JHPR

www.jhpr.birjand.ac.ir

Volume 7, Issue 1, March 2024, E-ISSN: 2588-6169

Journal of Horticulture and Postharvest Research

An International Journal

JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH
2024, VOL. 7(1), 1-14

Journal of Horticulture and Postharvest Research

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

University of Birjand

Evaluation of physico-chemical, microbial and sensory attributes of minimally processed litchi (*Litchi chinensis* Sonn.) under low temperature storage

Pushpendra Kumar[1,*], Shruti Sethi[2], Swarajya Laxmi Nayak[3], Eldho Varghese[4], Gireesh Chand[5]

[1]*Department of Post Harvest Management, College of Horticulture and Forestry, Central Agricultural University, Pasighat-791102 (Arunachal Pradesh), India*
[2]*Division of Food Science and Postharvest Technology, ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India*
[3]*ICAR-Directorate of Medicinal Aromatic Plants Research (ICAR-DMAPR), Boriavi, Gujarat, India*
[4]*Fishery Resources Assessment Division (FRAD), ICAR-Central Marine Fisheries Research Institute, Kochi-682 018, India*
[5]*Department of Plant Pathology, College of Agriculture, Central Agricultural University, Pasighat-791102 (Arunachal Pradesh), India*

ARTICLE INFO

Original Article

Article history:
Received 3 October 2023
Revised 5 December 2023
Accepted 8 December 2023
Available online 5 January 2024

Keywords:
Microbial changes
Organic acids
Peeled litchis
Peroxidase
Sensory attributes

DOI: 10.22077/jhpr.2023.6833.1336
P-ISSN: 2588-4883
E-ISSN: 2588-6169

***Corresponding author:**
Department of Post Harvest Management, College of Horticulture and Forestry, Central Agricultural University, Pasighat-791102 (Arunachal Pradesh), India.

Email: pushpendra.iari@yahoo.in

ABSTRACT

Purpose: The main aim of this study was to evaluate the changes in quality of minimally processed litchi fruit during storage at low temperatures (5-7°C). **Research method:** The study attempts to report the efficacies of seven different anti-browning compounds at various concentrations namely methionine (0.1%), cysteine (0.1%), EDTA (0.1%), oxaloacetic acid (1%), ascorbic acid (1%), citric acid (1%), and potassium metabisulfite (0.5%) on quality and shelf-life extension of minimally processed litchi aril. Treated litchi aril kept in trays wrapped with plastic film and stored under refrigerated conditions. Changes in total soluble solids, titratable acidity, ascorbic acid, total phenolics, sensory attributes, colour, weight loss, microbial and activities of polyphenol oxidase and peroxidase enzymes were evaluated. **Findings:** Treatments reduce the weight loss and sensory attributes with higher contents of TSS, titratable acidity, ascorbic acid, total phenolics and suppressed the increase in activities of polyphenol oxidase and peroxidase. Therefore, a postharvest dip of peeled litchi into solutions of chemical preservatives could be suggested to maintain the postharvest quality under refrigeration storage. Treated litchi arils were acceptable (sensory score >7) up to 8 days as compared to 3 days for the control samples during refrigerated storage. **Research limitations:** No limitations were found. **Originality/Value:** Further, 1% ascorbic acid and 1% citric acid were recorded to be the best to preserve the quality of minimally processed 'Mujaffarpur' litchi during refrigeration storage.

INTRODUCTION

Litchi (*Litchi chinensis* Sonn.) is a fruit of high commerce often preferred by the consumers for its sweet acidic taste, excellent aroma, attractive bright red peel colour and high nutritive value (Mphahlele et al., 2020; Phanumong et al., 2016). Though litchi fruit is relished for its nutritive value and claims high price when fresh, it is highly prone to pericarp browning and turns brown within 2–3 days after harvest (Jiang et al., 2012). This results in a short shelf life that narrows down the marketability of the fruit causing huge economic loss to the farmers. Enzymatic oxidation of ascorbic acid and action of polyphenol oxidase and peroxidase enzymes are the major culprits of litchi peel browning (Yun et al., 2020). However, the internal arils remain in good condition, in contrast to the dark brown colour pericarp. Thus, the deteriorated external appearance of the fruit masks the good internal quality of the pulp. This underpins the need for techniques to improve the acceptability of the fruit and prevent loss to the farmers.

In recent years, the demand for minimally processed litchi fruits by the consumers and restaurants has augmented because of the convenience and nutritive quality thus, creating new marketing opportunities (Sarkar & Sumi, 2023). Edible arils of unmarketable brown fruits can be stored for long time by merely peeling and stabilizing the fruit to retain fresh-like characteristics and good postharvest quality (Mphahlele et al., 2020). However, the absence of protective pericarp limits the shelf life of the minimally processed litchi fruit and requires additional treatment to preserve the quality of edible aril to allow its marketing for a sufficient period of time (Phanumong et al., 2015). Moreover, litchi aril is also very prone to dehydration during storage (Jiang & Fu, 2000). Therefore, postharvest technique for preservation of peeled litchi fruits is the need of the hour to maintain good quality of fruit during the entire supply chain till it reaches the consumer.

Hence, the use of GRAS and eco-friendly chemicals with practical applicability is required to control the problem. Organic acids are such GRAS compounds reported to maintain quality and enhance the shelf life of fresh-cut produce (Kumar et al., 2018b; Oms-Oliu et al., 2010; Gorny et al., 2002). Organic acids are also reported as antimicrobial and antioxidant agents that control the enzymatic activity and thereby inhibit the browning reactions (Ventura-Aguilar et al., 2017; Azevedo et al., 2018).

Previously some researchers have worked on minimal processing of litchi fruit to improve the shelf life and maintaining the postharvest quality of arils (Mphahlele et al., 2020; Dong et al., 2004; Bolanos et al., 2010; Kaushik et al., 2014; Phanumong et al., 2015, 2017; Phanumong et al., 2016). However, a comparative analysis of aforesaid chemicals with different concentrations on peeled litchi cv. ‘Mujaffarpur’ during refrigerated storage is reported for the very first time. Therefore, the objective of this study was to evaluate the effects of chemical preservatives on postharvest quality maintenance and shelf life extension of peeled litchi cv. ‘Mujaffarpur’.

MATERIALS AND METHODS

Plant material and sample processing

Litchi (*Litchi chinensis* Sonn.) cv. 'Muzaffarpur' was harvested when fully red, with TSS ≥ 16 from College of Horticulture and Forestry (CHF) farm. Later, the fruits were carefully transported in containers without any damage to the Department of Post Harvest Management laboratory located at CHF, CAU (I), Pasighat and Arunachal Pradesh, India for subsequent processing. Split and cracked fruits were manually separated and uniform sized fruit free from disease were selected.

Selected whole litchi fruits were sanitized in 200 ppm sodium hypochlorite solution for 2 min. After draining, the litchi fruits were manually peeled carefully with a sharp stainless steel knife. Arils obtained were then dipped into distilled water (control), methionine (0.1%), cysteine (0.1%), EDTA (0.1%), oxalic acid (OA) (1%), ascorbic acid (AA) (1%), citric acid (CA) (1%) and potassium metabisulfite (KMS) (0.5%) for 5 min. On the basis of preliminary trials, the concentrations of these individual chemicals were selected for final study. Approximately 12 pieces of arils (~ 150 g) were packed in a tray, shrink wrapped and immediately stored under refrigeration (5 °C) condition for 8 days. After processing of litchi fruits the baseline measurements (0 day) were conducted prior to packaging and storage. During storage period, different quality attributes were analysed including browning metabolism (phenolic content, polyphenol oxidase, peroxidase activity) and other quality factors namely mass loss, colour index, soluble solids content, titratable acidity, ascorbic acid at two days' interval.

Analysis of quality attributes

Weight loss

Weight loss of each package was taken before storage and at each sampling day using an electronic balance (Wensar Weighing Scales Limited, Chennai, India). The loss in weight was expressed in percentage (%) of the initial weight and calculated as follows (1) (Kumar et al., 2017):

$$WL = \frac{Wi - Wf}{Wi} \times 100 \qquad (1)$$

Where, WL is the weight loss (%), Wi is the initial weight (g) and Wf is the final weight (g) at the time of sampling during storage.

Colour attributes

Colour of the arils was measured by using a colourmeter (CS 3260, Analytical Technologies Ltd., Gujarat, India) in CIELAB (L*, a*, b*) coordinates where L* denotes the lightness, a*: red/green and b*: yellow/blue. The whiteness index (WI), as determined by the following equation (2), strongly correlates with consumer preferences, as a higher whiteness index indicates a fresher appearance of the product.

$$WI = 100 - [(100 - L*)^2 + (a*^2 + b*^2)]½ \qquad (2)$$

Browning index (BI), an important attribute in processed foods indicating extent of browning was calculated using the following equation (3).

$$BI = [100(x - 0.31)]/0.172 \qquad (3)$$

Where: $x = (a* + 1.75\, L*) / (5.646\, L* + a* - 3.012\, b*)$

From the L*, a* and b* values, total colour change (ΔE*) was calculated. ΔE* is an attribute that quantifies the overall colour difference of samples. It was calculated using the following equation (4):

$$\Delta E = 100 - [(100 - L*)^2 + (a*^2 + b*^2)]½ \qquad (4)$$

Soluble solids content, titratable acidity and pH

Soluble solids content (ºBrix) of arils was determined using digital refractometer (Milwaukee Digital Refractometer (MA 871), Milwaukee Instruments, Inc., United States). Titratable acidity (%) was measured by titration with 0.1 N NaOH (Nayak et al., 2019). pH value was estimated by extracting the juice from the litchi using a pH meter (EUTECH INSTRUMENTS).

Determination of ascorbic acid and phenolics content

Ascorbic acid was determined by titrating sample aliquot with 2,6-dichlorophenol indophenol dye and expressed in mg of ascorbic acid per 100 g (Ranganna, 2007). The total phenolic content was determined according to Folin-Ciocalteu assay (Kumar et al., 2017). Wherein methanolic extract of the pulp was mixed with double distilled water, Folin–Ciocalteu reagent and sodium carbonate and the absorbance of colour developed was recorded at 760 nm (Double Beam Spectrophotometer, Systronics 2206). The values obtained were expressed as mg GAE per 100 g of fresh weight.

Extraction and assay of polyphenol oxidase and peroxidase activity

The polyphenol oxidase (PPO) activity was measured as previously described by Kumar et al. (2018a). To determine the polyphenol oxidase activity, 2 g of sample was homogenized in 10 mL of 0.2 M sodium phosphate buffer (pH 6.8) followed by centrifugation at 10,000 rpm for 10 min. at 4 ºC. Aliquots were recovered as enzyme extracts for estimation of polyphenol oxidase activity. The increase in absorbance at 410 nm was recorded for 10 min. with the help of a spectrophotometer (Double Beam Spectrophotometer, Systronics 2206). Enzyme activity was explained as the change of 0.001 in absorbance at 410 nm and represented as min^{-1} g^{-1}.

Peroxidase (POD) activity was determined as per Kumar et al. (2018a). Two grams of sample was ground in 10 mL sodium phosphate buffer (0.1 M), containing 1% polyvinylpyrrolidone (PVP). It was then centrifuged at 10,000 rpm for 10 min. at 4 ºC. The assay mixture consisted of 0.1 M sodium phosphate buffer (pH 7.0), 0.042 % hydrogen peroxide (H_2O_2), 0.07 M guaiacol and 100 µl of enzyme extract in a final volume of 3 mL. The increase in absorbance at 436 nm was noted for 10 min. Enzyme (POD) activity was described as a change of 0.001 in absorbance at 436 nm (Double Beam Spectrophotometer, Systronics 2206) and expressed as min^{-1} g^{-1}. One unit of enzyme activity was defined as the amount of enzyme that caused a change of 0.001 in absorbance per minute.

Subjective Evaluation

The subjective assessment of peeled litchi arils after 8 days of storage was conducted using a 9-point hedonic scale, where a score of 1 represented poor quality, and a score of 9 denoted excellent qualities. A group of 30 panelists, comprising 15 males and 15 females including staff and students participated in the sensory analysis. An orientation program was organized to familiarize the panelists with the testing procedure, the attributes under examination, and the completion of the evaluation form. The samples were assigned three-digit codes and presented to the panelists in a randomized order at around 25°C to minimize any potential positional bias. Additionally, water was provided to the panelists for rinsing their mouths between sample tastings. Panelists were asked to rate their degree of liking for appearance, color, firmness, flavor, and overall acceptability using the 9-point hedonic scale, where a rating of 1 indicated strong dislike, and a rating of 9 indicated strong liking. The mean scores for each parameter were calculated.

Microbial analyses

The evolution of the microbial changes of fresh-cut litchi throughout storage was evaluated by the total aerobic plate counts. 10 g of litchi aril were removed aseptically from each tray and transferred into sterile plastic bags. Litchi samples were diluted with 90 ml of sterile normal saline and homogenized for 1 min. Dilutions were made and then spread plated onto plate count agar medium. Plates were incubated at 30±2 ºC for 48 h. Total aerobic plate counts were counted and the results expressed as log CFU/g (Cappuccino & Sherman 2008).

Statistical analysis

The data collected during the 8-day storage of litchi arils underwent statistical analysis using SAS 9.3 data analysis software. Analysis of Variance (ANOVA) was conducted employing a Completely Randomized Design (CRD) with three replications. The significance level was defined by Tukey's HSD for all tests. Statistical significance level was indicated at $P < 0.05$.

RESULTS AND DISCUSSION

Litchi fruits cv. 'Mujaffarpur' used for the study were found to have an average weight of 22 g and the edible portion accounted for approximately 55% of the total fruit weight. The soluble solids content (°B), pH, titratable acidity (%), ascorbic acid (mg 100 g^{-1}) and total phenols (mg 100 g^{-1}) were 17.4, 5.06, 0.50, 23.89 and 100, respectively. The L* (brightness index) value of aril was 49.55 at the start of the experiment.

Physical changes

Weight loss (WL)

Weight loss (WL) is mainly attributed to moisture loss from a respiring commodity. The weight of treated minimally processed litchi was measured to analyse the efficiency of the browning inhibitors used in the study to act as inhibitors of water loss over the experimental duration. As storage time elapsed, weight of peeled litchi arils decreased gradually (Fig. 1A). All the treatments demonstrated a good ability for minimizing water loss from the fruits when compared with the control litchi fruits. Further, ascorbic acid (1%), citric acid (1%) and methionine (0.1%) demonstrated a better performance for moisture loss inhibition. The findings were in agreement with Phanumong et al. (2015) and Shah and Nath (2008) who subjected the minimally processed litchi fruits with calcium salts and cysteine, ascorbic acid and 4-hexyl resorcinol and stored under low temperature.

Browning index (BI), whiteness index (WI) and total colour (ΔE) changes

Influence of browning inhibitors used in this study on colour attributes (BI, WI and ΔE*) are presented in Figure 1B, and Figure 2A & B. As evident, treatments had a significant effect on the colour changes of peeled litchi arils during storage. Browning of aril is the main issue in peeled litchi fruits. Browning is visible in minimally processed fruits due to tissue damage by cutting operations or exposure to air which results in a loss of natural colour (Chiumarelli & Hubinger, 2012; Oms-Oliu et al., 2010). The browning index (BI) which combines the colour parameters of CIE Lab is an indicator of the degree of brown colour (Olivas et al., 2007). Changes in BI of peeled litchi fruits during refrigerated storage are portrayed in Figure 1B. As shown, control fruits exhibited higher browning index. Treatment of peeled fruits with ascorbic acid (1%), cysteine (0.1%) and methionine (0.1%) curbed the browning till the last day of storage. The effectiveness of these chemicals in retarding the browning process are consistent with the results of Kumar et al. (2018a, b) in minimally processed apple and

Phanumong et al. (2015) in litchi stored under low temperature. A progressive increase in the browning with storage, as observed in our study has also been reported by these authors.

Whiteness index is closely associated with customer choices (Pathare et al., 2013) with a white appearance depicting better freshness of the commodity. WI of the peeled litchi fruit was significantly affected by treatments during storage (Fig. 2A). During 8 days of storage, whiteness index of the minimally processed fruit was higher in arils treated with ascorbic acid (1%) and cysteine (0.1%) and methionine (0.1%) compared to other samples. There was a significant decline (from 48.58 to 36.30) in WI for control fruits. The potential mechanism behind this effect lies in the ability of methionine, cysteine, and ascorbic acid to act as reducing agents, interacting with the active sites of PPO enzymes and consequently inhibiting their activity. This impediment of the enzymatic browning process helps in preserving the whiteness index of the minimally processed litchi (Silveira Alexandre et al., 2022).

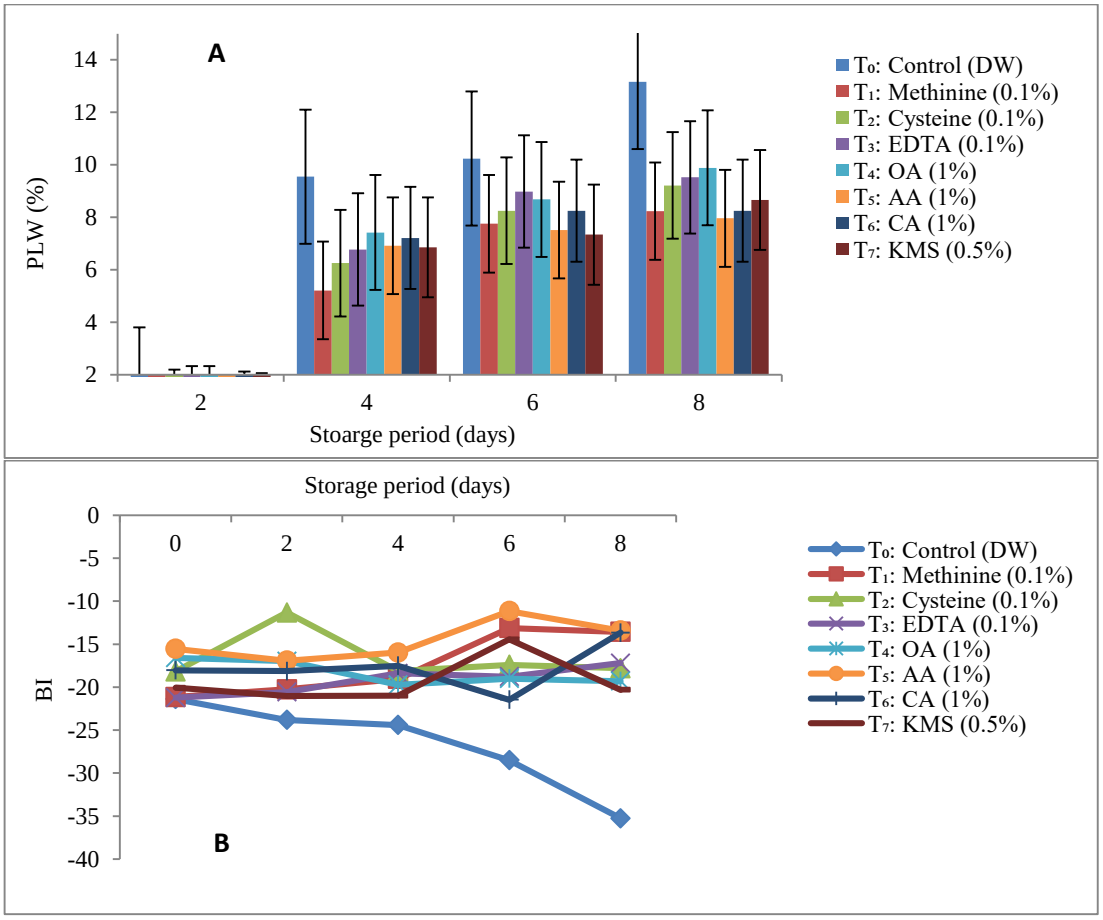

Fig. 1. Changes in physiological loss in weight (PLW) (A) and browning index (BI) (B) of peeled litchi arils given various dipping treatments. Values represent means ± S.E. of 3 replicates.

