 21*(1), 144-151. https://www.researchgate.net/publication/354679742

Ma, R. H., Ni, Z. J., Zhu, Y. Y., Thakur, K., Zhang, F., Zhang, Y. Y., Hu, F., Zhang, J. G., & Wei, Z. G., & Wei, Z. J. (2021). A recent update on the multifaceted health benefits associated with ginger and its bioactive components. *Food & Function, 12*(2), 519-542. https://doi.org/10.1039/d0fo02834g

Mehdikhha, M., Onsinejad, R., Ilkaee, M., & Kaviani, B. (2016). Effect of salicylic acid, citric acid and ascorbic acid on postharvest quality and vase life of Gerbera (*Gerbera jamesonii)* cut flowers. *Journal of Ornamental Plants, 6*(3), 181-191.

Meperanum, R. D., Muttulani, M. A. J. J., & Turnos-Milagrosa, L. J. N. (2025). Plant-based hydrating solutions for extending vase life and postharvest quality of chrysanthemum (*Dendrathema x grandiflorum*). *Current Agricultural Research Journal, 13*(1), 263-270. https://doi.org/10.12944/CARJ.13.1.20

Mortazavi, N., Naderi, R., Khalighi, A., Babalar, M., & Allizadeh, H. (2007). The effect of cytokinin and calcium on cut flowers quality in rose *(Rosa hyrida* L.) cv. Illona. *Journal of Food Agriculture and Environment, 5*(3&4), 311-313. https://agris.fao.org/search/fr/records/647760bb5eb437ddff77b0d8

Mwelase, S., Adeyemi, J. O., & Fawole, O. A. (2024). Recent advances in postharvest application of exogenous phytohormones for quality preservation of fruits and vegetables. *Plants, 13*(22), 1-22. https://doi.org/10.3390/plants13223255

Nguyen, T. K., Jung, Y. O., & Lim, J. H. (2020). Tools for cut flower for export: Is it a genuine challenge from growers to customers? *Flower Research Journal, 28*(4), 241–249. https://doi.org/10.11623/frj.2020.28.4.02

Poonsri W. (2020). Effect of modified and controlled atmosphere storage on enzyme activity and senescence of *Dendrobium* orchids. *Heliyon, 6*(9), 1-6. https://doi.org/ 10.1016/j.heliyon.2020.e05070

Rihn, A. L., Yue, C., Behe, B. K., & Hall, C. (2015). Consumer preferences for cut flower guarantees. *Acta Horticulturae,* 1090(1090), 45-54. https://doi.org/10.17660/ActaHortic.2015.1090.8

Scariot, V., Paradiso, R., Rogers, H., & de Pascale, S. (2014). Ethylene control in cut flowers: Classical and innovative approaches. *Postharvest Biology and Technology, 97*, 83–92. https://doi.org/10.1016/j.postharvbio.2014.06.010

Shu, C., Ge, L., Li, Z., Chen, B., Liao, S., Lu, L., Wu, Q., Jiang, X., An, Y., Wang, Z., & Qu, M. (2024). Antibacterial activity of cinnamon essential oil and its main component of cinnamaldehyde and the underlying mechanism. *Frontiers in Pharmacology, 15*, 1-14. https://doi.org/10.3389/fphar.2024.1378434

Singh, A., & Kumar, P. (2008). Influence of post-harvest treatments on modified atmosphere low temperature stored Gladiolus cut spikes. *International Journal of Postharvest Technology and Innovation, 1*(3), 267-277. https://doi.org/10.1504/IJPTI.2008.021461

Sudaria, M. A. M., Lungsod, E. M., Robles, A. C, Gepte, C. L., & Yabao, R. M. (2016). Post-harvest treatment of pyroligneous acid on solanaceous crops at different storage condition. *Journal of Agriculture and Ecology*, *2*(1), 44-56. https://doi.org/10.53911/JAE.2021.12203

Tinebra, I., Sortino, G., Inglese, P., Fretto, S., & Farina, V. (2021). Effect of different modified atmosphere packaging on the quality of mulberry fruit *(Morus alba)*. *International Journal of Food Science*, 1-10. https://doi.org/10.1155/2021/8844502

van Doorn, W. G., & Woltering, E. J. (2008). Physiology and molecular biology of petal senescence. *Journal of Experimental Botany*, *59*(3), 453-480. https://doi.org/10.1093/jxb/erm356

Vehniwal, S. S., & Abbey, L. (2019). Cut flower vase life – influential factors, metabolism and organic formulation. *Horticulture International Journal*, *3*(6), 275–281. https://doi.org/10.15406/hij.2019.03.00142