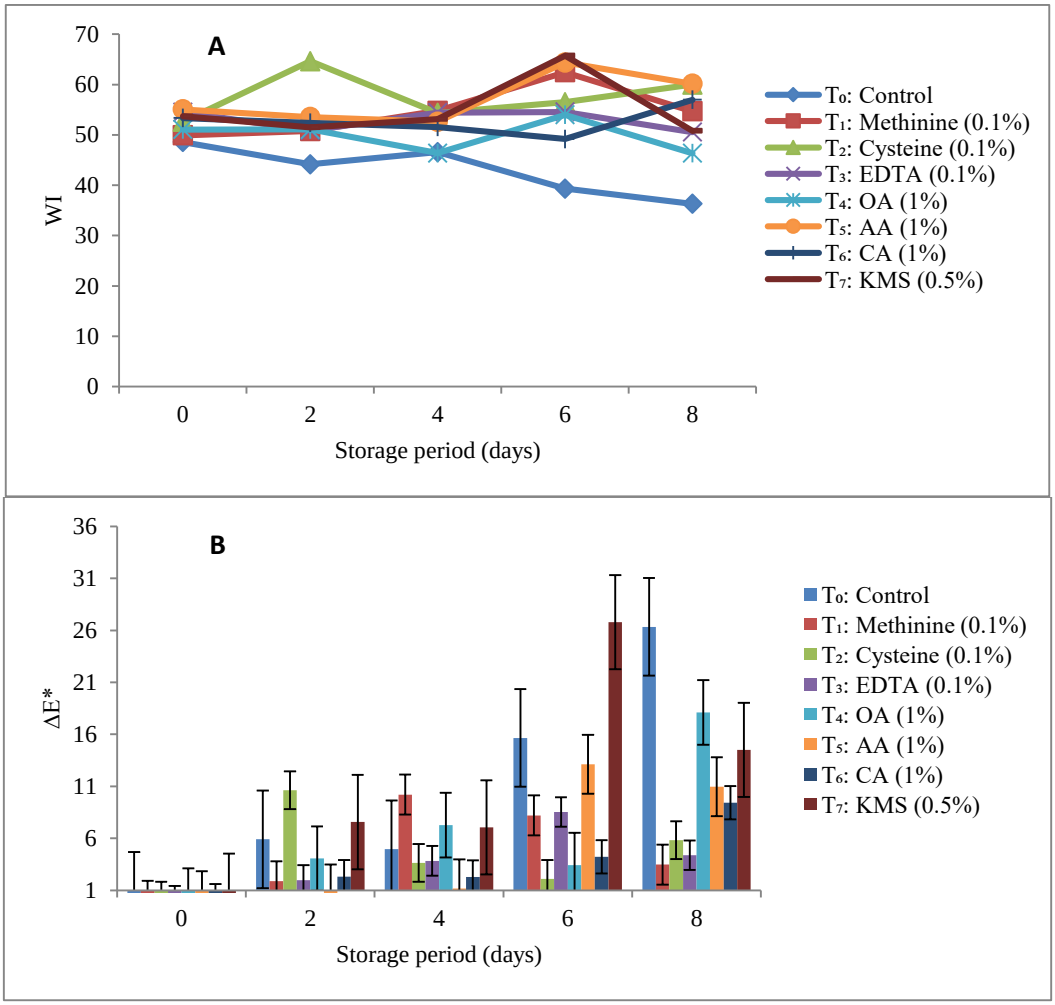

Fig. 2. Changes in whiteness index (WI) (A) and total colour (ΔE*) (B) of peeled litchi arils given various dipping treatments. Values represent means ± S.E. of 3 replicates.

Total colour change (ΔE) of minimally processed litchi fruit was significantly affected by treatments (Fig. 2B). However, samples from untreated lot showed higher colour change (26.34) after 8 days compared to the treated ones. Citric acid treatment of peeled arils resulted in the least ΔE* values (~3.65) in comparison to other browning inhibitors on day 8. For the peeled arils treated with methionine, the increase in ΔE* values was significant till 4th day as shown in Figure 2B, after which rate of increase of ΔE* reduced. Increase in ΔE* is a result of physicochemical and enzymatic changes in fruit (Lamikanra & Watson, 2001) though at a slower rate due to chilled temperature storage. The increase in browning of arils can be attributed to the activity of enzymes such as polyphenol oxidase and peroxidise as observed in our experiments. Samples treated with ascorbic acid, citric acid and cysteine also showed comparatively lesser change in ΔE* over the storage period and thus good colour stability.

Chemical changes

Soluble solids content and titratable acidity

Soluble solids content of litchi arils decreased significantly during storage from an initial value of 17.4-17.8 to 9.1-13.1 ºBrix (Table 1). Arils treated with ascorbic acid (1%), citric acid (1%), methionine (0.1%) and cysteine (0.1%) maintained higher total soluble solids content throughout the storage period. The decrease in the total soluble solids and titratable acidity during storage occurs as a result of utilization of sugars and organic acids in various

metabolic activities (Tassou & Boziaris, 2002). These results are in accordance with Shah and Nath (2008), who reported that the total soluble solids significantly reduced during storage of 20 days at 4 °C in minimally processed litchi fruits subjected to osmo-vacuum, moderate vacuum packing and browning inhibitors. Similar observations were also made by Dong et al. (2004), Kumar et al. (2018a) and Soliva-Fortuny et al. (2004) yielding a significant reduction in soluble solids content in treated minimally processed fruit under low temperature storage.

Titratable acidity (TA) was significantly influenced by the different chemicals applied on the peeled litchi fruits (Table 1). At day 0, the titratable acidity level was 0.52% across all treatments and declined gradually with storage period irrespective of treatment. A significant decline in titratable acidity (~57 %) was observed in control samples after 8 days of storage. Treated litchi fruits exhibited a non-significant change in titratable acidity during storage. Fruit treated with ascorbic acid (1%) and citric acid (1%) had slightly higher titratable acidity than other treatments after 8 days of storage. At the termination of experiment, the titratable acidity was 0.38, 0.37 and 0.35 % for arils subjected to ascorbic acid (1%), citric acid (1%) and KMS (0.5%), respectively. However, there were no significant differences between treatments. Similarly, Dong et al. (2004) observed a significant reduction in titratable acidity in peeled litchi treated with chitosan coating.

Ascorbic acid (AA) content

Ascorbic acid is one of the important nutrients in horticultural produce with biological significance (Paciolla et al., 2019). In all the treated arils, AA content was higher than that of control during the 8 days of storage (Table 2). This suggests that all the treatments possessed the ability to maintain ascorbic acid content in litchi arils. Ascorbic acid (1%), methionine (0.1%) and cysteine (0.1%) treatments performed best among all. This may be attributed to prevention of the enzymatic oxidation of AA. Ascorbic acid content initially was 23.86–24.35 mg 100^{-1} g (Table 2). Higher initial AA content in litchi fruits treated with ascorbic acid (1%) was due to the inhibitions of AA into the fruits. However, in general, ascorbic acid content reduced significantly across all the treatments at end of 8 days of cold storage. Similar decline in AA content during low temperature storage has been reported by Kaushik et al. (2014) in minimally processed litchi. The loss of AA has been attributed to its high reactivity to oxygen and its degradation has been associated with browning process (Gimnez et al., 2003).

Table 1. Changes in total soluble solids and titratable acidity of peeled litchi arils given various dipping treatments and stored under refrigeration condition.

Treatment	TSS (°B)					Titratable acidity (%)				
	Storage period (days)					Storage period (days)				
	0	2	4	6	8	0	2	4	6	8
Control (DW)	17.4^{a}	15.3^{ab}	13.3^{ab}	11.2^{ab}	9.1^{b}	0.501^{ab}	0.389^{ab}	0.326^{ab}	0.289^{ab}	0.211^{b}
Methinine (0.1%)	17.8^{a}	16.3^{a}	14.1^{ab}	12.8^{ab}	11.4^{ab}	0.523^{ab}	0.456^{a}	0.412^{a}	0.385^{ab}	0.336^{ab}
Cysteine (0.1%)	17.7^{a}	16.1^{ab}	13.9^{ab}	12.5^{ab}	11.3^{ab}	0.523^{a}	0.451^{a}	0.405^{ab}	0.336^{ab}	0.312^{ab}
EDTA (0.1%)	17.6^{a}	16.2^{ab}	14.0^{ab}	13.2^{ab}	12.2^{ab}	0.512^{a}	0.506^{a}	0.412^{ab}	0.342^{ab}	0.321^{ab}
OA (1%)	17.4^{a}	16.3^{a}	14.4^{ab}	12.7^{ab}	12.2^{ab}	0.532^{a}	0.500^{a}	0.514^{a}	0.386^{a}	0.337^{ab}
AA (1%)	17.7^{a}	16.5^{a}	14.4^{ab}	13.6^{ab}	13.1^{ab}	0.534^{a}	0.502^{a}	0.465^{a}	0.398^{a}	0.379^{ab}
CA (1%)	17.5^{a}	16.4^{a}	14.1^{ab}	13.2^{ab}	13.0^{ab}	0.524^{a}	0.489^{a}	0.456^{a}	0.375^{a}	0.366^{ab}
KMS (0.5%)	17.4^{ab}	16.1^{a}	13.8^{ab}	13.1^{ab}	12.9^{ab}	0.514^{a}	0.478^{a}	0.435^{a}	0.375^{ab}	0.354^{ab}

*Means with same superscript are not significantly different.

Table 2. Change in ascorbic acid content of peeled litchi arils given various dipping treatments and stored under refrigeration condition.

Treatment	Ascorbic acid (mg/100 g)				
	Storage period (days)				
	0	2	4	6	8
Control (DW)	23.89^{a}	21.56^{a}	18.65^{a}	15.32^{ab}	12.68^{b}
Methinine (0.1%)	24.15^{a}	23.63^{a}	22.14^{a}	20.24^{a}	19.21^{a}
Cysteine (0.1%)	24.20^{a}	23.55^{a}	21.99^{a}	19.85^{a}	18.98^{ab}
EDTA (0.1%)	23.87^{a}	22.65^{a}	21.56^{a}	19.21^{a}	18.23^{ab}
OA (1%)	23.86^{a}	22.12^{a}	21.12^{a}	19.86^{a}	17.82^{ab}
AA (1%)	24.32^{a}	23.75^{a}	22.56^{a}	20.28^{a}	20.12^{a}
CA (1%)	24.25^{a}	23.14^{a}	21.85^{a}	20.21^{a}	19.78^{a}
KMS (0.5%)	24.35^{a}	22.62^{a}	21.45^{a}	19.58^{a}	17.89^{ab}

*Means with same superscript are not significantly different.

Total phenolic content

Mechanical damage and oxidative damage due to the minimal processing triggers physical stresses owing to alteration in phenolic metabolism in minimally processed tissues. Figure 3A represents the variations in total phenolic content of control and treated peeled litchi stored under refrigerated conditions. The initial phenolic content in litchi arils was 100-100.91 mg GAE 100 g^{-1} which reduced as storage progressed. However, the declining trend was not similar in all the litchi samples. At the termination of experiment, total phenolic content corresponding to control, methionine treated and ascorbic acid treated samples were 61.36, 93.18 and 90.91 mg GAE 100^{-1} g FW, respectively. It is evident that methionine and ascorbic acid treatments were more effective in retaining phenolic compounds. The minimal changes in total phenolics recorded in treated samples may have been associated with the low polyphenol oxidase activity. Aquino-Bolanos and Mercado-Silva (2004) and Bolanos et al. (2010) also reported decline in phenolics with the progress of time in minimally processed litchi and cut jicama, respectively. Higher phenolic content in ascorbic acid treated samples may be because of the ability of ascorbic acid to reduce quinones to phenolic compounds that led to inhibition of browning in tissue. Nonetheless, the extent of potential for browning inhibition could be affected by the amount and nature of phenolic compounds (Altisent et al., 2014).

Polyphenol oxidase (PPO) and peroxidase (POD) activity

Minimally processed products deteriorate faster than intact produce because of browning of the cut surface. Enzymatic browning degrades the slices visual appeal, marketability, and nutritional and sensory value (Farooq et al., 2023; Kumar et al., 2018a). As soon as the fruits are subjected to minimal processing operations, deteriorative changes are initiated and decompartmentalization of the cell constituents is initiated. Oxidative reactions between polyphenolic substrates with enzymes such as polyphenol oxidase (PPO) and peroxidase (POD) take place causing browning. Effect of different treatments on polyphenol oxidase activity of peeled litchi is shown in Figure 3B. Polyphenol oxidase (PPO) activity in untreated samples was 106 U g^{-1} min on day 0, which increased as storage time progressed till day 4 (142 U g^{-1} min) then sharply showing a decline. The maximum increase in PPO activity throughout the storage period was observed in control fruit as compared to treated ones. The highest mean value of 119 U g^{-1} min was found in control peeled fruit and lowest 107, 108 and 110 U g^{-1} for ascorbic acid (1%), methionine (0.1%) and CA (1%) and KMS (0.5%) treated samples, respectively during 8 days of storage. The results indicate the reduction of polyphenol oxidase activity by the treatments applied. However, the extent of reduction varied with the chemical used. Similarly, Dong et al. (2004) also recorded an increase in polyphenol oxidase activity in minimally processed litchi fruit stored under low temperature.

Organic acids including ascorbic acid and citric acid have been reported to exhibit an inhibitory effect on polyphenol oxidase and its anti-browning activity in minimally processed produce (Ahvenaien, 1996; Altunkaya et al., 2008).

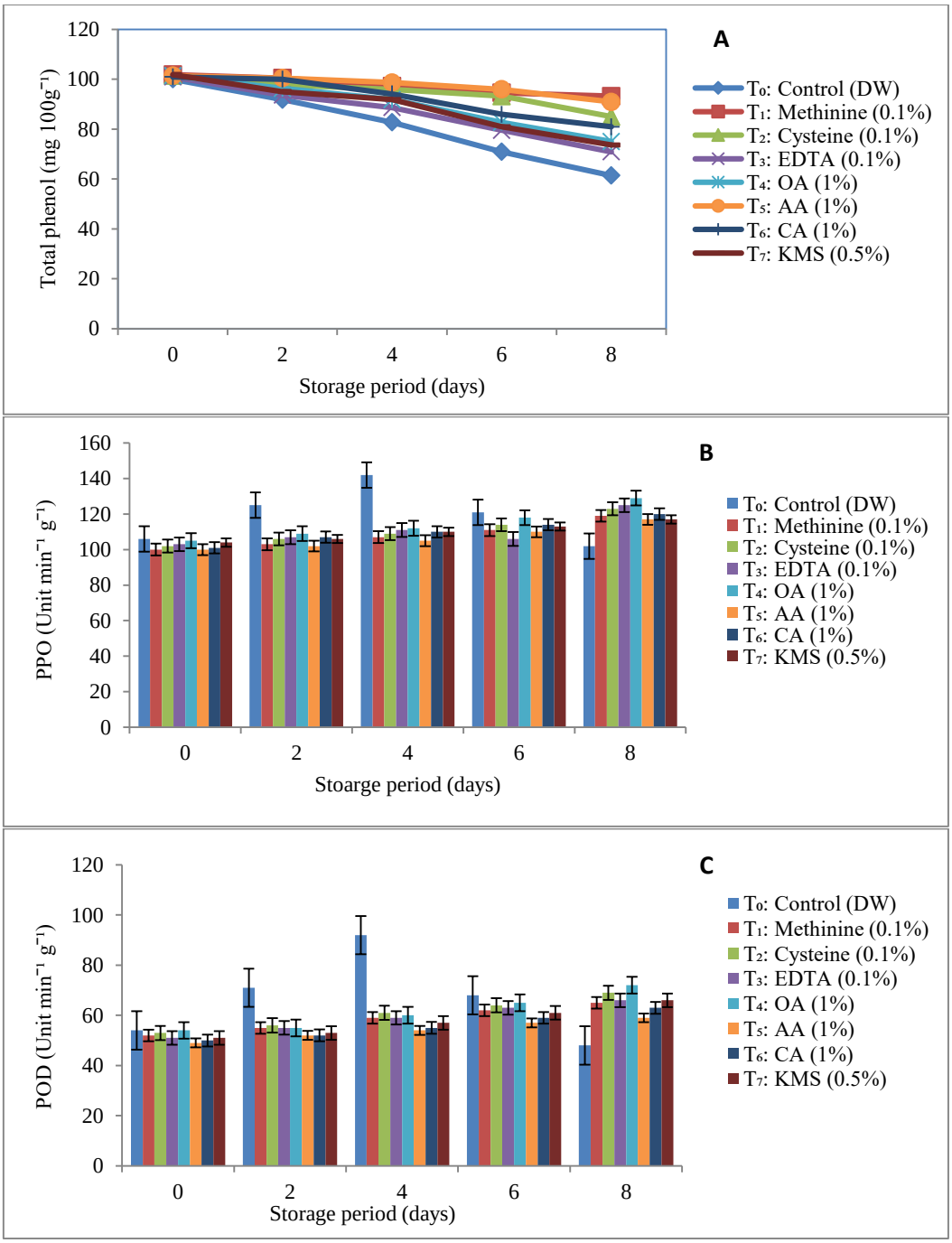

Fig. 3. Changes in total phenolics (A), polyphenol oxidase (PPO) (B) and peroxidase (C) activity of peeled litchi arils subjected to various chemical dips. Vertical bars represent the standard error of the means.

Peroxidase activity is involved in the defence system against oxidative stress and damage repair mechanisms in fresh-cuts leading to colour changes on the exposed surface (Aquino-Bolaños & Mercado-Silva, 2004). Peroxidase activity of control fruit increased rapidly up to day 4 but showed a sudden decline from day 6 of storage. Similar behaviour of elevation in peroxidase activity during the initial storage period and a decline in the latter part of storage of control slices of peach was recorded by Li-Qin et al. (2009). The treated samples showed a gradual increase in peroxidase activity throughout the storage and differed significantly from the control samples (Fig. 3C). The maximum activity of peroxidase enzyme at the end of the storage was observed in control fruit (48 U min^{-1} g^{-1}). The overall analyses of the results suggested that all the chemical treatments were effective in reducing the peroxidase activity. Similar observations of controlling the peroxidase activities by ascorbic acid were noted by Lamikanra and Watson (2001), and Kuwar et al. (2015) in case of minimally processed cantaloupe and papaya, respectively. Both polyphenol oxidase and peroxidase activities are essential factors having influence on the quality of tissue as they affect content of phenolics and the rate and intensity of enzymatic browning.

Sensory quality

Changes in sensory parameters such as appearance, colour, firmness, flavour and overall acceptability of peeled litchi arils as influenced by different treatments were analyzed during storage for 8 days (Fig. 4). Colour scores for arils significantly decreased during the 8-day storage. Litchi fruit treated with ascorbic acid (1%), citric acid (1%), and methionine (0.1%) had significantly higher colour score due to lesser. Firmness scores were also recorded to decline during 8 days of storage. This decline may be attributed to the loss of drip from arils during storage which gave a shrink appearance to litchi fruit and hence loss in turgidity. At termination of the experiment, the flavour scores of arils were found to range from 4.3 to 8.7 which reduced during the storage for all the samples due to loss in sugars. Arils dipped in citric acid (1%), methionine (0.1%) and ascorbic acid (1%) had significantly better flavour during storage due to higher sugar content. Based on the overall acceptability, arils dipped in ascorbic acid (1%), citric acid (1%) and methionine (0.1%) were acceptable up to 8 days. These findings are in agreement with previous studies by Shah and Nath (2008) who treated the peeled litchi arils with cysteine, ascorbic acid and 4-hexyl resorcinol and stored under low temperature (4±2 °C).

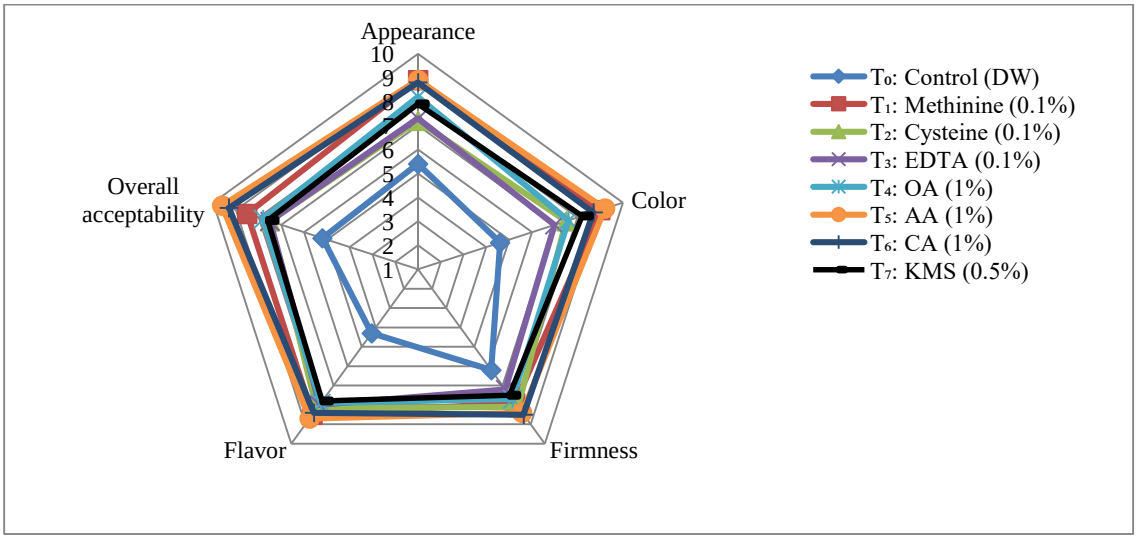

Fig. 4. Subjective quality evaluation of minimally processed litchi stored under refrigeration condition for 8 days.

Microbiological analysis

Microbial growth in all treatments increased as the storage period progressed which was consistent with the results reported by Kumar et al. (2018b). Total microbial counts increased by 5 log CFU/g on untreated litchi arils while microbial loads on treated fruit increased by 3.0-4.0 log CFU/g throughout the storage regardless the applied treatment. Hence, untreated control litchi arils showed markedly higher microbial counts at 8 days of storage than minimally processed litchi subjected to different treatments. Ascorbic acid (1%) treatment was markedly effective in inhibiting the growth of microorganisms in minimally processed litchi at refrigerated conditions (<5 log CFU/g on day 8) and maintained lowest level among all treatments throughout 8 days of the storage. After eight days of storage, for fresh-cut litchi treated by Methinine (0.1%) microbial counts did not exceed 5 log CFU/g while in the control microbial counts were over 5 log CFU/g.

CONCLUSION

Chemical treatments were found to be effective in stabilizing the colour, biochemical and sensorial changes in peeled litchi arils stored under refrigerated condition. The shelf life was extended to 8 days by ascorbic acid (1%), citric acid (1%) and methionine (0.1%) treatments as compared to 3 days for untreated ones. Considering health issues and consumer acceptance, organic acids are perceived as natural additives and can be used at industrial level. The use of organic acids is a feasible and convenient method of preservation for fresh-cut produce. On the basis of overall sensory quality, enzyme activities and effect on nutritional constituents of arils, 1% ascorbic acid and 1% citric acid were recorded to be the best to preserve the quality during refrigeration storage.

Conflict of interest

The authors have no conflict of interest to report.

Acknowledgements

We thank Department of Fruit Science for supplying litchi fruits for the study. We also thank Osi M. for her technical assistance.

REFERENCES

Ahvenaien, R. (1996). New approaches in improving the shelf life of minimally processed fruits and vegetables. *Trends in Food Science and Technology*, *7*(6), 179–187. https://doi.org/10.1016/0924-2244(96)10022-4

Altisent, R., Plaza, L., Alegre, I., Vin, I., & Abadias, M. (2014). Comparative study of improved vs. traditional apple cultivars and their aptitude to be minimally processed as 'ready to eat' apple wedges. *LWT*, *58*, 541–549. https://doi.org/ 10.1016/j.lwt.2014.03.019

Altunkaya, A., & Gokmen, V. (2008). Effect of various inhibitors on enzymatic browning, antioxidant activity and total phenol content of fresh lettuce (*Lactuca sativa*). *Food Chemistry*, *107*(3), 1173–1179. https://doi.org/10.1016/j.foodchem.2007.09.046

Aquino-Bolanos, E.N., & Mercado-Silva, E. (2004). Effects of polyphenol oxidase and peroxidase activity, phenolics and lignin content on the browning of cut jicama. *Postharvest Biology and Technology*, *33*(3), 275–283. https://doi.org/10.1016/j.postharvbio.2004.03.009

Azevedo, V.M, Dias, M.V., Siqueira, H.H., Fukushima, K.L., Silva, E.K., Carneiro, J.D.D.S., & Borges, S.V. (2018). Effect of whey protein isolate films incorporated with montmorillonite and citric acid on the preservation of fresh-cut apples. *Food Research International*, *107*, 306–313. https://doi.org/10.1016/j.foodres.2018.02.050

Bolanos, E. N. A., Velazquez, R. C., Cardenaz, A. V., Santamaría, I. R., Vera, N. G., Fuentes, A. D. H., & Silva, E. M. (2010). Effect of storage temperature and time on quality in minimally processed litchi fruit (*Litchi Chinensis* Sonn.). *Journal of Food Quality*, *33*(3), 299–311. https://doi.org/10.1111/j.1745-4557.2010.00324.x

Cappuccino, J. G., & Sherman, N. (2008). Microbiology: a laboratory manual, Vol 9. Benjamin Cummings, Boston.

Chiumarelli, M., & Hubinger, M. D. (2012). Stability, solubility, mechanical and barrier properties of cassava starch - Carnauba wax edible coatings to preserve fresh-cut apples. *Food Hydrocolloids*, *28*(1), 59-67. https://doi.org/10.1016/j.foodhyd.2011.12.006.

Dong, H., Cheng, L., Tan, J., Zheng, K., & Jiang, Y. (2004). Effects of chitosan coating on quality and shelf life of peeled fruit. *Journal of Food Engineering*, *64*, 355–358. https://doi.org/10.1016/j.jfoodeng.2003.11.003

Farooq, S., Dar, A. H., Dash, K. K., Srivastava, S., Pandey, V. K., Ayoub, W. S., Pandiselvam, R., Manzoor, S., & Kaur, M. (2023). Cold plasma treatment advancements in food processing and impact on the physiochemical characteristics of food products. *Food Science and Biotechnology*, *32*(5), pp.621-638. https://doi.org/10.1007/s10068-023-01266-5

Gimnez, M., Olarte, C., Sanz, S., Lomas, C., Echavarri, L., & Ayala, F. (2003). Influence of packaging films on the sensory and microbiological evolution of minimally processed borage (*Borrago officinalis*). *Journal of Food Science*, *68*(3), 1051–1058. https://doi.org/10.1111/j.1365-2621.2003.tb08286.x

Gorny, J. R., Pierce, B. H., Cifuentes, R. A., & Kader, A. A. (2002). Quality changes in fresh cut pear slices as affected by controlled atmosphere and chemical preservatives. *Postharvest Biology and Technology*, *24*, 271–278. https://doi.org/10.1016/S0925-5214(01)00139-9

Jiang, Y. M., & Fu, J. R. (2000). A review of advances in the study of postharvest physiology and technology of storage and transport of litchi fruit. *Subtropical Plant Research Communication*, *29*(3), 64–70. https://doi.org/10.17660/ActaHortic.2001.558.62

Jiang, Y., Gao, H., & Zhang, M. (2012). Lychee (Litchi). In M. Siddig, J. Ahmed, M. G. Lobo, & F. Ozadali (Eds.), Tropical and Subtropical Fruits: Postharvest Physiology, Processing and Packaging (pp. 241-258). New Delhi, India: Wiley Press.

Kaushik, N., Kaur, B. P., & Rao, P. S. (2014). Application of high pressure processing for shelf life extension of litchi fruits (*Litchi chinensis* cv. Bombai) during refrigeration. *Food Science and Technology International*, *20*, 527–541. https://doi.org/10.1177/1082013213496093

Kumar, P., Sethi, S., Sharma, R. R., Singh, D., & Varghese, E. (2018a). Improving the shelf life of fresh-cut 'Royal Delicious' apple with edible coatings and anti-browning agents. *Journal of Food Science and Technology*, *55*, 3767–3778. https://doi.org/10.1007/s13197-018-3308-6

Kumar, P., Sethi, S., Sharma, R. R., Srivastav, M., & Varghese, E. (2017). Effect of chitosan coating on postharvest life and quality of plum during storage at low temperature. *Scientia Horticulturae*, *226*, 104-109. https://doi.org/10.1016/j.scienta.2017.08.037

Kumar, P., Sethi, S., Sharma, R. R., Srivastav, M., Singh, D., & Varghese, E. (2018b). Edible coatings influence the cold-storage life and quality of 'Santa Rosa' plum (*Prunus salicina* Lindell). *Journal of Food Science and Technology*, 55, 2344–2350. https://doi.org/10.1007/s13197-018-3130-1

Kuwar, U., Sharma, S., & Tadapaneni, V. R. R. (2015). Aloe vera gel and honey-based edible coatings combined with chemical dip as a safe means for quality maintenance and shelf life extension of fresh-cut papaya. *Journal of Food Quality*, *38*(5), 347–358. https://doi.org/10.1111/jfq.12150

Lamikanra, O., & Watson, M. A. (2001). Effects of ascorbic acid on peroxidase and polyphenoloxidase activities in fresh-cut cantaloupe melon. *Journal of Food Quality*, *66*(9), 1283–1286. https://doi.org/10.1111/j.1365-2621.2001.tb15202.x

Li-Qin, Z., Jie, Z., Shu-Hua, Z., & Lai-Hui, G. (2009). Inhibition of browning on the surface of peach slices by short-term exposure to nitric oxide and ascorbic acid. *Food Chemistry*, *114*(1), 174–179. https://doi.org/10.1016/j.foodchem.2008.09.036

Mphahlele, R. R., Caleb, O. J., & Ngcobo, M. E. K. (2020). Effects of packaging and duration on quality of minimally processed and unpitted litchi cv. 'Mauritius' under low storage temperature. *Heliyon*, *6*, 03229. https://doi.org/10.1016/j.heliyon.2020.e03229

Nayak, S. L., Sethi, S., Sharma, R. R., Sharma, R. M., Singh, S., & Singh, D. (2019). Aqueous ozone controls decay and maintains quality attributes of strawberry (*Fragaria× ananassa* Duch.). *Journal of Food Science and Technology*, *57*, 319-326. https://doi.org/10.1007/s13197-019-04063-3

Olivas, G. I., Mattinson, D. S., & Barbosa-Canovas, G. V. (2007). Alginate coatings for preservation of minimally processed "Gala" apples. *Postharvest Biology and Technology*, *45*(1), 89-96. https://doi.org/10.1016/j.postharvbio.2006.11.018

Oms-Oliu, G., Rojas-Graü, M. A., Gonzalez, L. A., Varela, P., Soliva-Fortuny, R., Hernando, M. I. H., Munuera, I. P., Fiszman, S., & Martín-Belloso, O. (2010). Recent approaches using chemical treatments to preserve quality of fresh-cut fruit: A review. *Postharvest Biology and Technology*, *57*(3), 139-148. https://doi.org/10.1016/j.postharvbio.2010.04.001

Paciolla, C., Fortunato, S., Dipierro, N., Paradiso, A., De Leonardis, S., Mastropasqua, L., de Pinto, & M. C. (2019). Vitamin C in plants: from functions to biofortification. *Antioxidants* (Basel). 29, *8*(11), 519. https://doi.org/10.3390/antiox8110519

Pathare, P. B., Opara, U. L., & Al-Said, F. A. (2013). Colour measurement and analysis in fresh and processed foods: a review. *Food and Bioprocess Technology*, *6*(1), 36–60. https://doi.org/10.1007/s11947-012-0867-9

Phanumong, P., Rattanapanone, N., & Haewsungcharern, M. (2015). Quality and shelf-life of minimally-processed litchi fruit. *Chiang Mai University Journal of Natural Sciences*, *4*, 35–51. https://doi.org/10.12982/CMUJNS.2015.0070

Phanumong, P., Sangsuwan, J., & Rattanapanone, N. (2017). Effect of modified atmosphere packaging with varied gas combinations and treatment on the quality of minimally processed litchi fruits. *Songklanakarin Journal of Science and Technology*, *39*, (6), 715–722.

Phanumong, P., Sangsuwan, J., Kim, S. M., & Rattanapanone, N. (2016). The improvement of texture and quality of minimally processed litchi fruit using various calcium salts. *Journal of Food Processing and Preservation*, *40*, 1297–1308. https://doi.org/10.1111/jfpp.12715

Ranganna, S. (2007). Handbook of Analysis and Quality Control for Fruits and Vegetable Products 3rd edition. Tata McGraw-Hill Publishing Company Ltd.

Sarkar, A., & Sumi, M. (2023). Impact of chemicals and modified atmosphere packaging (MAP) on postharvest quality of litchi cv. *China*. *Journal of Postharvest Technology*, *11*(1), 115-124.

Shah, N. S., & Nath, N. (2006). Effect of calcium lactate, 4-hexyl resorcinol, and vacuum packing on physico-chemical, sensory and microbiological qualities of minimally processed litchis (*Litchi chinensis* Sonn.). *International Journal of Food Science & Technology*, *41*, 1073-1081. https://doi.org/10.5851/kosfa.2017.37.2.313

Shah, N. S., & Nath, N. (2008). Changes in qualities of minimally-processed litchis: Effect of antibrowning agents, osmo-vacuum drying and moderate vacuum packaging. *LWT*, *41*, 660-668. https://doi.org/10.1016/j.lwt.2007.04.012

Silveira Alexandre, A. C., Ferreira Gomes, B. A., Duarte, G. N., Piva, S. F., Barros Zauza, S., & de Barros Vilas Boas, E. V. (2022). Recent advances in processing and preservation of minimally processed fruits and vegetables: A review–Part 1: Fundamentals and chemical methods. *Journal of Food Processing and Preservation*, *46*(8), e16757. https://doi.org/10.1111/jfpp.16757

Soliva-Fortuny, R. C., Elez-Martinez, P., & Martin-Belleso, O. (2004). Microbiological and biochemical stability of fresh cut apples preserved by modified atmosphere packaging. *Innovative Food Science and Emerging Technologies*, *5*, 215–224. https://doi.org/10.1016/j.ifset.2003.11.004

Tassou, C. C., & Boziaris, J. S. (2002). Survival of Salmonella enteritidis and changes in pH and organic acids in grated carrots inoculated or not inoculated with Lactobacillus sp. and stored under different atmospheres at 4°C. *Journal of the Science of Food and Agriculture*, *82*, 1122–1127. https://doi.org/10.1002/jsfa.1157

Ventura-Aguilar, R. I., Colinas-Leon, M. T., & Bautista-Banos, S. (2017). Combination of sodium erythorbate and citric acid with MAP, extended storage life of sliced oyster mushrooms. *LWT*, 79, 437–444. https://doi.org/10.1016/j.lwt.2017.01.053

Yun, Z., Gao, H., Chen, X., Chen, Z., Zhang, Z., Li, T., Qu, H., & Jiang, Y. (2020). Effects of hydrogen water treatment on antioxidant system of litchi fruit during the pericarp browning. *Food Chemistry*, 127618. https://doi.org/10.1016/j.foodchem.2020.127618

JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH
2024, VOL. 7(1), 15-30

Journal of Horticulture and Postharvest Research

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

University of Birjand

Enhancing mineral uptake and antioxidant enzymes activity of kiwifruit via foliar application of brown macroalga extract

Mahshid Ghafouri[1], Farhang Razavi[1]*, Masoud Arghavani[1] and Ebrahim Abedi Gheshlaghi[2]

[1]Department of Horticultural Sciences, Faculty of Agriculture, University of Zanjan, Zanjan, Iran
[2]Department of Horticulture Crops Research, Guilan Agricultural and Natural Resources Research and Education Center, AREEO, Rasht, Iran

ARTICLE INFO

Original Article

Article history:
Received 4 November 2023
Revised 15 January 2024
Accepted 17 January 2024
Available online 18 February 2024

Keywords:
Catalase
Hydrogen peroxide
Malondialdehyde
Preharvest treatment
Superoxide dismutase

DOI: 10.22077/jhpr.2024.6926.1343
P-ISSN: 2588-4883
E-ISSN: 2588-6169

***Corresponding author:**
[1]Department of Horticultural Sciences, Faculty of Agriculture, University of Zanjan, Zanjan, Iran.

Email: razavi.farhang@znu.ac.ir

ABSTRACT

Purpose: The application of natural organic compounds without harmful environmental effects in the production of horticultural and agricultural products is considered as a new method to reduce waste before and after harvesting, increase the storage life and maintain antioxidant activity in developed agriculture. As regards, this study investigated the impact of foliar applications of brown macroalga extract on antioxidant enzyme activity and mineral uptake in 'Hayward' kiwifruits. **Research method**: Four treatment levels of brown macroalga extract (0, 1, 2, and 3 g/L) were applied at three distinct phases, occurring 110, 125, and 140 days after full bloom. Fruit samples were stored at 1±0.5°C with 95% relative humidity for 90 days, with measurements taken every 30 days. **Findings:** Results revealed that the 3 g/L extract treatment significantly increased the uptake of calcium (47.82 %), nitrogen (20.52 %), potassium (12.06 %), phosphorus (19.81 %), and iron (25.77 %) compared to the control. The extract demonstrated a substantial effect on all recorded traits. Among the applied treatments, 3 g/L of brown macroalga extract concentration had the best effect in reducing electrolyte leakage (25.10%), malondialdehyde accumulation (96.73%), hydrogen peroxide content (54.54%) and increasing activities of antioxidant enzymes including superoxide dismutase (50.42%), catalase (84.90%), ascorbate peroxidase (79.02%), and peroxidase (49.40%) compared to the control in 90 days of storage. **Research limitations:** No limitations were found. **Originality/Value:** The results suggest that the 3 g/L brown macroalga extract concentration holds promise for enhancing the quality of 'Hayward' kiwifruits.

INTRODUCTION

Kiwifruits (*Actinidia deliciosa*) are revered for their significant antioxidant content, boasting compounds such as ascorbic acid, carotenoids, and flavonoids. Their nutritional richness and potential health benefits, including anticancer properties due to their high vitamin C and mineral content, have earned them a well-deserved place in the realm of healthy dietary choices (Cassano et al., 2006).

The global production of kiwifruits, as reported by FAO (2021), now exceeds 4 million tons, with Iran ranking fifth, contributing approximately 289,000 tons. As agriculture worldwide embraces organic and sustainable farming, the reduction in chemical fertilizers and synthetic inputs becomes a pivotal goal. In pursuit of this eco-conscious approach, the use of bio stimulants, particularly seaweed extracts, has emerged as a promising avenue to enhance crop growth and development. An increasingly organic-driven agricultural sector has unfolded, fueled by the remarkable rise in global organic food consumption over the past decade (Rana & Paul, 2017).

Seaweeds, brimming with growth stimulators, vitamins, antioxidants, organic acids, organic nitrogen, phosphorous, and potassium compounds, have gained attention as a source of plant hormones derived from *Ascophyllum*. Notably, pre-harvest applications of nutrient solutions, like seaweed extracts, have demonstrated an ability to bolster both the quantity and quality of horticultural crops. Such interventions can also enhance post-harvest storage properties and marketability (Keyrouz et al., 2011).

A compelling body of evidence indicates that liquid seaweed extracts wield a positive influence on crop yield and quality across various species. Mandarins, oranges, strawberries, grapes, apples, melons, and other fruits have all exhibited improved productivity and quality with the application of these extracts. These outcomes are attributed to the presence of cell metabolism-enhancing compounds, including plant growth regulators such as auxin, gibberellin, cytokinin, as well as organic osmolytes like betaine, amino acids, minerals, polysaccharides, and vitamins (Zodape et al., 2001; Fornes et al., 2002, Masny et al., 2004, Geny et al., 2007; Lai et al., 2007, Abdel-Mawgoud et al., 2010).

Furthermore, the study of commercial brown macroalgae extracts has demonstrated their potential in enhancing the storage and nutritional quality of vegetables such as spinach. The application of these extracts not only boosts plant tolerance to various environmental stresses but also augments the activity of antioxidant enzymes, fortifying the plants' defense mechanisms against diseases (Jayaraman et al., 2011). Nikoogoftar-Sedghi et al. (2023) showed that foliar application of *Ascophyllum nodosum* (L.) seaweed extract in pistachio enhanced carbohydrate, protein, total phenol and flavonoid levels, and improved antioxidant enzymes activity.

A study by Rathore et al. (2008) further exemplified the benefits of seaweed applications, revealing an increase in nutrient uptake by rain-fed soybeans, particularly with regard to calcium. This heightened calcium content is integral in enhancing cell wall resistance in plants and fruits, further emphasizing the agricultural advantages of seaweed extracts. Building upon this background, our research embarks on an exploration of the effects of foliar applications of brown macroalgae extract to kiwifruit vines, 'Hayward' cultivar. We investigate how this application influences nutrient uptake and the activity of antioxidant enzymes in kiwifruits during post-harvest cold storage.

MATERIALS AND METHODS

Plant material and experimental setup

Ten-year-old kiwifruit vines (*Actinidia deliciosa* cv. 'Hayward') cultivated in a commercial vineyard located in Astara County, Iran, were selected for this study. The experiment adopted a factorial design based on a randomized complete block format, encompassing three replicates. The first factor pertained to the application of brown macroalga extract at varying concentrations: 0, 1, 2, and 3 g/L. The second factor involved storage duration with four levels: 0, 30, 60, and 90 days' post-storage. The foliar application of the brown macroalga extract was conducted at three distinct phases, occurring 110, 125, and 140 days after full bloom. Ripe fruits were collected, and subsequent assessments were carried out in the Postharvest Physiology Laboratory of the Department of Horticulture at the University of Zanjan. The fruits were subjected to cold storage, maintaining a temperature of 1±0.5°C and 95% relative humidity for a span of 90 days. Evaluations were performed at 30, 60, and 90 days' post-storage. Also, to replicate conventional shelf-life conditions, the fruits were stored at 25°C for 72 hours before the measurement of the traits.

Measurement of traits

Extraction of nutrients

To extract and quantify nutrients, plant samples were subjected to drying before analysis. After weighing, the fruits were sliced into 20-mm sections, encompassing both flesh and skin. These slices were subsequently oven-dried at 75°C for 72 hours, followed by grinding. The concentrations of various nutrients were analyzed separately. The dried kiwifruit samples were powdered using a Chinese mortar. Subsequently, 1 gram of the powdered sample was placed into a porcelain crucible and incinerated at temperatures ranging from 500-550°C for 5-6 hours. The resultant ash was rinsed with 11 mL of 2N hydrochloric acid until the digestion process was completed. Following this, they were transferred to a 100 mL beaker, heated for 5-10 minutes to induce a change in solution color, and subsequently filtered with filter paper. The volume was adjusted to 100 mL using distilled water (Westerman, 1990).

Nutrient measurement methods

The nitrogen content of the powdered samples was determined through the Kjeldahl method. Calcium and potassium levels were measured using a complexometric method and a flame photometer, respectively. Phosphorus content was determined through the molybdenum vanadate method and the application of a yellow reagent. Iron, manganese, and zinc levels were quantified using an atomic absorption device (Westerman, 1990). Selenium accumulation was determined in treated kiwifruits with various concentrations of brown macroalga extract by the method outlined by Zasoski and Burau (1977). All data were expressed in mg kg^{-1} dry weight.

Measurement of oxidative stress and antioxidant enzymes

Malondialdehyde (MDA)

The concentration of malondialdehyde, a marker of membrane peroxidation, was assessed following the procedure described by Heath and Packer (1968). In this method, 1 gram of fresh tissue was homogenized with 5 mL of 1% trichloroacetic acid (TCA). To this mixture, 4 mL of TCA (20%) containing 0.5% thiobarbituric acid was added to 1 mL of the supernatant. The mixture was then heated to 95°C, cooled, and the MDA concentration was determined by measuring the absorbance at 532 nm and 600 nm, utilizing an extinction coefficient of 155 $mmol^{-1}cm^{-1}$.

Electrolyte leakage (EL)

Electrolyte leakage was estimated according to the method proposed by Lim et al. (1998) and calculated using the formula: "EL (%) = (Final EL - Initial EL) / Initial EL × 100."

Hydrogen peroxide (H_2O_2)

The measurement of H_2O_2 content involved homogenizing 1 gram of fruit tissue, including the skin, with 5 mL of 1% TCA in an ice bath. The mixture was centrifuged, and 500 µL of the supernatant was combined with 500 µL of 10 mM potassium phosphate buffer (pH = 7) and 1 mL of 1 M potassium iodide (KI). The absorbance was recorded at 765 nm, and the H_2O_2 content was calculated from a standard curve and reported in nM/g fresh weight (FW) (Alexieva et al., 2001).

Antioxidant enzymes

To determine the activity of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX), 1 gram of frozen kiwifruit tissue was homogenized with 50 mM phosphate buffer (pH = 7) containing 2% PVP. The resulting extract was centrifuged at 12,000 × g at 4°C for 20 minutes, and the supernatant was employed as an enzymatic extract (Zhang et al., 2013). Enzyme activity was measured at specific wavelengths using a spectrophotometer, and specific enzyme activities were calculated in units per gram of fresh weight (U/g FW).

Experimental design and data analysis

The experimental design involved a factorial arrangement within a randomized complete block design with three replications, with each replication containing one vine. The various concentrations of nutrients were analyzed as completely randomized blocks, while the biochemical traits and antioxidant enzyme activities were treated as a factorial. Data were analyzed using the SPSS software (ver. 20). Statistical comparisons were conducted through Duncan's multiple range tests at a significance level of $P < 0.05$. Graphs and tables were generated using Excel (ver. 2016).

RESULTS AND DISCUSSION

Our analysis of variance (ANOVA) revealed significant effects of foliar application of brown macroalga extract in reducing EL, MDA and H_2O_2 accumulation and increasing activities of antioxidant enzymes including SOD, CAT, APX and POD at the $P<0.01$ level. The application had a profound impact on the uptake of nitrogen (N), potassium (K), and iron (Fe), exhibiting statistical significance at the $P < 0.01$ level. Additionally, it had significant effects, albeit at the $P < 0.05$ level, on the uptake of calcium (Ca) and phosphorus (P). In contrast, its effect was not statistically significant concerning the uptake of selenium (Se), magnesium (Mg), zinc (Zn), and manganese (Mn) (Table 1 and 2).

Table 1. Analysis of variance (ANOVA) for the effect of macroalga (*Ascophylum nodosum*) extract on nutrient contents of kiwifruits at harvest time.

Sources of variation	df	Mean of squares								
		Se	N	P	K	Ca	Mg	Fe	Zn	Mn
Block	2	2.5005	2737.583	1525	2708.333	700	75	0.32	0.006	0.481
Concentration of brown algae	3	0.001^{ns}	437589.639**	2866.667*	235719.444**	1800*	163.889^{ns}	1.001**	0.381^{ns}	1.159^{ns}
Error	30	0.001	6878.139	1291.667	5919.444	500	130.556	0.084	0.256	0.633
C.V (%)		24.32	1.73	9.45	2.07	8.60	8.96	7.33	18.45	29.35

**, *, ns: significant at the 1% and 5 % of probability level and non-significant.

Table 2. Analysis of variance (ANOVA) for the effect of macroalga (*Ascophylum nodosum*) extract on evaluated characteristics of kiwifruit cv. 'Hayward'during storage period.

Sources of variation	df	Mean of squares						
		EL	MDH	H_2O_2	CAT	POD	APX	SOD
Block	2	0.682	0.008	0.100	0.150	2.543	33.90	0.658
Concentration of brown algae (CBA)	3	136.23**	5.749**	0.51**	18.91**	362.33**	452.27**	10012.52**
Storage time (ST)	3	914.05**	6.023**	0.548**	66.585**	1439.85**	1456.13**	8281.02**
CBA × ST	9	3.58**	0.815**	0.136**	0.958**	27.47**	102.82^{*8}	171.21**
Error	30	1.077	0.011	0.001	0.158	3.25	10.9	1.929
C.V (%)		4.48	4.90	2.37	7.47	5.95	11.47	0.670

**, *, ns: significant at the 1% and 5 % of probability level and non-significant.

Effect of brown macroalga extract on nutrient contents

The comparison of means demonstrated the significance of foliar application of brown macroalga extract on the Ca content. The highest Ca content (340 mg/kg DW) was achieved with the application of 3 g/L of the brown macroalga extract, while the lowest (230 mg/kg DW) was observed in the control group (Fig. 1a). There was a clear positive relationship between Ca content and extract concentration, with higher extract rates resulting in greater Ca content.

Similarly, the K content in the fruit tissue exhibited an increase with higher extract rates. The application of the macroalga extract at a rate of 3 g/L led to the highest K content (3936.66 mg/kg DW), while the control group showed the lowest K content (3306.66 mg/kg DW) (Fig. 1b). Although different extract rates did not display significant differences, the effect was statistically significant ($P < 0.01$) compared to non-use.

The analysis of data revealed a rising trend in P content within the fruit tissue with increasing brown macroalga extract rates. The various extract treatments displayed significant differences in P content, with the highest and lowest P contents (423.33 and 353.33 mg/kg DW) observed at the 3 g/L extract rate and in the control group, respectively (Fig. 1c).

The positive effect of the extract on N content in the fruit tissue was evident through the increasing trend observed. Significant differences were identified among the various brown macroalga extract treatments. The highest N content (5109.66 mg/kg DW) was recorded in plants treated with 3 g/L of the brown macroalga extract, while the untreated plants had the lowest content (4239 mg/kg DW) (Fig. 1d).

The results also revealed an increasing trend in Fe content within the vines subjected to the foliar application of brown macroalga extract. These treatments showed significant differences, with the 3 g/L extract rate associated with the highest Fe content (4.06 mg/kg DW), and the control group with the lowest (3.23 mg/kg DW) (Fig. 2).

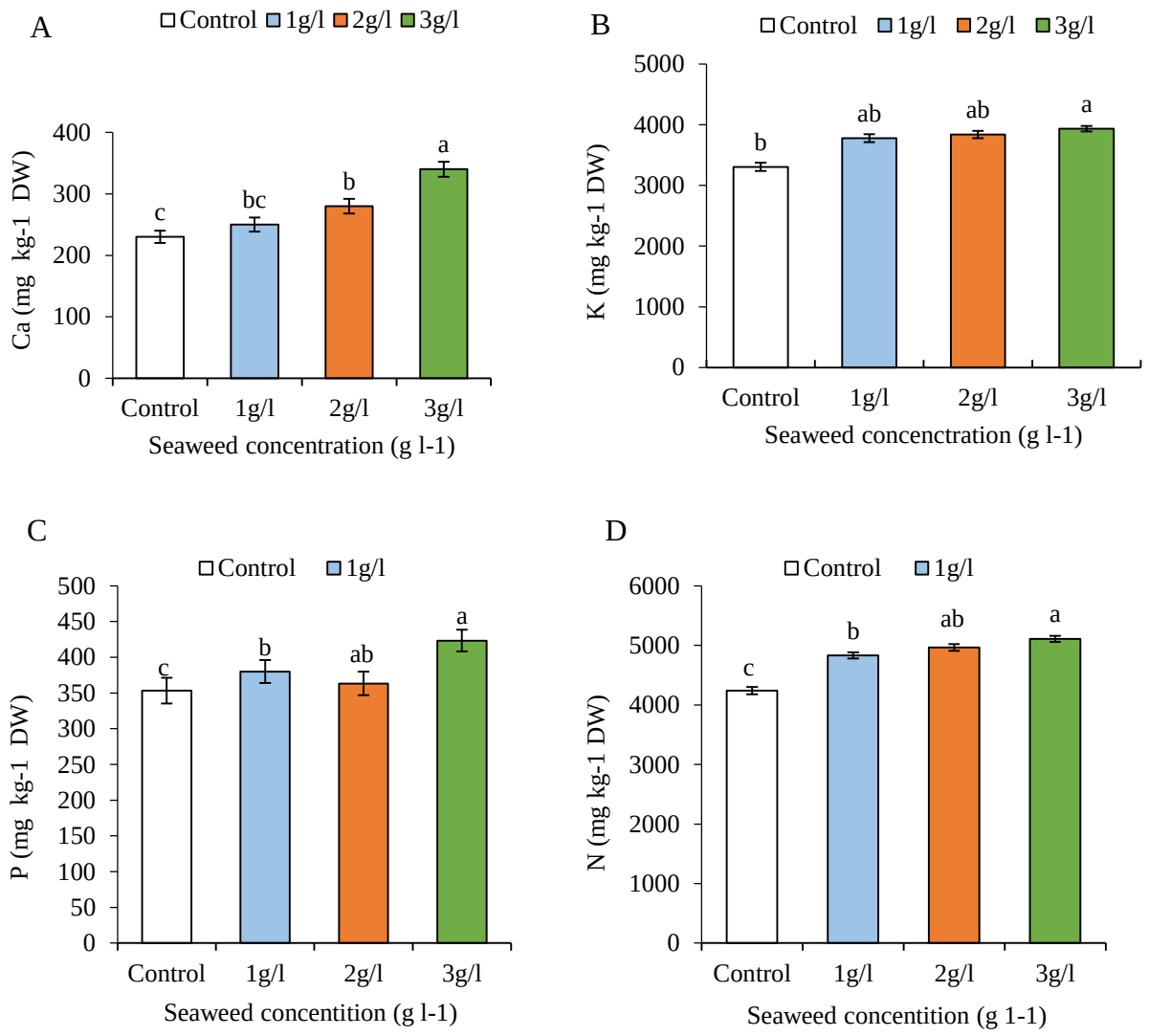

Fig. 1. The effect of *Ascophyllum nodosum* seaweed extract at various concentrations (0, 1, 2, and 3 g/L) on the content of (A) calcium, (B) potassium, (C) phosphorus, and (D) nitrogen in 'Hayward' kiwifruit at the time of harvest. Each value represents the mean ± standard error of three replications.

The nutrition of plants during the fruit growth stage significantly impacts post-harvest crop quality (Ramezanian et al., 2009). Optimal nutrition is a fundamental requirement for enhancing both crop quantity and quality. Beyond ensuring the availability of all necessary nutrients in adequate quantities, maintaining a balanced nutrient profile is of paramount importance. Nutritional imbalances can not only fail to enhance yields but can also disrupt plant growth, ultimately leading to reduced crop yields. Effective nutrient management that ensures the availability of all required nutrients is essential. Even a short-term nutrient deficiency during the growing season can diminish potential crop yields. Mismanagement of nutrients, even at trace levels (ppm) found in fruit trees, can result in adverse economic consequences. In contrast, proper nutrient management represents an efficient strategy in horticulture (Malkouti & Homai, 2005).

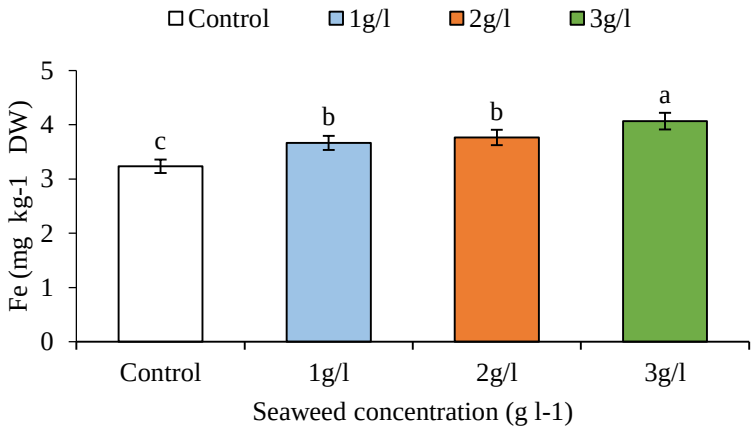

Fig. 2. The effect of *Ascophyllum nodosum* seaweed extract at various concentrations (0, 1, 2, and 3 g/L) on iron content in 'Hayward' kiwifruit at the time of harvest. Each value represents the mean ± standard error of three replications.

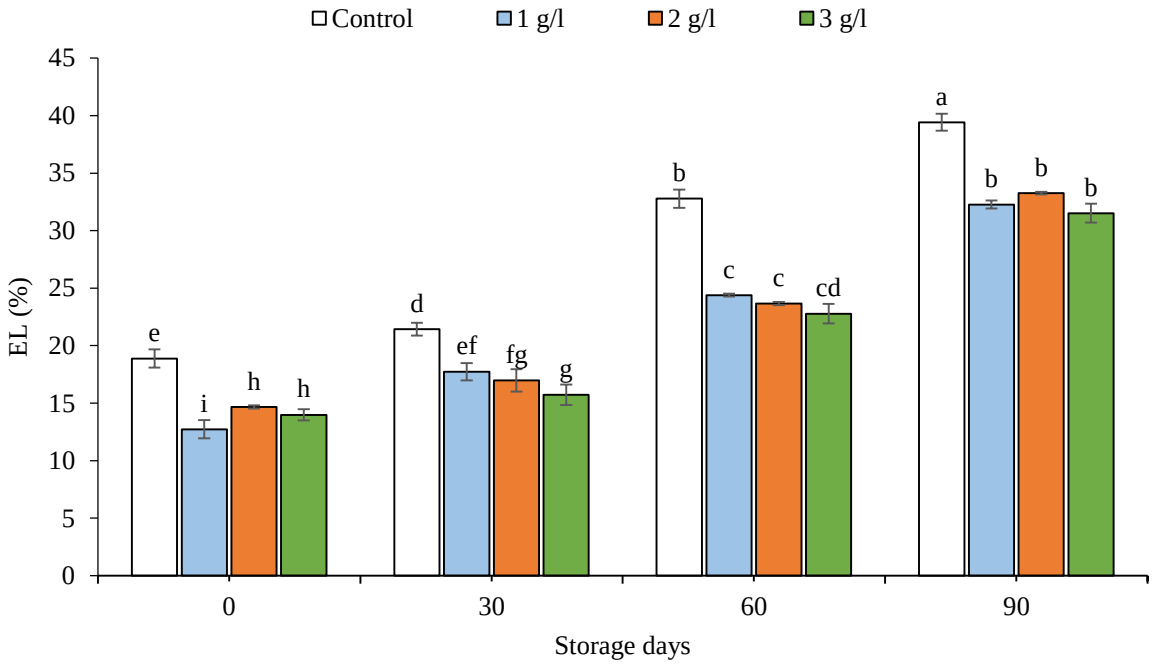

Fig. 3. The effect of *Ascophyllum nodosum* seaweed extract at different concentrations (0, 1, 2, and 3 g/L) on the electrolyte leakage of 'Hayward' kiwifruit during storage (0, 30, 60, and 90 days after storage). Each value represents the mean ± standard error of three replications.

The macroalgal extract from *Ascophyllum nodosum* contains minerals such as N, P, K, Ca, Fe, Mg, Zn, Na, and S (Rayorath et al., 2009). These minerals are readily absorbed through the stomata and hydrophilic pores of cuticles on the leaves. The uptake of these minerals from the leaf surface is influenced by environmental factors including temperature, humidity, and light intensity, which, in turn, affect stomatal opening and the permeability of cuticles and cell walls.

Bio-stimulators, in general, are compounds that induce and optimize plant metabolism and metabolic processes to enhance plant efficiency (Starck, 2005). Seaweed extracts serve as bio-stimulators and are officially recognized in agriculture for their ability to improve plant

growth. The focus of our research was the foliar application of seaweed extract. Foliar application is a method that efficiently delivers nutrients to higher plants by spraying a solution containing these nutrients onto the plant foliage. This method allows plants to acquire nutrients much more rapidly than they would through root uptake from the soil, though it is not without its drawbacks. Despite these limitations, foliar application remains the preferred practice in specific conditions (Marschener, 2022). Seaweeds contain growth stimulators, organic macro- and micro-nutrients, vitamins, antioxidants, organic acids, organic NPK, organic acids of seaweed origin, and plant hormones extracted from *Ascophyllum* algae (Hurtado et al., 2009).

Among the various nutrients, calcium (Ca) has garnered considerable attention in recent years with respect to nutrient management. Calcium application is particularly beneficial during the storage phase, as it contributes to prolonging crop shelf-life and reducing physiological disorders and rot (Rathore et al., 2008). Achieving an optimal balance in nutrient solutions is a vital goal for all agricultural systems (Dong et al., 2005). A study investigating the effect of seaweed extract on nutrient uptake in rainfed soybeans reported an increase in nutrients such as Ca, further highlighting the significance of calcium in plant nutrition (Rathore et al., 2008). Increased calcium content in plants enhances cell wall resistance and the pectin content in both plants and fruits (Rathore et al., 2008).

Electrolyte leakage (EL) and malondialdehyde (MDA)

The comparison of means unveiled an upward trend in EL over the 90-day storage period. The lowest EL (12.72%) was associated with the treatment involving 1 g/L of brown macroalga extract recorded at the time of harvest, whereas the highest EL (39.42%) was observed in the control group, recorded 90 days after storage.

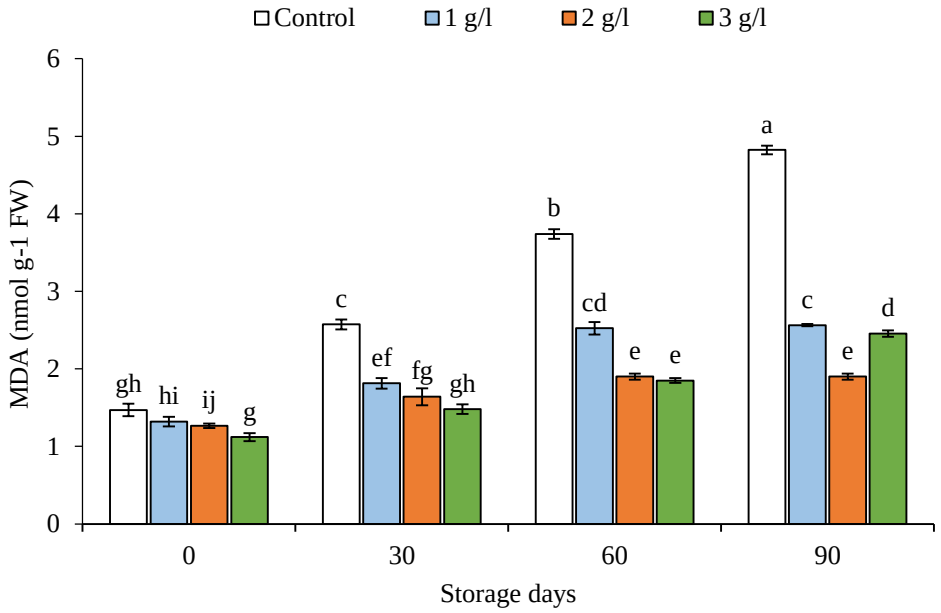

Fig. 4. The impact of *Ascophyllum nodosum* seaweed extract at varying concentrations (0, 1, 2, and 3 g/L) on the malondialdehyde (MDA) content of 'Hayward' kiwifruit during storage (0, 30, 60, and 90 days after storage). Each value represents the mean ± standard error of three replications.

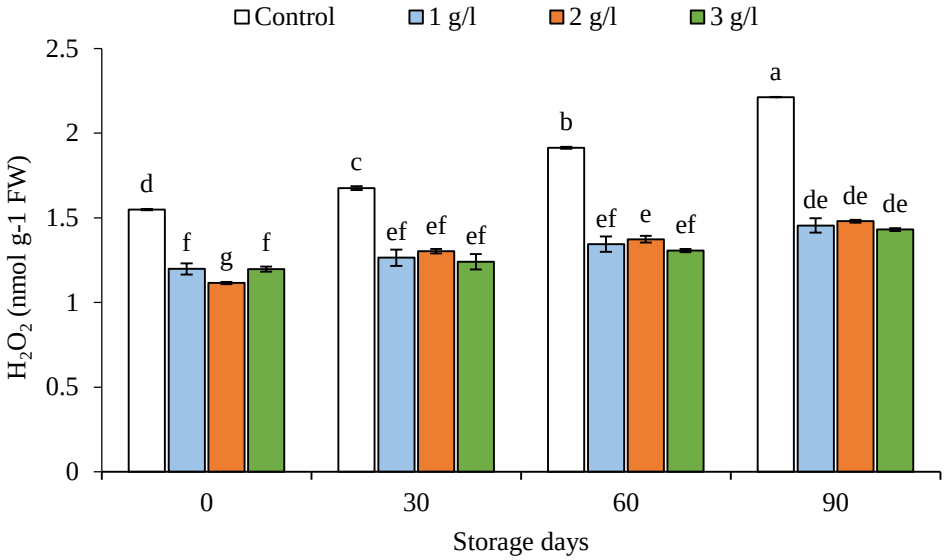

Fig. 5. Influence of *Ascophyllum nodosum* seaweed extract at different concentrations (0, 1, 2, and 3 g/L) on the hydrogen peroxide (H_2O_2) content of 'Hayward' kiwifruit during storage (0, 30, 60, and 90 days after storage). Each value represents the mean ± standard error of three replications.

Furthermore, the comparison of means demonstrated a consistent increase in MDA levels in all fruits throughout the storage period. This trend was more pronounced in the control samples, while MDA accumulation was notably suppressed in the treated fruits over the three months of storage. At the conclusion of the storage period (90 days) plus an additional three days of shelf-life at 25°C, the highest MDA accumulation (4.82 nmol/g FW) was attributed to the control group, while the lowest (1.12 nmol/g FW) was recorded in fruits treated with 3 g/L of seaweed extract at the time of harvest.

The results clearly indicate that the brown macroalga extract has a beneficial impact on reducing EL and inhibiting MDA accumulation. Cell membrane integrity, as the foremost cellular structure influenced by chilling (Rui et al., 2010), is of paramount importance. Chilling induces the phase transition of the cell membrane from a flexible crystalline liquid to a solid gel-like structure, leading to a loss of membrane permeability (Aghdam and Bodbodak, 2013). Prolonged exposure to chilling stress results in the rupture of cell membranes, leading to the leakage of intracellular water, ions, and metabolites, a phenomenon that can be accessed via EL (Sharom et al., 1994).

EL is an effective indicator for measuring cell membrane integrity and thus serves as a reliable indicator of the same. Moreover, lipid peroxidation, a factor that diminishes cell membrane integrity, can be gauged through the assessment of MDA synthesis. The accumulation of MDA, the final product of lipid peroxidation, is indicative of lipid peroxidation and cell membrane damage (Luo et al., 2012; Cheng et al., 2011). Free radicals, when present in excess, can target the double bonds of fatty acids, resulting in MDA production. They can also replace the methyl groups of fatty acids, thereby increasing membrane peroxidation (Radotic et al., 2000).

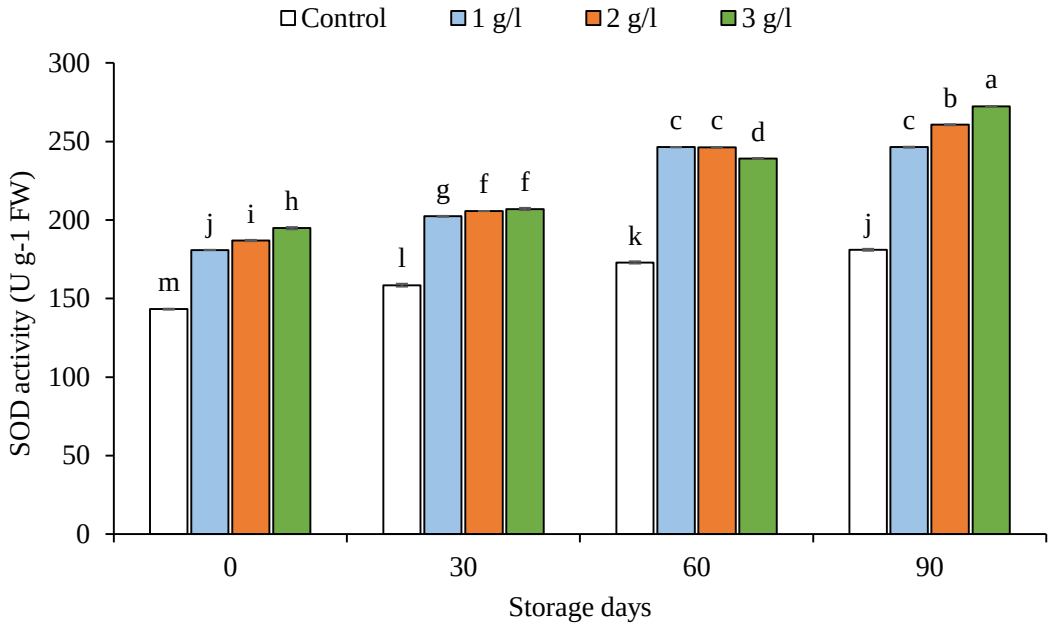

Fig. 6. The effect of *Ascophyllum nodosum* seaweed extract at varying concentrations (0, 1, 2, and 3 g/L) on the superoxide dismutase (SOD) activity in 'Hayward' kiwifruit during storage (0, 30, 60, and 90 days after storage). Each value represents the mean ± standard error of three replications.

In fruits, chilling alters membrane structure through peroxidation, and MDA content is an indicator of declining cell membrane integrity and the onset of chilling-induced damage (Shewfelt & Purvis, 1995). As observed in the EL trend, MDA content increased in control fruits as chilling stress intensified and membrane damage accumulated during storage. However, EL and MDA content in kiwifruits treated with the brown macroalga extract decreased, owing to the positive influence of the extract on enhancing the activity of antioxidant enzymes. The key mechanisms for scavenging reactive oxygen species (ROS) in plants involve enzymatic elements such as ascorbate peroxidase, peroxidase, and catalase, which can neutralize ROS.

Higher membrane peroxidation may result from the inability of the plants enzymatic or non-enzymatic antioxidant systems to effectively neutralize ROS (Allen & Ort, 2001). Similar results regarding the impact of brown macroalga extract on EL and MDA accumulation have been reported in different crops. The application of 15% seaweed liquid fertilizer to wheat reduced MDA content compared to the control over an eight-day storage period at 25°C (GhaffariZadeh et al., 2015).

Hydrogen peroxide (H_2O_2)

Maximum velocity (Vmax) and constant of Michaelis-menton (Km) were estimated by Sigma plot software. Results of analysis showed that Vmax and Km in the *in vivo* and *in vitro* experiment were 18.75; 38.65 and 68.03 109.3 respectively.

The comparison of means revealed an increase in H_2O_2 content during storage. However, the brown macroalga extract treatments effectively restrained the rise in H_2O_2 levels during storage. The lowest amount of H_2O_2 (1.115 nmol/g FW) was related to the treatment with 2g/L of the extract at harvest, while the highest (2.212 nmol/g FW) was observed in the control group during the third month of storage (Fig. 5). H_2O_2 accumulation in fruits typically increases during the post-harvest period due to various stresses, including chilling. Cold temperatures induce a phase transition from a liquid to a rigid solid gel, leading to reduced

cell membrane selectivity. This transformation results in an over synthesis of H_2O_2 radicals and superoxide, leading to membrane damage and membrane lipid peroxidation (Zhang et al., 2015). H_2O_2 is generated through the beta-oxidation of fatty acids in glyoxysomes and/or by photorespiration in peroxisomes (Foyer & Noctor, 2000). Free radicals, including H_2O_2, have a dual role in plants. At low concentrations, they function as signaling molecules in the plant's defense system against stress (Zhou et al., 2012) and can enhance the plant's antioxidant capacity by increasing the activity of antioxidant enzymes. However, at higher concentrations, they become detrimental to plants, prompting the antioxidant system to scavenge excess radicals (Hu et al., 2012). Seaweed extracts can reduce ROS synthesis due to their composition (Farvin & Jacobsen, 2013). A. nodosum, in particular, plays a crucial role in suppressing the formation of free radicals at the initiation of oxidation and in expanding free radical chain reactions as an electron donor (Kindleysides et al., 2012). Similar results have been reported regarding the decrease in H_2O_2 levels as influenced by the application of brown macroalga extract (marine) at rates of 1000 and 2000 mg/L (ppm) in grapes cv. 'Rasheh' under drought stress (Amani et al., 2018), corroborating our findings.

Superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and peroxidase (POD) activity

Based on the comparison of means, SOD, CAT, APX, and POD activities increased in all fruits during the storage period. The rate of increase was more pronounced in the treated fruits, while it was less rapid in the control group, becoming even more marginal in the final 30 days. The highest SOD activity (272.39 units/g FW) was recorded in fruits treated with 3 mg/L of the brown macroalga extract after 90 days of storage, while the lowest (143.28 units/g FW) was observed in the control group at the time of harvest (Fig. 6). The comparison of means indicated an increase in CAT activity throughout the 90-day storage period in all fruits. However, the increase was less prominent in the control group. The highest and lowest CAT activities were observed in the fruits treated with 3 g/L of the extract after three months of storage and the control fruits at the time of harvest (9.8 and 1.8 units/g FW, respectively) (Fig. 7).

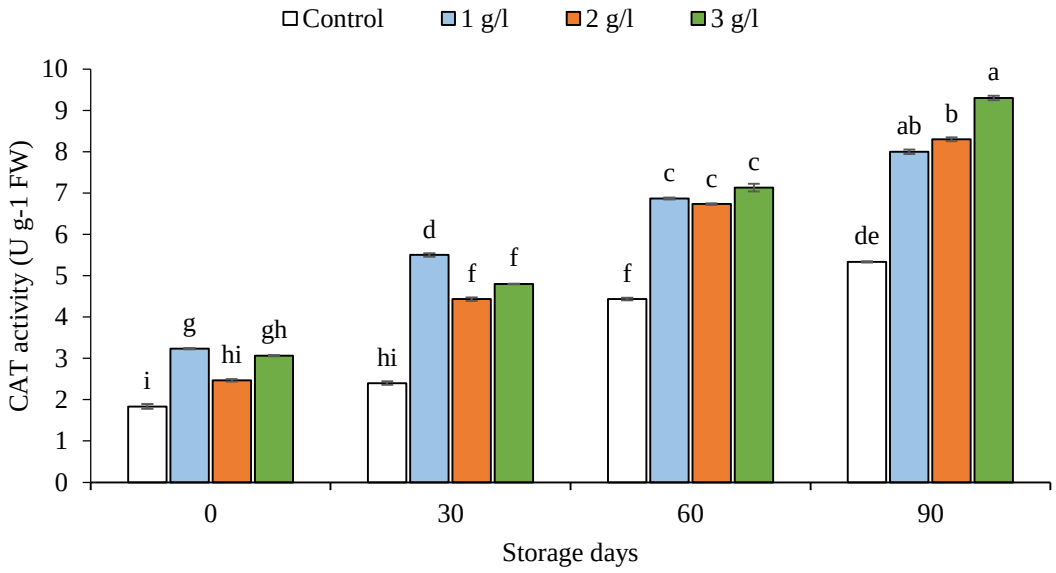

Fig. 7. The impact of *Ascophyllum nodosum* seaweed extract at different concentrations (0, 1, 2, and 3 g/L) on the catalase (CAT) activity in 'Hayward' kiwifruit during storage (0, 30, 60, and 90 days after storage). Each value represents the mean ± standard error of three replications.

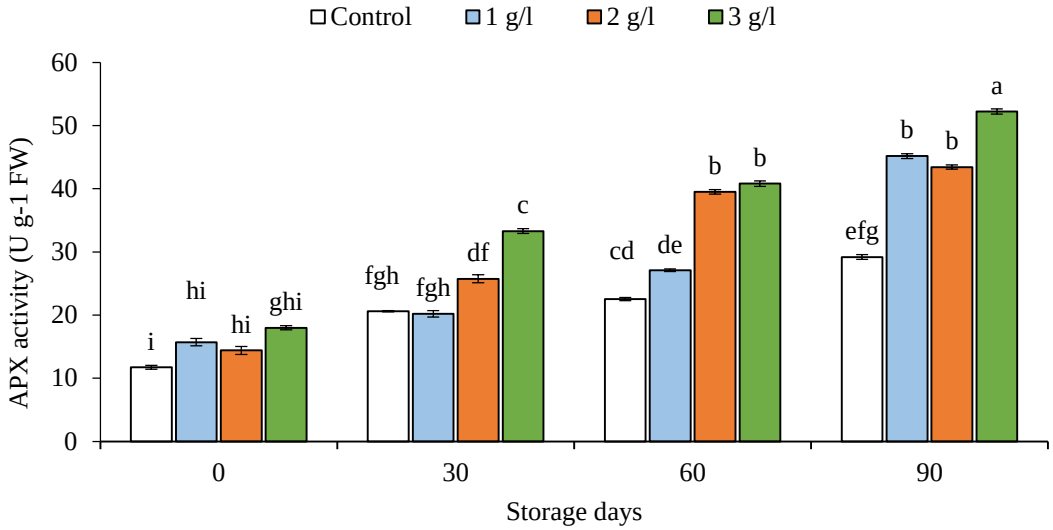

Fig. 8. Influence of *Ascophyllum nodosum* seaweed extract at varying concentrations (0, 1, 2, and 3 g/L) on the ascorbate peroxidase (APX) activity in 'Hayward' kiwifruit during storage (0, 30, 60, and 90 days after storage). Each value represents the mean ± standard error of three replications.

Our research also revealed a steady increase in APX activity during the storage period in all fruit samples. However, the treated fruits exhibited higher APX activity than the control group. The highest APX activity (52.24 units/g FW) was recorded in fruits treated with 3 g/L of brown macroalga extract after 90 days of storage, while the lowest (11.75 units/g FW) was associated with the control fruits at the time of harvest (Fig. 8). Furthermore, the comparison of means indicated a consistent increase in POD activity during storage in all fruit samples. Once again, the treated fruits exhibited notably higher POD activity compared to the control group. The highest POD activity (49.69 units/g FW) was recorded in fruits treated with 3 g/L of the brown macroalga extract after 90 days of storage, while the control group displayed the lowest POD activity (14.97 units/g FW) at the time of harvest (Fig. 9).

The upsurge in SOD, CAT, APX, and POD activities is indicative of enhanced antioxidant enzyme systems in fruits treated with brown macroalga extract. These enzymes play a crucial role in scavenging reactive oxygen species (ROS) and preventing oxidative damage to cellular structures. SOD catalyzes the dismutation of superoxide radicals into oxygen and H_2O_2, which is further detoxified by CAT and APX. The coordinated action of these enzymes helps in maintaining cellular redox homeostasis. Similarly, POD catalyzes the breakdown of H_2O_2, reducing its potential to cause cellular damage (Rui et al., 2010; Zhang et al., 2015).

The significant increase in antioxidant enzyme activities in treated fruits during storage is a testament to the extract's ability to enhance the fruit's defense mechanisms against oxidative stress and maintain the integrity of cellular structures. The importance of these antioxidant enzymes in preventing chilling injury and maintaining fruit quality has been well-established in previous studies. For example, in bananas, increased SOD, CAT, and POD activities were associated with reduced chilling injury (Chen et al., 2011). Similar findings were reported in studies on peaches, where higher SOD, CAT, and POD activities were linked to enhance chilling tolerance and reduced membrane damage (Wang et al., 2019). Therefore, our findings underscore that increased antioxidant enzyme activity reduces ROS levels, ultimately contributing to the preservation of cell wall structure and the extension of fruit longevity.

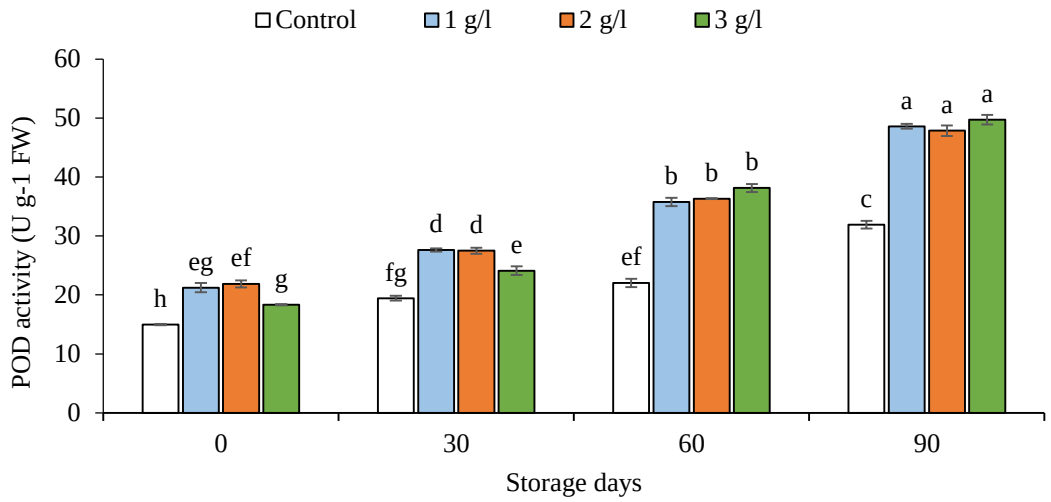

Fig. 9. The effect of *Ascophyllum nodosum* seaweed extract at different concentrations (0, 1, 2, and 3 g/L) on the peroxidase (POD) activity in 'Hayward' kiwifruit during storage (0, 30, 60, and 90 days after storage). Each value represents the mean ± standard error of three replications.

CONCLUSION

In conclusion, the foliar application of brown macroalga extract resulted in significant improvements in nutrient uptake, reduced chilling injury, and delayed ripening during the storage of kiwifruits. Notably, it contributed to the preservation of cell membrane integrity, as evidenced by reduced electrolyte leakage, lower malondialdehyde levels, and enhanced antioxidant enzyme activities, including superoxide dismutase, catalase, ascorbate peroxidase, and peroxidase. The results of this study provide insights into the potential applications of brown macroalga extract in fruit preservation and contribute to our understanding of the mechanisms underlying its effects on nutrient uptake and post-harvest quality. Among the various extract rates studied in this research, the application of 3 g/L was notably the most effective treatment. Hence, we recommend this concentration as the optimal choice for achieving the desired improvements in kiwifruit quality and longevity.

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

Abdel-Mawgoud, A. M. R., Tantaway, A. S., Magda, M. H., & Hoda, A. M. (2010). Seaweed extract improves growth, yield and quality of different watermelon hybrids. *Research Journal of Agriculture and Biological Sciences*, *6*(2), 161–168.

Aghdam, M. S., & Bodbodak, S. (2013). Physiological and biochemical mechanisms regulating chilling tolerance in fruits and vegetables under postharvest salicylates and jasmonates treatments. *Scientia Horticulturae, 156,* 73-85. https://doi.org/10.1016/j.scienta.2013.03.028.

Alexieva, V., Sergiev, I., Mapelli, S. & Karanov, E. (2001). The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. *Plant Cell and Environment*, *24*(12), 1337-1344. https://doi.org/10.1046/j.1365-3040.2001.00778.x.

Allen, D. J., & Ort, D. R. (2001). Impacts of chilling temperatures on photosynthesis in warm-climate plants. *Trends Plant Science*, *6*(1), 36-42. https://doi.org/10.1016/S1360-1385(00)01808-2.

Amani, P., Javadi, T. & Ghaderi. N. (2018). *The effect of Ascophyllum nodosum (marmarine) on the quality and quantity of grape cv. Rasheh in drought and not drought.* University of Kurdistan. Kurdistan.

Cassano, A., Figoli, A., Tagarelli, A., Sindona, G., & Drioli, E. (2006). Integrated membrane process for the production of highly nutritional kiwifruit juice. *Desalination*, *189*(1-3), 21–30. https://doi.org/10.1016/j.desal.2005.06.009.

Chen, T. H., & Murata. N. (2011). Glycine betaine protects plants against abiotic stress: mechanisms and biotechnological applications. *Plant, Cell & Environment*, *34*(1), 1-20. https://doi.org/10.1111/j.1365-3040.2010.02232.x.

Dong, S., cheng, L., Scagel., C & Fuchigami, L. (2005). Timing of urea application affects leaf and root N uptake in young Fuji/M9 apple trees. *The Journal of Horticultural Science and Biotechnology*, *80*(1), 116-120. https://doi.org/ 10.1080/14620316.2005.11511901.

FAO. org. (2021). Statistical Database/ faostat/collections. Production crop.

Farvin, K. S. & Jacobsen, C. (2013). Phenolic compounds and antioxidant activities of selected species of seaweeds from Danish coast. *Food Chemistry*, *138*(2-3), 1670-1681. https://doi.org/10.1016/j.foodchem.2012.10.078.

Fornes, F., Sanchez-Perales, M. & Guardiola, J. L. (2002). Effect of a seaweed extract on the productivity of 'de Nules' Clementine mandarin and Navelina orange. *Botanica Marina*, *45*(5), 486-489. https://doi.org/10.1515/BOT.2002.051.

Foyer, C. H. & Noctor, G. (2000). Oxygen processing in photosynthesis: regulation and signalling. *New Phytologist*, *146*(3), 359-388. https://doi.org/10.1046/j.1469-8137.2000.00667.x.

Geny, L., Bernardon Mery, A., & Larrive, G. (2007). A physiological pathway source of agronomic progress: The activation of flowering hormones. An algae filtrate acts on grapevines and apple trees. *Physio Activator Technology*, 609, 37-40.

GhaffariZadeh, A., Seyed Nejad, S. M., & Gilani, A. (2015). The effect of different levels of urea fertilizer and brown seaweed extract on physiological traits and grain yield of wheat. *Crop Physiology Journal*, *7*(27), 69-83.

Heath, R. L., & Packer, L. (1968). Photoperoxidation in isolated chloroplasts. *Archives Biochemistry Biophysics*, *125*, 850-857. https://doi.org/10.1016/0003-9861(68)90654-1.

Hu, L. Y., Hu, S. L., Wu, J., Li, Y. H., Zheng, J. L, Wei, Z. J., Liu, J., Wang, H. L., Liu, Y. S., & Zhang, H. (2012). Hydrogen sulfide prolongs postharvest shelf life of strawberry and plays an antioxidative role in fruits. *Journal of Agricultural and Food Chemistry*, *60*, 8684-8693. https://doi.org/10.1021/jf300728h.

Hurtado, A. Q., Yunque, D. A., Tibubos, K., & Critchley, A. T. (2009). Use of Acadian marine plant extracts powder from *Ascophyllum nodosum* in tissue culture of Kappaphycus varieties. *Journal of Applied Phycology*, *21*(6), 633-639. https://doi.org/ 10.1007/s10811-008-9395-4.

Jayaraman, J., Norrie, J., & Punja, Z. (2011). Commercial extract from the brown seaweed *Ascophyllum nodosum* reduces fungal diseases in greenhouse cucumber. *Journal of Applied Phycology*, *23*(3), 353-361. https://doi.org/10.1007/s10811-010-9547-1.

Keyrouz, R., Abasq, M. L., Le Bourvellec, C., Blanc, N., Audibert, L., ArGall, E., & Hauchard, D. (2011). Total phenolic contents, radical scavenging and cyclic voltammetry of seaweeds from Brittany. *Food Chemistry*, *126*, 831–836. https://doi.org/10.1016/j.foodchem.2010.10.061.

Kindleysides, S., Quek, S. Y., & Miller, M. R. (2012). Inhibition of fish oil oxidation and the radical scavenging activity of New Zealand seaweed extracts. *Food Chemistry*, *133*(4), 1624-1631. https://doi.org/10.1016/j.foodchem.2012.02.068.

Lai, A., Santangelo, E., Soressi, G. P., & Fantoni, R. (2007). Analysis of the main secondary metabolites produced in tomato (*Lycopersicon esculentum*, Mill) epicarp tissue during fruit ripening using fluorescence techniques. *Postharvest Biology and Technology*, *43*(3), 355-342. https://doi.org/10.1016/j.postharvbio.2006.09.016.

Lim, C. C., Arora, R., & Townssenal, E. C. (1998). Comparing comports and richards function to estimate freezing injury in rhododendron using electrolyte leakage. *Journal of the American Society for Horticultural Science*, *123*(2), 246-252. https://doi.org/10.21273/JASHS.123.2.246.

Luo, Z. S., Wu, X., Xie, Y., & Chen, C. (2012). Alleviation of chilling injury and browning of postharvest bamboo shoot by salicylic acid treatment. *Food Chemistry*, *131*(2), 456-61. https://doi.org/10.1016/j.foodchem.2011.09.007.

Malkouti, M. J., & Homai, M. (2005). Fertility of soils in arid and semi-arid regions (problems and solutions) Tehran: Tarbiat Modares University, Scientific Publication Office. (In Persian).

Marschener, P. (2022). *Marschner's mineral nutrition of higher plants*. Third Edition, Academic Press is an imprint of Elsevier.

Masny, A., Basak, A., & Zurawicz, E. (2004). Effect of foliar application of Kelpak and Goemar BM 86 preparations on yield and fruit quality in two strawberry cultivars. *Journal of Fruit and Ornamental Plant Research*, *12*, 23-27.

Nikoogoftar-Sedghi, M., Rabiei, V., Razavi, F., Molaei, S., & Khadivi, A. (2023). The effect of foliar application of *Ascophyllum nodosum* (L.) Le Jol. seaweed extract on biochemical traits related to abiotic stresses in pistachio (*Pistacia vera* L. cv. Kaleh-Ghoochi). *BMC Plant Biology*, *23*(1), 635. https://doi.org/10.1186/s12870-023-04654-5.

Radotic, K., Ducic, T., & Mutavdzic, D. (2000). Changes in peroxidase activity and isoenzymes in spruce needles after exposure to different concentrations of cadmium. *Environmental and Experimental Botany*, *44*(2), 105-113. https://doi.org/10.1016/s0098-8472(00)00059-9.

Ramezanian, A., Rahemi, M., & vazifehshenas, M. R. (2009). Effects of foliar application of calcium chloride and urea on quantitative and qualitative characteristics of pomegranate fruits. *Scientia Horticulturae*, *121*(2), 171-175. https://doi.org/10.1016/j.scienta.2009.01.039.

Rana, J., & Paul, J. (2017). Consumer behavior and purchase intention for organic food: A review and research agenda. *Journal of Retailing and Consumer Services*, *38*, 157-165. https://doi.org/10.1016/j.jretconser.2017.06.004.

Rathore, S. S., Chaudhary, D. R., Boricha, A., Ghosh, B. P., Bhatt, S. T., & Zodape, J. S. (2008). Effect of seaweed extract on the growth, yield and nutrient uptake of soybean (*Glycine max*) under rainfed conditions. *South African Journal of Botany*, *75*, 351–355. https://doi.org/10.1016/j.jretconser.2017.06.004.

Rayorath, P., Benkel, B., Hodges, D. M., Allan-Wojtas, P., MacKinnon, S., Critchley, A. T., & Prithiviraj, B. (2009). Lipophilic components of the brown seaweed, *Ascophyllum nodosum*, enhance freezing tolerance in Arabidopsis thaliana. *Planta*, *230*, 135–147. https://doi.org/ 10.1007/s00425-009-0920-8.

Rui, H., Cao, S., Shang, H., Jin, P., Wang, K., & Zheng, Y. (2010). Effects of heat treatment on internal browning and membrane fatty acid in loquat fruit in response to chilling stress. *Journal of the Science of Food and Agriculture*, *90*(9), 1557-1561. https://doi.org/10.1002/jsfa.3993.

Sharom, M., Willemot, C., & Thompson, J. E. (1994). Chilling injury induces lipid phase changes in membranes of tomato fruit. *Plant Physiology*, *105*(1), 305-308. https://doi.org/10.1104/pp.105.1.305.

Shewfelt, R. L. & Purvis, A. C. (1995). Toward a comprehensive model for lipid peroxidation in plant tissue disorders. *Horticulture Science*, *30*(2), 213-218.

Starck, Z. (2005). Application of growth regulators and biostimulators in modern plant cultivation (in Polish). Rolnik Dzierawca. *Growing Assistant*, 2, 74-86.

Wang, L., Bokhary, S. U. F., Xie, B., Hu, S., Jin, P., & Zheng, Y. (2019). Biochemical and molecular effects of glycine betaine treatment on membrane fatty acid metabolism in cold stored peaches. *Postharvest Biology and Technology*, *154*, 58–69. https://doi.org/10.1016/j.postharvbio.2019.04.007.

Westerman, R. L. (1990). *Soil testing and plant analysis*. 3rd Edition, Soil Science Society of America Book Series, Number 3, Madison, Wisconsin, USA.

Zasoski, R. J., & Burau, R. G. (1977). A rapid nitric perchloric acid digestion method for multielement tissue analysis. *Communications in Soil Science and Plant Analysis*, *8*(5), 425-436. https://doi.org/10.1080/00103627709366735.

Zhang, Z., Huber, D. J., & Rao, J. (2013). Antioxidant systems of ripening avocado (*Persea americana* Mill.) fruit following treatment at the pre climacteric stage with aqueous 1-methylcyclopropene. *Postharvest Biology and Technology*, *76*, 58–64. https://doi.org/10.1016/j.postharvbio.2012.09.003.

Zhang, Z., Huber, D. J., Qu, H., Yun, Z., Wang, H., Huang, Z., Huang, H., & Jiang, Y. (2015). Enzymatic browning and antioxidant activities in harvested litchi fruit as influenced by apple polyphenols. *Food Chemistry*, *171*, 191-199. https://doi.org/10.1016/j.foodchem.2014.09.001.

Zhou, J., Wang, K., Shi, X., Xia, Y., J. Zhouand, J., & Yu. Q. (2012). Hydrogen peroxide is involved in the cold acclimation-induced chilling tolerance of tomato plants. *Plant Physiology and Biochemistry*, *60*, 141-149. https://doi.org/10.1016/j.plaphy.2012.07.010.

Zodape, S.T. (2001). Seaweeds as a bio fertilizer. *Journal of Scientific and Industrial Research*, *60*, 378–389.

JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH
2024, VOL. 7(1), 31-44

Journal of Horticulture and Postharvest Research

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

University of Birjand

Salinity tolerance of five ornamental species from the Asteraceae family in seed germination and early seedling growth stages

Maryam Davoudi[1] and Hassan Bayat[1*]

[1]*Department of Horticultural Science, College of Agriculture, University of Birjand, Birjand, Iran*

ARTICLE INFO

Original Article

Article history:
Received 14 October 2023
Revised 18 December 2023
Accepted 9 January 2024

Keywords:
Ageratum
Coreopsis
Radicle
Seed vigor
Sodium chloride

DOI: 10.22077/jhpr.2024.6778.1332
P-ISSN: 2588-4883
E-ISSN: 2588-6169

***Corresponding author:**

Department of Horticultural Science, College of Agriculture, University of Birjand, Birjand, Iran.

Email: hassanbayat@birjand.ac.ir, hassanbayat55@gmail.com

ABSTRACT

Purpose: Seed germination and seedling growth are recognized as the most sensitive stages of salinity for the majority of plant species. This experiment aimed to characterize the impact of various salinity levels on selected ornamental plants from the Asteraceae family including *Gazannia splendens*, *Rudbeckia hirta*, *Ageratum houstonianum, Gaillardia aristata*, and *Coreopsis grandiflora* during the seed germination stage. **Research method**: Five independent experiments were conducted in a completely randomized design (CRD), comprising seven treatments with different concentrations of sodium chloride (0, 20, 40, 60, 80, 100, and 120 mM) in four replications using Petrie plates. **Findings:** The salinity of 120 mM significantly reduced the seed germination percentage in *G. splendens, G. aristata,* and *C. grandiflora,* compared to the other species. *A. houstanianum* exhibited a stimulating effect on seed germination percentage with salinity levels ranging from 20 mM to 80 mM. Salinity stress at a concentration of 120 mM had negatively affected vegetative traits in *A. houstonianum, R. hirta, G. aristata*, and *C. grandiflora,* inhibiting the growth of plumule in *A. houstonianum and C. grandiflora.* However, traits like plumule length and seedling length in *G. splendens* remained unaffected by salinity. **Research limitations:** No limitations were identified. **Originality/Value**: Results demonstrate varied responses of ornamental plants to different salinity levels. *R. hirta* exhibited superior performance in seed germination and early seedling stages compared to other plants.

INTRODUCTION

Soil salinity is a serious abiotic stress limiting agricultural yield in arid and semi-arid areas (FAO, 2019). Approximately 20% of the world's agricultural land has saline and or sodium soils, with 25% to 30% of irrigated land being commercially unproductive because of the soil salinity. Salt stress affects plants differently across the developmental stages (Shahid et al., 2018). Understanding the threshold of salt damage at all developmental stages is crucial for studying salt tolerance during different growth phases (Hannachi & Van Labeke, 2018).

In numerous plants, the most sensitive stages to salinity stress are seed germination and seedling growth (Li et al., 2020). Plants are particularly vulnerable to environmental stress during these stages, making the sensitivity of plant species to high salt concentrations in the soil critical for successful plant establishment (Yasemin et al., 2020). Salt stress induces toxic effects in germinating seeds, significantly reducing seed germination, seedling growth, and establishment and root and shoot length under high salinity stress (Murillo-Amador et al., 2002; Liang et al., 2018; Saadatfar et al., 2023).

Seasonal flowers may encounter stress during their growth and development. The level of salt tolerance of ornamental plants depends on the species, their development, salt stress level, and environmental factors (Guo et al., 2022). Salinity diminishes growth indices in various ornamental flowers, including snapdragon, sage, petunia, violet (Villarino & Mattson, 2011), zinnia (Bizhani et al., 2013), cockscomb (Carter et al., 2005) and in marigold (Chaparzadeh et al., 2004). *Carthamus tinctorius* has been suggested as a suitable crop for saline-stressed soils, exhibiting the most significant germination (30%) at the maximum salinity level (32 dS/m) (Green et al., 2019). Gola et al. (2019) investigated the effect of salt stress at different levels of 0, 1, 3, 5, and 7 dS/m on the *G. aristata* plant and showed that salinity negatively affected seed germination and seeding growth. Zivdar et al. (2011) reported that the percentage of seed germination, germination rate, and radicle length decreased in zinnia under salinity stress. Aboutalebi Jahromi and Hosseini Farahi (2016) demonstrated that different salinity concentrations (0, 2, 4, 6, and 8 mmhos/cm) significantly decreased the parameters like plant length, number of lateral branches, fresh and dry weight of radicle, fresh and dry weight of plumule in French marigold plant. Salinity stress at different levels (0, 2, 4, 6, and 8 dS/m) reduced some biochemical characteristics including net photosynthesis, chlorophyll content, and soluble proteins while increasing proline and malondialdehyde (MDA) in sunflowers (*Helianthus annuus* L.*)* genotypes (Morsali Aghajari et al., 2020). In landscape design, annual and perennial ornamental flowers play a crucial role, offering a variety of colors and shapes (Dole & Wilkins, 2005). The Asteraceae family holds significant economic importance because of considerable advantageous species suited to marginal stressed lands (Garcia-Oliveira et al., 2021). *Gazannia splendens* from the Asteraceae family, native to Africa, serves as a perennial plant but is used annually in temperate regions because of winter cooling (Salehi et al., 2014). Gloriosa daisy (*Rudbeckia hirta),* is an annual summer flower that has copious value in the landscape because of the extended flowering period (Mcdonald, 2002). Gaillardia *(Gaillardia aristata)* is an ornamental flower with a height of 30 to 50 cm. The flowers are single or double with yellow, red, or brown color. This flower is one of the hardiest garden plants that have annual and perennial varieties. *G. aristata*'s flowering period is relatively long and lasts from early summer to mid-autumn (Gawade, 2018). Ageratum (*Ageratum houstonianum)* is a summer ornamental plant native to Central and South America and usually planted on the edges of gardens or parks (Wiedenfeld & Andrade-Cetto, 2001). *Coreopsis grandiflora*, a perennial ornamental flower, exceeds 60 cm in height, producing yellow ray and disc flowers (Dole & Wilkins, 2005).

Given the critical role of salinity in seed germination and seeding growth, this study aims to evaluate the effects of different concentrations of NaCl on seed germination and seeding growth stages in several selected ornamental plants from the Asteraceae family.

MATERIALS AND METHODS

Plant materials and germination assays

Seeds of *G. splendens* cv.'Sundance Red', *R. hirta* cv. 'Indian Summer', *A. houstonianum* cv. 'Blue Puffs', *G. aristate* cv. 'SpinTop Mango', and *C. grandiflora* cv. 'Early Sunrise', was purchased from Agriplus Co, Iran. The germination study was conducted under laboratory conditions. Seeds were sterilized for 20 minutes in a sodium hypochlorite solution (5%), and then they were rinsed three times with distilled water for two minutes. After sterilization, 20 seeds of each cultivar were randomly distributed into a Petrie plate on Watman filter paper and then wetted with 10 ml distilled water, or intended solutions were added to each Petrie plate depending on the treatment After closing the Petri plates with para-film, they were placed in the growth chamber at a temperature of 25°C. Seeds were considered germinated if their radicle length was at least two mm or more (Allahverdi et al., 2012)

Salinity stress treatments and experimental design

The experiment was implemented in a completely randomized design (CRD) with four replications in the Horticulture Laboratory of the Faculty of Agriculture, University of Birjand, Iran, in 2022. Irrigation water salinity stress was imposed at seven levels: zero (distilled water), 20, 40, 60, 80, 100, and 120 mM sodium chloride, with electrical conductivities equal to 0.6, 1.82, 3.65, 5.48, 7.31, 9.14, and 10.96 dS/m respectively.

Studied parameters

Various traits, including germinated seeds, radicle length, and plumule length were evaluated at 24-hour intervals up to day nine. At the end of the germination test (10th day), five randomly selected seedlings were taken from each Petrie plate to measure radicle and plumule lengths (mm) and radicle and plumule fresh weights (mg). Radicle weight, plumule weight, and dry weight of seedlings were measured using a sensitive scale with an accuracy of 0.0001 grams. Radicle and plumule length were measured with a ruler. Seed germination rate was measured using Maguire's method (Maguire, 1962) according to the following formula (1):

$$GR = \Sigma Ni / Di \quad (1)$$

Where: GR: germination rate (number of seeds per day)
Ni: number of germinated seeds per count
Di: number of days until the i^{th} count
Germination percentage was calculated according to the following formula (2):

$$GP = (Ni/N)\times 100 \quad (2)$$

In this formula, GP is the percentage of germination, Ni is the number of germinated seeds till i^{th} day, and N is the total number of seeds (Bajji et al., 2002).
The mean germination time was calculated based on the following equation (3) (Ruan et al., 2002):

$$MGT=\Sigma (D\times N)/\Sigma N \quad (3)$$

In this equation, MGT is the mean germination time, N is the number of seeds that germinated till the D^{th} day, and D is the number of days that have passed since the beginning of the germination time.
The seed vigor index (SV) was obtained by multiplying the sum of radicle length (RL), and plumule length (PL) by germination percentage (GP) according to the following equation (4) (Bajji et al., 2002).

$$SV=(PL+RL)\times GP \quad (4)$$

Data analysis
Data were subjected to analysis of variance (ANOVA) using JMP 13 software (SAS Campus, Cary, NC, USA) and a mean comparison was conducted with the LSD test at a 5% probability level.

RESULTS

G. splendens
The analysis of variance (Table 1) showed that, except for plumule and seedling length, which were not significant, the other investigated traits showed a significant difference at the 1% probability level. The comparison of mean data (Table 2) demonstrated that with the increase in salt concentration, the percentage and rate of seed germination decreased. The lowest seed germination percentage (6.25%) and rate (1 seed per day) were observed in 120 mM treatment. The mean germination time at 80 mM concentration showed a significant increase compared to the control. The highest (2.59) and the lowest (0.21) seed germination index was observed in the control and 120 mM NaCl treatments, respectively. The increase in salinity concentration decreased the fresh and dry weight of the radicle. So the lowest fresh and dry weight of the radicle (0.007 and 0.0004 grams) was obtained from the 120 mM treatment. The control treatment had the highest fresh (0.0207 g) and (0.0015 g) dry weights of the plumule, and the lowest fresh weight (0.005 g) and dry weight (0.0002 g) of plumule was obtained from 120 mM treatment. The highest salinity level decreased radicle length by 42.28% compared to the control (Table 2).

R. hirta
Variance analysis of the obtained data (Table 1) revealed that the effect of salinity levels was significant at the 1% probability level for all investigated traits. The comparison of mean data (Table 2) showed that the percentage of seed germination at salinity levels of 20, 40, 60, and 80 mM did not differ significantly from the control. However, with an increase in the salinity level to 100 and 120 mM, a striking decrease in seed germination percentage was observed. The seed germination rate descended with an increase in salinity level, reducing by 67 and 55% at the levels of 100 and 120 mM, respectively, compared to the control. At 100 and 120 mM salinity levels, there was a 58 and 44% increase in mean germination time, respectively, compared to the control, while the rest of the salinity levels did not show any significant difference from the control. The highest (5.22) and lowest (0.16) seed germination index were obtained from the control and 120 mM NaCl treatment, respectively. The highest radicle (0.015 g) and plumule (0.0096 g) fresh weights were obtained from 20 mM NaCl treatment, whereas the lowest fresh weight of radicle (0.009 g) and plumule (0.004 g) were obtained from 120 mM NaCl treatment. Also, the highest and the lowest dry weight of the radicle (0.001 g and 0.0007 g) were obtained from the control and 120 mM NaCl concentrations, respectively. The highest and the lowest dry weight of the plumule (0.0009 g and 0.00032 g)

were recorded from the control and 100 mM NaCl concentrations, respectively. Radicle length decreased with increasing salinity, reaching a 98% decrease at 100 mM NaCl compared to the control. Plumule length decreased by 78% in the 120 mM treatment compared to the control. Seedling length also declined by 94% at 100 mM NaCl compared to the control (Table 2).

Table 1. Variance analysis of salinity stress effects on germination characteristics of some ornamental plants of the Asteraceae family.

	Mean of squares							
G. splendens	Source of variance	df	Germination percentage	Germination rate	Mean germination time	Seed vigor index	Radicle fresh weight	Radicle dry weight
	Salinity	6	943.24^{**}	25.01^{**}	0.77^{**}	2.07^{**}	$6.74e^{-7**}$	$1.99e^{-6**}$
	Error	21	59.89	3.12	0.18	0.25	0.0003	$4.58e^{-8}$
	Source of variance	df	Plumule fresh weight	Plumule dry weight	Radicle length	Plumule length	Seedling length	
	Salinity	6	0.00018^{**}	1.01^{**}	1.25^{**}	0.084^{ns}	1.77^{ns}	
	Error	21	$17.34e^{-6}$	$2.1e^{-8}$	0.31	0.064	0.96	
R. hirta	Source of variance	df	Germination percentage	Germination rate	Mean germination time	Seed vigor index	Radicle fresh weight	Radicle dry weight
	Salinity	6	112.81^{**}	18.74^{**}	0.90^{**}	15.83^{**}	0.00002^{**}	$5.59e^{-8**}$
	Error	21	861.3	1.52	0.088	0.21	$1.38e^{-6}$	$6.52e^{-9}$
	Source of variance	df	Plumule fresh weight	Plumule dry weight	Radicle length	Plumule length	Seedling length	
	Salinity	6	$9.22e^{-6**}$	$1.42e^{-7**}$	20.76^{**}	0.23^{**}	25.01^{**}	
	Error	21	$1.03e^{-6}$	$1.58e^{-8}$	0.05	0.0019	0.05	
A. houstonianum	Source of variance	df	Germination percentage	Germination rate	Mean germination time	Seed vigor index	Radicle fresh weight	Radicle dry weight
	Salinity	6	502.97^{**}	25.26^{**}	0.59^{*}	2.47^{**}	$1.38e^{-6**}$	$2.44e^{-10**}$
	Error	21	38.09	2.04	0.17	0.04	$3.13e^{-8}$	$5.86e^{-9}$
	Source of variance	df	Plumule fresh weight	Plumule dry weight	Radicle length	Plumule length	Seedling length	
	Salinity	6	$2.56e^{-8**}$	$1.40e^{-10**}$	0.02^{**}	0.33^{**}	5.86^{**}	
	Error	21	$7.58e^{-10}$	$5.67e^{-12}$	4.98	0.004	0.03	
G. aristata	Source of variance	df	Germination percentage	Germination rate	Mean germination time	Seed vigor index	Radicle fresh weight	Radicle dry weight
	Salinity	6	1926.69^{**}	37.88^{**}	2.51^{**}	8.44^{**}	0.0002^{**}	1926.69^{**}
	Error	21	144.58	4.03	5.41	0.46	5.00001	$5.17e^{-8}$
	Source of variance	df	Plumule fresh weight	Plumule dry weight	Radicle length	Plumule length	Seedling length	
	Salinity	6	37.88^{**}	2.51^{**}	8.44^{**}	0.0002^{**}	1926.69^{**}	
	Error	21	0.000014	$7.52e^{-8}$	0.23	0.008	0.29	
C. grandiflora	Source of variance	df	Germination percentage	Germination rate	Mean germination time	Seed vigor index	Radicle fresh weight	Radicle dry weight
	Salinity	6	3189.58^{**}	48.5^{**}	0.83^{**}	4.72^{**}	$6.74e\text{-}6^{**}$	$6.17e\text{-}8^{**}$
	Error	21	21.46	11.98	0.17	0.105	2.2e-7	1.36e-8
	Source of variance	df	Plumule fresh weight	Plumule dry weight	Radicle length	Plumule length	Seedling length	
	Salinity	6	3.6^{**}	$2.82e\text{-}9^{**}$	4.14^{**}	0.25^{**}	6.23^{**}	
	Error	21	4.11e-7	4.12e-11	0.11	0.006	0.16	

** Significance at the 1% probability level, * Significance at the 5% probability level, and ^{ns} is non-significance.

Table 2. Mean comparison of salinity effects on seed germination properties of *G. splendens* and *R. hirts.*

G. splendens

Salinity (mM)	Germination percentage (%)	Germination rate (seed per day)	Mean germination time (seed per day)	Seed vigor index	Radicle fresh weight (g)	Radicle dry weight (g)
0	57.5^{a}	9.16^{a}	1.61^{c}	2.59^{a}	0.035^{a}	0.0025^{a}
20	41.25^{b}	4.5^{b}	2.24^{abc}	1.73^{b}	0.034^{a}	0.0022^{b}
40	36.25^{b}	4.09^{b}	2.31^{ab}	1.13^{b}	0.024^{b}	0.0018^{c}
60	36.66^{b}	5.48^{b}	1.93^{bcd}	1.1^{b}	0.022^{bc}	0.0017^{c}
80	37.5^{b}	3.35^{bc}	2.83^{a}	1.38^{b}	0.019^{cd}	0.0012^{d}
100	42.5^{b}	5.75^{b}	1.96^{bcd}	1.44^{b}	0.017^{d}	0.001^{d}
120	6.25^{c}	1.00^{c}	1.5^{d}	0.21^{c}	0.007^{e}	0.0004^{e}

Salinity (mM)	Plumule fresh weight (g)	Plumule dry weight (g)	Radicle length (cm)	Plumule length (cm)	Seedling length (cm)
0	0.0207^{a}	0.0015^{a}	3.24^{a}	1.05^{a}	4.29^{a}
20	0.0205^{a}	0.0014^{a}	3.31^{a}	0.9^{a}	4.21^{a}
40	0.0130^{b}	0.001^{b}	3.30^{a}	0.72^{a}	4.02^{a}
60	0.0202^{a}	0.0014^{a}	3.32^{a}	0.67^{a}	4.00^{a}
80	0.008^{c}	0.0007^{c}	3.07^{a}	0.67^{a}	3.75^{a}
100	0.007^{c}	0.0006^{c}	2.60^{ab}	0.77^{a}	3.37^{a}
120	0.005^{c}	0.0002^{d}	1.87^{b}	0.66^{a}	2.53^{a}

R. hirta

Salinity (mM)	Germination percentage (%)	Germination rate (seed per day)	Mean germination time (seed per day)	Seed vigor index	Radicle fresh weight (g)	Radicle dry weight (g)
0	83.75^{a}	9.02^{a}	2.22^{b}	5.22^{a}	0.014^{ab}	0.001^{a}
20	70.00^{ab}	7.63^{ab}	2.29^{b}	4.38^{b}	0.015^{a}	0.0009^{a}
40	72.50^{a}	7.11^{b}	2.37^{b}	3.79^{b}	0.013^{bc}	0.0009^{ab}
60	82.50^{a}	7.37^{ab}	2.44^{b}	4.31^{b}	0.013^{bc}	0.0008^{bc}
80	81.25^{a}	7.36^{ab}	2.36^{b}	2.81^{c}	0.012^{c}	0.0008^{bc}
100	56.25^{bc}	4.04^{c}	3.20^{a}	0.36^{b}	0.01^{d}	0.0007^{cd}
120	45.00^{c}	3.00^{c}	3.40^{a}	0.16^{d}	0.009^{d}	0.0007^{d}

Salinity (mM)	Plumule fresh weight (g)	Plumule dry weight (g)	Radicle length (cm)	Plumule length (cm)	Seedling length (cm)
0	0.009^{ab}	0.0009^{a}	5.28^{a}	0.93^{a}	6.21^{a}
20	0.0096^{a}	0.0006^{b}	5.37^{a}	0.86^{b}	6.23^{a}
40	0.008^{ab}	0.00055^{bc}	4.42^{b}	0.80^{b}	5.22^{b}
60	0.007^{b}	0.00055^{bcd}	4.53^{b}	0.69^{c}	5.22^{b}
80	0.008^{ab}	0.00050^{bcd}	2.82^{c}	0.65^{c}	3.47^{c}
100	0.007^{b}	0.00032^{d}	0.12^{d}	0.52^{d}	0.65^{d}
120	0.004^{c}	0.00037^{cd}	0.16^{d}	0.20^{e}	0.36^{d}

In each column, the means with at least one similar letter are not significant based on the LSD test at the 5% probability level.

A. houstonianum

Variance analysis of the obtained data (Table 1) demonstrated that the effect of salinity levels on all investigated traits was significant at the 1% and 5% probability levels. The comparison of mean data (Table 3) showed that the highest seed germination percentage (75.75%) was obtained from 80 mM NaCl concentration, while the lowest (43.75%) was observed at 120 mM concentration. The 60 mM NaCl concentration had the highest seed germination rate, which did not show a statistically considerable difference with 80 mM NaCl concentration. The lowest and highest mean germination times were related to 40 and 120 mM salinity, respectively. With the increase in salinity, the seed germination index significantly decreased, so the control and 20 mM salinity with 2.24 had the highest and 120 mM had the lowest seed germination index. With increasing salinity, the fresh and dry weight of radicles decreased from 0.0021 and 0.00014 g in control to 0.0004 and 0.00003 g at 120 mM salinity, respectively. The highest fresh weight (0.00019 g) and dry weight (0.000000 g) of the plumules were obtained from the 60 mM NaCl concentration, showing no significant difference from the control treatment, while the lowest was obtained at 120 mM NaCl.

Treatment with 120 mM salinity reduced the length of the radicle and seedling by 85% and 87%, respectively, compared to the control (Table 3).

G. aristata

Variance analysis of the obtained data (Table 1) showed that the effect of salinity levels on all investigated traits was significant. The comparison of mean data (Table 3) revealed that with increasing salinity levels, seed germination percentage and rate decreased. Treatment with 120 mM salinity decreased the seed germination percentage and germination rate by 60 and 96%, respectively, compared to the control. The mean germination time did not show a significant difference from the control to 100 mM NaCl, while it showed a considerable increase when the control compared to the salinity level of 120 mM NaCl. The highest seed vigor index (3.93) was related to the control treatment, and the lowest (0.007) was observed in the 120 mM NaCl treatment. The lowest fresh and dry weights of radicle were observed at 120 mM salinity level. The fresh and dry weight of the plumule decreased with increasing salinity levels, although in some cases, this decrease did not show a significant difference compared to the control. The length of radicle and seedling at salinity levels of 20, 40, 60, and 80 mM NaCl did not differ significantly compared to the control but showed a noticeable decline at higher salinity levels. With the increase in salinity concentration, the plumule length decreased, and the lowest value (0.11 cm) was obtained from the 120 mM treatment (Table 3).

C. grandiflora

Variance analysis of the obtained data (Table 1) demonstrated that the effect of salinity levels on all investigated traits was significant. The comparison of mean data (Table 4) revealed that the seed germination percentage and rate decreased with increasing salinity. The highest mean germination time was obtained from the level of 80 mM NaCl. The seed vigor index showed no significant difference with the control up to the salinity level of 40 mM, but other levels illustrated a significant difference with the control. The lowest seed seed vigor index was obtained at the salinity of 120 mM. As salinity levels increased, the fresh and dry weights of radicle and plumule decreased, reaching the lowest values for these traits at 120 mM salinity. With increasing salinity, the length of the radicle, plumule, and seedling respectively decreased from 3, 0.8, and 3.8 cm in the control to 0.7, 0.0, and 0.7 cm in 120 mM salinity (Table 4).

Table 3. The effects of salinity stress on the seed germination characteristics of *A. houstonianum* and *G. aristata*.

A. houstonianum

Salinity (mM)	Germination percentage (%)	Germination rate (seed per day)	Mean germination time (seed per day)	Seed vigor index	Radicle fresh weight (g)	Radicle dry weight (g)
0	58.57^{bc}	8.52^{b}	1.88^{ab}	2.24^{a}	0.0021^{a}	0.00014^{a}
20	67.50^{ab}	10.35^{ab}	1.69^{bc}	2.24^{a}	0.0018^{b}	0.00012^{ab}
40	53.75^{bc}	9.68^{ab}	1.26^{c}	1.56^{b}	0.0016^{bc}	0.00011^{b}
60	67.5^{ab}	11.57^{a}	1.45^{bc}	1.55^{b}	0.0014^{c}	0.0001^{b}
80	73.75^{a}	11.11^{a}	1.85^{abc}	1.31^{b}	0.0011^{d}	0.00008^{c}
100	47.5^{de}	6.3^{c}	2.06^{ab}	0.48^{c}	0.0009^{d}	0.00007^{c}
120	43.75^{e}	4.78^{c}	2.43^{a}	0.21^{c}	0.0004^{e}	0.00003^{d}

Salinity (mM)	Plumule fresh weight (g)	Plumule dry weight (g)	Radicle length (cm)	Plumule length (cm)	Seedling length (cm)
0	0.00019^{a}	0.00001^{5a}	3.47^{a}	0.35^{b}	3.82^{a}
20	0.00016^{ab}	0.000013^{ab}	2.97^{b}	0.32^{bc}	3.30^{b}
40	0.00012^{bc}	0.00001^{bc}	2.65^{c}	0.25^{cd}	2.9^{c}
60	0.00019^{a}	0.000014^{a}	1.55^{d}	0.90^{a}	2.45^{d}
80	0.00008^{c}	0.000008^{c}	1.4^{d}	0.22^{d}	1.77^{e}
100	0.00001^{d}	0.000003^{d}	0.92^{e}	0.1^{e}	1.02^{f}
120	0.0 ^{d}	0.0^{d}	0.5^{f}	0.0 ^{f}	0.5^{g}

G. aristata

Salinity (mM)	Germination percentage (%)	Germination rate (seed per day)	Mean germination time (seed per day)	Seed vigor index	Radicle fresh weight (g)	Radicle dry weight (g)
0	66.25^{a}	10.05^{a}	1.64^{b}	3.93^{a}	0.03^{a}	0.002^{a}
20	63.75^{a}	6.13^{b}	2.32^{b}	3.69^{a}	0.027^{ab}	0.0018^{ab}
40	45.00^{b}	5.92^{b}	1.82^{b}	2.45^{b}	0.027^{ab}	0.0016^{bc}
60	45.00^{b}	5.55^{b}	2.31^{b}	2.36^{b}	0.023^{bc}	0.0015^{bc}
80	41.66^{b}	4.66^{bc}	2.15^{b}	2.17^{b}	0.02^{c}	0.0013^{cd}
100	18.75^{c}	2.3^{cd}	2.24^{b}	0.56^{c}	0.11^{d}	0.001^{d}
120	6.25^{c}	0.36^{d}	4.33^{a}	0.037^{c}	0.007^{d}	0.0005^{e}

Salinity (mM)	Plumule fresh weight (g)	Plumule dry weight (g)	Radicle length (cm)	Plumule length (cm)	Seedling length (cm)
0	0.018^{b}	0.0011^{ab}	4.69^{a}	1.22^{a}	5.92^{a}
20	0.018^{b}	0.0010^{ab}	4.76^{a}	0.97^{b}	5.73^{a}
40	0.019^{b}	0.0010^{ab}	4.63^{a}	0.87^{bc}	5.51^{a}
60	0.02^{a}	0.0013^{a}	4.57^{a}	0.76^{cd}	5.34^{a}
80	0.0015^{b}	0.001^{a}	4.52^{a}	0.69^{d}	5.21^{a}
100	0.009^{c}	0.0008^{b}	2.49^{b}	0.52^{e}	3.01^{b}
120	0.003^{c}	0.0002^{c}	0.28^{c}	0.11^{f}	0.39^{c}

Table 4. The effects of salinity stress on the seed germination characteristics of *C .grandiflora*.

C. grandiflora

Salinity (mM)	Germination percentage (%)	Germination rate (seed per day)	Mean germination time (seed per day)	Seed vigor index	Radicle fresh weight (g)	Radicle dry weight (g)
0	71.25^{bc}	8.9^{a}	2.01^{c}	2.71^{a}	0.0045^{a}	0.00038^{a}
20	80.00^{a}	9.37^{a}	2.05^{c}	2.77^{a}	0.0044^{a}	0.00039^{a}
40	78.33^{ab}	7.67^{a}	2.49^{bc}	2.34^{a}	0.004^{ab}	0.00031^{ab}
60	65.00^{c}	7.39^{a}	2.10^{c}	1.68^{b}	0.0034^{bc}	0.00024^{ab}
80	48.75^{d}	4.02^{b}	3.17^{a}	1.03^{c}	0.003^{cd}	0.00036^{a}
100	30.00^{e}	2.65^{b}	2.96^{ab}	1.50^{d}	0.0026^{d}	0.00019^{bc}
120	2.50^{f}	0.20^{c}	2.5^{abc}	0.005^{e}	0.0008^{e}	0.00005^{c}

Salinity (mM)	Plumule fresh weight (g)	Plumule dry weight (g)	Radicle length (cm)	Plumule length (cm)	Seedling length (cm)
0	0.0009^{a}	0.000072^{ab}	3^{a}	0.8^{a}	3.8^{a}
20	0.0008^{b}	0.000075^{a}	2.92^{ab}	0.53^{b}	3.46^{a}
40	0.00077^{bc}	0.00067^{ab}	2.45^{bc}	0.4^{c}	2.85^{b}
60	0.00074^{cd}	0.000063^{b}	2.1^{cd}	0.47^{bc}	2.57^{bc}
80	0.00065^{d}	0.00005^{c}	1.63^{de}	0.47^{bc}	2.11^{cd}
100	0.0004^{e}	0.00003^{d}	1.42^{e}	0.23^{d}	1.65^{d}
120	0.0 ^{f}	0.0 ^{e}	0.7^{f}	0.0 ^{e}	0.7^{e}

In each column, the means with at least one similar letter are not significant based on the LSD test at the 5% probability level.

DISCUSSION

The percentage and rate of germination of plant seeds are crucial for an agronomic perspective. Salinity stress significantly impacts the germination and early growth of seedlings. The decline in seed germination percentage and rate, along with the increase in the time required to reach the final germination due to salinity stress, poses critical limits in semi-arid areas with limited favorable conditions around the seeds. Plants that can germinate and thrive in saline conditions play an essential role in enhancing growth and productivity (Carpýcý et al., 2009).

In this study, salinity reduced the percentage and rate of seed germination in all tested ornamental plants, and with increasing salinity, the percentage and rate of germination decreased. For instance, the salinity of 120 mM NaCl in *G. splendens*, *G. aristata*, and *C. grandiflora* significantly reduced the percentage of germination compared to other ornamental species. However, *A. houstonianum* and *R. hirta* exhibited favorable germination under salt-stress conditions. Similar findings were reported by Gholizadeh et al., (2016), indicating that increased salinity led to the lowest germination percentage of *Echinacea purpurea* seeds obtained from a salinity of 12 dS/m. The germination rate of Zinnia decreased from 92.17% to 58% with an increase in salinity from zero to 12 dS/m (Zivdar et al., 2011), aligning with the observed trends in this study. Numerous studies on sunflowers also support the correlation between increased salinity stress and decreased germination rate (Hafeez et al., 2017; Kaya et al., 2019; Li et al., 2020). The reduction in seed germination with increased salt concentration is attributed to physicochemical and the toxic-osmotic effects of the solutes in the saline solution. In fact, with the increase in osmotic pressure (more negative osmotic potential), which results from the increase in salinity, the seed water absorption stage is disturbed. Slow water absorption affects the activities within the seed, leading to an increased time for radicle emergence and a subsequent decrease in the germination rate (Ajmal Khan et al., 2006). On the other hand, the presence of a high concentration of anions and cations (especially sodium and chlorine) in the growth medium prevent seed germination by toxicity effect (Rajabi Dehnavi et al., 2020). Besides, salinity prevents the movement of essential reserve materials to the embryo (Duarte et al., 2006; Xiong et al., 2024).

The seed vigor index reflects a seed's ability to germinate and sprout after planting and to maintain this potential during storage after harvesting (Wang et al., 2018). In all the tested ornamental plants, with increasing salinity levels, the seed vigor decreased compared to the control. The further reduction of seed vigor at higher levels of salinity is most likely because of the low osmotic potential of the root that prevent water absorption, the toxicity of chlorine and sodium ions, or the disruption of enzyme activity (Qian et al., 2000; Rouhi et al., 2011).

In *C. grandiflora* and *A. houstonianum*, 120 *mM* salinity inhibited plumule growth. In addition, the fresh and dry weight of radicles and plumules in various plants decreased with increasing salinity levels. The present results are consistent with the decrease in dry weight of *G. aristata* aerial parts caused by irrigation with NaCl solution (Niu & Rodriguez, 2006). Torbaghan (2012) on marigolds reported that salinities of 3 and 10 dS/m had the highest and lowest plumule weight, respectively. With the increase in salinity level to 7 dS/m, the fresh weight of *G. aristata*'s plumule decreased (Gola et al., 2019). In another experiment, the fresh and dry weight of the *Calendula* radicle declined with a rising salinity level to 8 dS/m (Jamali et al., 2021). Also, a report demonstrated that the salinity stress of 6 dS/m decreased the fresh weight of French marigold radicle and plumule (Aboutalebi Jahromi & Hosseini Farahi, 2016). Adding NaCl to the nutrient solution reduced the dry weight of *G. splendens* plants, so that the salinity of 7.5 dS/m reduced the dry weight of the plant by 32% compared to the

salinity of 2 dS/m (García-Caparrós et al., 2017). This study's results are consistent with the findings of researchers on *Oenothera bennise* L. (Sikha et al., 2014; Moosavi et al., 2012). Salinity stress disrupts cell membranes, reduces enzyme activities, and decreases accessible water in growing plants, preventing the normal growth of radicles and plumules (Farooq & Azam, 2006). Also, salinity stress reduces the growth of plants by disrupting water absorption and toxicity caused by ions (Yokoi, 2002; Okcu et al., 2005; Taiz & Zeiger, 2006).

Except for the *G. splendens* plant, whose plumule and seedling length were not affected by salinity stress, other ornamental species decreased their radicle, plumule, and seedling length with increasing salinity. In *A. houstonianum* and *C. grandiflora* plants, salinity at 120 mM prevented the growth of the plumule. Reduction of radicle and plumule length with increasing salt stress has been proven by numerous researchers. In this regard, it has been reported that the radicle length of zinnia and Catharanthus decreased with increasing salinity levels (Marković et al., 2022). The results of Shila et al. (2016) illustrated a significant effect of salinity on the radicle length of sunflower, and the maximum radicle length was obtained from a concentration of 20 mM sodium chloride and the minimum radicle length was obtained from a concentration of 320 mM (Shila et al., 2016). Salinity changes the anatomical structure of the radicle and leads to a decrease in the number of cells in the xylem and the number of layers of the parenchyma cortex (Benidire et al., 2015). By limiting the activity of the cell wall and changing the amount of effective proteins in the wall, salt stress reduces the activity of meristems and consequently decreases cell division (Kaya et al., 2006). On the other hand, the high accumulation of salts in the cell wall changes the metabolic activities and limits the elastic property of the cell wall. In addition, the secondary cell wall is formed earlier and the cell wall becomes stiff. For this reason, the effect of osmotic pressure on cell wall elongation is less and the plumule length is reduced (Naseer et al., 2001).

CONCLUSION

According to the obtained results, the response of ornamental plants to different salinity levels varied. *R. hirta* exhibited superior performance in both seed germination and seedling growth traits than other plants. Thus, it had high germination up to an 80 mM salinity level and continued to grow at 120 mM salinity. *A. Houstonianum* showed high seed germination at 80 mM salinity, but seedlings did not thrive at 120 mM salinity. *G. splendens* and *G. aristata* did not have high seed germination, but seedling growth occurred at 60 mM salinity. *C. grandiflora* did not show proper seed germination and seedling growth at 20 mM salinity. Therefore, *R. hirta* appears to be more resistant to salt stress compared to other plants.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

Aboutalebi Jahromi, A., & Hosseini Farahi, M. (2016). Seed germination, vegetative growth, and concentration of some elements in French marigold (*Tageta patula*) as influenced by salinity and ammonium nitrate. *International Journal of Horticultural Science and Technology*, *3*(2), 199-209. https://doi.org/10.22059/ijhst.2017.212510.145

Ajmal Khan, M., Zaher Ahmed, M., & Hameed, A. (2006). Effect of salt and ascorbic acid on the seed germination of halophytes. *Journal of Arid Environments*, *67*(3), 535-540. https://doi.org/10.1016/j.jaridenv.2006.03.001

Allahverdi Mamqani, B., Sharifi Ashourabadi, I., Makizadeh Tafti, M., Hosni, J., & Bakhtiari Ramezani, M. (2012). Investigating the response to light of some thyme species in the germination stage. *Plant Environmental Physiology*, *8*, 1-16.

Bajji, M., Kine, J. M., & Lutts, S. (2002). Osmotic and ionic effect of NaCl on germination, early seedling growth and ion content of *Atriplex halimus*. *Canadian Journal of Botany*, *80*(3), 297-304. https://doi.org/10.1139/B02-008

Benidire, K., Daoui, Z., Fatemi, A., Achouak, W., Bouarab, L., & Oufdou K. (2015). Effect of salt stress on the germination and development of seedlings of *Vicia faba* L. *Journal of Materials and Environmental Science*, *6*, 840-851.

Bizhani, S., Jowkar, A., & Abdolmaleki, M. (2013). Growth and antioxidant response of *Zinnia elegans* under salt stress conditions. *Technical Journal of Engineering and Applied Sciences*, *3*(13), 1285-1292.

Carpýcý, E. B., Celýk, N., & Bayram, G. (2009). Effects of salt stress on germination of some maize (*Zea mays* L.) cultivars. *African Journal of Biotechnology*, *8*(19), 4918-4922. https://doi.org/10.4314/ajb.v8i19.65187

Carter, C. T., Grieve, C. M., Poss, J. A., & Suarez, D. L. (2005). Production and ion uptake of *Celosia argentea i*rrigated with saline wastewaters. *Scientia Horticulturae*, *106*(3), 381-394. https://doi.org/10.1016/j.scienta.2005.04.007

Chaparzadeh, N., D'Amico, M. L., Khavari-Nejad, R. A., Izzo, R., & Navari-Izzo, F. (2004). Antioxidative responses of Calendula officinalis under salinity conditions. *Plant Physiology and Biochemistry*, *42*(9), 695-701.

Dole, J. M., & Wilkins, H. F. (2005). *Floriculture. Principles and Species*. Pearson/Prentice Hall.

Duarte, G. L., Lopes, N. F., Moraes, D. M. D., & Silva, R. N. D. (2006). Physiological quality of wheat seeds submitted to saline stress. *Revista Brasileira de Sementes*, *28*, 122-126.

Farooq, S., & Azam, F. (2006). The use of cell membrane stability (CMS) technique to screen for salt tolerance wheat varieties. *Journal of Plant Physiology*, *163*(6), 629-637. https://doi.org/10.1016/j.jplph.2005.06.006

Food ana Agricultural Organization of the United Nations- FAO. (2019). Salt-affected soils. Rome, Italy. Available in: http://www.fao.org/soils-portal/soilmanagement/management-of-some-problem-soils/saltaffected-soils/en/. Access in: September, 15, 2019.

Garcia-Oliveira, P., Barral, M., Carpena, M., Gullón, P., Fraga-Corral, M., Otero, P., & Simal-Gandara, J. (2021). Traditional plants from Asteraceae family as potential candidates for functional food industry. *Food & Function*, *12*, 2850-2873. https://doi.org/10.1039/D0FO03433A

García-Caparrós, P., Llanderal, A., & Lao, M. T. (2017). Effects of salinity on growth, water-use efficiency, and nutrient leaching of three containerized ornamental plants. *Communications in Soil Science and Plant Analysis*, *48*(10), 1221-1230. https://doi.org/10.1080/00103624.2017.1341915

Gawade, N., Bhalekar, S. G., Bhosale, P., Katwate, S. M., & Wadekar, V. (2018). Studies on Different Genotypes of Gaillardia (*Gaillardia pulchella* L.) for Quantitative and Qualitative Performance. *International Journal of Current Microbiology and Applied Sciences*, *7*(3), 1030-1039. https://doi.org/10.20546/ijcmas.2018.703.123

Gholizadeh, F., Manzari-Tavakkoli, A., & Pazoki, A. (2016). Evaluation of salt tolerance on germination stage and morphological characteristics of some medicinal plants artichoke, flax, safflower and coneflower. *International Journal of Farming and Allied Sciences*, *5*(3), 229-237.

Gola, A. Q., Wahocho, M. H. U. N. A., Aziz, T., Abro, J. A., Khan, Z., Jakhro, M. I., & Bugti, G. H. (2019). 61. Effect of salt NaCl stress on seed germination and vegetative growth of *Gaillardia* (*Gaillardia puchella* L.). *Pure and Applied Biology* (PAB), *8*(1), 593-600. http://dx.doi.org/10.19045/bspab.2018.700221

Green, A. W., Meehan, M. A., & DeSutter, T. M. (2020). Seed germination of selected crop and graminoid species in response to treatment with sodium chloride and oil-field brine solutions. *Canadian Journal of Plant Science*, *100*, 495-503.

Guo, J., Shan, C., Zhang, Y., Wang, X., Tian, H., Han, G., & Wang, B. (2022). Mechanisms of salt tolerance and molecular breeding of salt-tolerant ornamental plants. *Frontiers in Plant Science*, *13*, 854116.

Hafeez, A., Arshadullah, M., Rasheed, M., Mahmood, I. A., Hyder, S. I., Aamir, S. S., Shaaban, M., & Mahmood, T. (2017). Effect of soil salinity on germination and growth of sunflower (*Helianthus annuus* L.). *Journal Innovation of Bio-Research*, *1*(1), 46-51.

Hannachi, S., & Van Labeke, M. C. (2018). Salt stress affects germination, seedling growth, and physiological responses differentially in eggplant cultivars (*Solanum melongena* L.). *Scientia Horticulturae*, *228*, 56-65.

Jamali, M. F., Jamali, F. A., Miano, T. F., Abbasi, Z. A., Otho, S. A., Talpur, K. H., ... & Jakhro, M. I. (2021). Growth and flowering response of marigold (*Tagetes erecta*) to salt stress. *Pakistan Journal of Agricultural Research*, *34*(4), 792-798.

Kaya, M. D., Akdoğan, G., Kulan, E. G., Dağhan, H. A. T. İ. C. E., & Sari, A. (2019). Salinity tolerance classification of sunflower (*Helianthus annuus* L.) and safflower (*Carthamus tinctorius* L.) by cluster and principal component analysis. *Applied Ecology and Environmental Research*, *17*(2), 3849-3857. http://dx.doi.org/10.15666/aeer/1702_38493857

Kaya, M. D., Okçu, G., Atak, M., Çıkılı, Y., & Kolsarıcı, Ö. (2006). Seed treatments to overcome salt and drought stress during germination in sunflower (*Helianthus annuus* L.). *European Journal of Agronomy*, *24*, 291-295. https://doi.org/10.1016/j.eja.2005.08.001

Li, W., Zhang, H., Zeng, Y., Xiang, L., Lei, Z., Huang, Q., ... & Cheng, Q. (2020). A salt tolerance evaluation method for sunflower (*Helianthus annuus* L.) at the seed germination stage. *Scientific Reports*, 10(1), 1-9. https://doi.org/10.1038/s41598-020-67210-3

Liang, W., Ma, X., Wan, P., & Liu, L. (2018). Plant salt-tolerance mechanism: a review. *Biochemical and Biophysical Research Communications*, *495*(1), 286– 291. https://doi.org/10.1016/j.bbrc.2017.11.043.

Maguire, J. D. (1962). Speed of germinationaid in selection and evaluation for seed vigor. *Crop Science*, *2*, 176-177. https://doi.org/10.2135/cropsci1962.0011183X000200020033x

Marković, M., Šoštarić, J., Kojić, A., Popović, B., Bubalo, A., Bošnjak, D., & Stanisavljević, A. (2022). Zinnia (*Zinnia elegans* L.) and periwinkle (*Catharanthus roseus* (L.) G. Don) responses to salinity stress. *Water*, *14*(7), 1066. https://doi.org/10.3390/w14071066

Mcdonald, E. (2002). *The 400 best garden plants*. Quantum Publishing Ltd.

Moosavi, S. G., Seghatoleslami, M. J., Jouyban, Z., & Ansarinia, E. (2012). Germination and growth parameters of seedlings of *Oenothera biennis* L. as affected by salinity stress. *Technical Journal of Engineering and Applied Sciences*, *2*(5), 123-127.

Morsali Aghajari, F., Darvishzadeh, R., & Razi, M. (2020). The effects of sodium chloride stress on some biochemical characteristics and antioxidative enzyme activities in two sunflowers (*Helianthus annuus* L.) genotypes. *Journal of Plant Molecular Breeding*, *8*(2), 10-20. https://doi.org/10.22058/jpmb.2022.533895.1237

Murillo-Amador, B., López-Aguilar, R., Kaya, C., Larrinaga-Mayoral, J., & Flores-Hernández, A. (2002). Comparative effects of NaCl and polyethylene glycol on germination, emergence and seedling growth of cowpea. *Journal of Agronomy and Crop Science*, *188*(4), 235–247. https://doi.org/10.1046/j.1439-037X.2002.00563.x.

Naseer, Sh., Nisar, A., & Ashrsf. M. (2001). Effect of salt stress on germination and seedling growth of barley (*Hordeum vulgare* L.). *Pakistan Journal of Biological Science*, *4*, 359-360. https://doi.org/10.3923/pjbs.2001.359.360

Niu, G., & Rodriguez, D. S. (2006). Relative salt tolerance of five herbaceous perennials. *Hort Science*, 41(6), 1493-1497. https://doi.org/10.21273/HORTSCI.41.6.1493

Okcu, G., Kaya, M. D., & Atak, M. (2005). Effect of salt and drought stress on germination and seedling growth of pea (*Pisum sativum*). *Turkish Journal of Agriculture and Forestry*, *29*(4), 137-243.

Qian Y. L., Engelke M. C., & Foster, M. J. V. (2000). Salinity effects on *Zoysia grass* cultivars and experimental lines. *Crop Science*, *40*, 488-492. https://doi.org/10.2135/cropsci2000.402488x

Rajabi Dehnavi, A., Zahedi, M., Ludwiczak, A., Cardenas Perez, S., & Piernik, A. (2020). Effect of salinity on seed germination and seedling development of sorghum (*Sorghum bicolor* (L.) Moench) genotypes. Agronomy, *10*, 859-871. https://doi.org/10.3390/agronomy10060859

Rouhi, H. R., Aboutalebian, M. A., & Sharifzadeh, F. (2011). Seed priming improves the germination traits of tall fescue (*Festuca arundinacea*). *Notulae Scientia Biologicae*, *3*(2), 57-63.

https://doi.org/10.15835/nsb325409
Ruan, S., Xue, Q., & Tylkowska, K. (2002). The influence of priming on germination of rice seeds and seedling emergence and performance in flooded soil. *Seed Science and Technology*, *30*(1), 61-67.
Saadatfar, A., Ahmadi Mousavi, E., & Tavassolian, I. (2023). Physical, plant growth regulators and TiO_2 nanoparticles priming treatments to improve seed germination of endangered asafoetida (*Ferula assafoetida* L.). *Journal of Horticulture and Postharvest Research*, *6*(3), 235-246. https://doi.org/10.22077/jhpr.2023.6064.1309
Salehi, S. A., Shahdadneghad, M., RohanyYazdi, M., & Mohammadi, T. (2014). Effects of zinc sulfate and ascorbic acid on flowering characteristics of ornamental plant *Gazannia splendens* cv. daybreak red stripe. *International Journal of Advanced Biological and Biomedical Research*, *2*(2), 392-398.
Shahid, S. A., Zaman, M., & Heng, L. (2018). Soil Salinity: Historical Perspectives and A World Overview of the Problem (Eds.) In *Guideline for Salinity Assessment, Mitigation, and Adaptation Using Nuclear and Related Techniques.* Springer Cham, p. 43-52. https://doi.org/10.1007/978-3-319-96190-3
Shila, A., Haque, M. A., Ahmed, R., & Howlader, M. H. K. (2016). Effect of different levels of salinity on germination and early seedling growth of sunflower. *World Research Journal of Agricultural Sciences*, *3*(1), 048-053.
Sikha, S., Sunil, P., Arti, J., Sujata, B., Navdeep, D., & Kranti, T. (2014). Effect of salt stress on seedling growth and survival of *Oenother bennise* L. *International Research Journal of Environment Sciences*, *3*(9), 70-74.
Taiz, L., & Zeiger, E. (2006). *Stress physiology. Plant Physiology*, 4. Sinauer Associates. Sunderland, MA.
Torbaghan, M. E. (2012). Effect of salt stress on germination and some growth parameters of marigold (*Calendula officinalis* L.). *Plant Science Journal*, *1*(1), 7-19.
Villarino, G. H., & Mattson, N. S. (2011). Assessing tolerance to sodium chloride salinity in fourteen floriculture species. *HortTechnology*, *21*, 539-545. https://doi.org/10.21273/HORTTECH.21.5.539
Wang, L., Patrick, J. W., & Ruan, Y. L. (2018). Live long and prosper: roles of sugar and sugar polymers in seed vigor. *Molecular Plant*, *11*, 1-3. https://doi.org/10.1016/j.molp.2017.12.012
Wiedenfeld, H., & Andrade-Cetto, A. (2001). Pyrrolizidine alkaloids from *Ageratum houstonianum* Mill. *Phytochemistry*, 57(8), 1269-1271. https://doi.org/10.1016/S0031-9422(01)00192-3
Xiong, M., Xu, J., Zhou, Z., Peng, B., Shen, Y., Shen, H., ... & Feng, G. (2024). Salinity inhibits seed germination and embryo growth by reducing starch mobilization efficiency in barley. *Plant Direct*, *8*(2), e564. https://doi.org/10.1002/pld3.564
Yasemin, S., Deger, A. G., & Koksal, N. (2020). The effects of salt stress in Zinnia (*Zinnia* sp.) cultivars during seed germination and at the early stages of seedling growth. *Turkish Journal of Agricultural Research*, *7*(3), 253-265.
Yokoi, S., Bressan, R. A., & Hasegawa, P. M. (2002). Salt stress tolerance of plants. *JIRCAS Working Report*, *23*(1), 25-33.
Zivdar, S., Khaleghi, E., & Dehkordi, F. S. (2011). Effect of salinity and temperature on seed germination indices of *Zinnia elegans* L.. *Journal of Applied Horticulture*, *13*(1), 48-51.

JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH
2024, VOL. 7(1), 45-58

Journal of Horticulture and Postharvest Research

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

University of Birjand

Evaluation of clay pot cooler storage for preserving postharvest quality of leafy vegetables

Weerahetti Arachchilage Buddhika Hanshani Hettiarachchi[1], Konara Mudiyanselage Shyamali Dilrukshika Sandarenu[1], Supurni Nimalshani Wiriththamulla Gamage[1], Rathnayake Mudiyanselage Tharangi Disnika Attanayaka[1], Yapa Mudiyanselage Hasanika Madhubhashini Dassanayaka[1], Dharshani Dammika Kumari Galahitiyawa[2] and Herath Mudiyanselage Prathibhani Chamidha Kumarihami[*1]

[1]Department of Crop Science, Faculty of Agriculture, University of Peradeniya, 20400, Peradeniya, Sri Lanka.
[2]Department of Agricultural Engineering, Faculty of Agriculture, University of Peradeniya, 20400, Peradeniya, Sri Lanka.

ARTICLE INFO

Original Article

Article history:
Received 11 December 2023
Revised 15 February 2024
Accepted 16 February 2024

Keywords:
Clay pot cooler
Leafy vegetables
Quality
Shelf-life

DOI: 10.22077/jhpr.2024.7033.1347
P-ISSN: 2588-4883
E-ISSN: 2588-6169

***Corresponding author:**
Department of Crop Science, Faculty of Agriculture, University of Peradeniya, 20400, Peradeniya, Sri Lanka.

Email: prathibhani@agri.pdn.ac.lk

ABSTRACT

Purpose: The effectiveness of the evaporative clay pot coolers was studied to preserve the postharvest quality of leafy vegetables, including kankun (*Ipomoea aquatica*), gotukola (*Centella asiatica*), lettuce (*Lactuca sativa*) and thampala (*Amaranthus spp*.). **Research method:** The bundles of leafy vegetables weighed about 200 g and were stored for seven days under room temperature storage, refrigerated storage, and clay pot cooler storage. **Findings:** The average temperatures were 27.7 °C, 7.1 °C, and 25.6 °C, and relative humidity values were 76.1%, 58.2%, and 93.6% in room temperature storage, refrigerated storage, and clay pot cooler, respectively. The average cooling efficiency of clay pot coolers was 66.7%. The physiological weight losses of leafy vegetables were significantly reduced during clay pot cooler storage. The chlorophyll content, soluble solids content, color changes, and visual quality of leafy vegetables were significantly maintained in clay pot cooler storage compared to room temperature storage. **Research limitations:** The main limitation of this study was the seepage of water into the inner pot of the clay pot cooler which enhanced the decay of leafy vegetables. This was successfully controlled by avoiding overwatering the sand that was used as the lining material of the clay pot cooler. **Originality/Value:** The clay pot cooler is one of the alternative low-cost storage methods to preserve the quality of leafy vegetables during storage.

INTRODUCTION

Leafy vegetables are a cheap and readily available food item that is widely consumed as an essential part of a balanced diet and an important source of micronutrients, minerals, fiber, and vitamins, especially vitamin A and vitamin C (Ambuko et al., 2017; Bihon et al., 2020). Further, leafy vegetables are used in Ayurvedic medicinal treatment due to their medicinal value (Kumara & Beneragama, 2020). Leafy vegetables deteriorate very quickly after harvesting owing to their high perishability. They tend to decay early when stored in high-temperature and low-humidity storage conditions (Liberty et al., 2013; Ronoh et al., 2018). The rate of deterioration depends on environmental factors, including storage temperature and relative humidity (Ambuko et al., 2017). Cold storage at low temperatures and high relative humidity is the most commonly used method to prolong fresh produce's storage life by reducing its respiration rate (Ambuko et al., 2017; Ronoh et al., 2020).

Relative humidity and temperature are key factors to consider during storage to extend the shelf-life of perishables. Reducing the temperature and increasing the relative humidity during storage slows down pathological activity and suppresses enzymatic activity and respiratory activity. Thereby it makes the storage environment suitable for the safe preservation of perishables. Further, it reduces the rate of water loss and the respiration rate which slows or inhibits the growth of spoilage microorganisms and minimizes metabolic activities (Chinenye et al., 2013; Liberty et al., 2013; Ambuko et al., 2017; Ronoh et al., 2020).

The use of mechanical/ domestic refrigerators is a common method in handling and preserving perishables. However, it is difficult due to high purchase and maintenance costs and inaccessibility to electricity in low-income rural communities in developed/ developing countries (Yahaya & Akande, 2018). For example, the price of mechanical refrigerators in Sri Lanka is very high (> 85,000 LKR), and increasing electricity bills add to the misery of Sri Lankans. Thus, many people are looking for alternative approaches to reduce electricity use at home. Therefore, there has been an increased focus on non-electrical sources that can be used for food preservation, including renewable energy sources such as water and wind. Among these technologies, a non-electrical method based on the principle of evaporative cooling has also attracted much attention (Odesola & Onyebuchi, 2009; Basediya et al., 2013; Rehman et al., 2020). An evaporative cooling system is an economical, efficient, and environmentally friendly method of maintaining the postharvest quality of perishables with the principle of evaporating water through the passive cooling system (Basediya et al., 2013; Deoraj et al., 2015). These systems do not require electricity to operate. They are more beneficial in terms of energy requirements relative to standard refrigeration systems, lower capital costs, and more potential savings. Moreover, with the increase in global warming and the high inflation rate, the demand for low-cost cooling systems will rise in the future (Manyozo et al., 2018; Rehman et al., 2020). Furthermore, it is beneficial to use simple passive cooling systems to achieve low temperatures for better storage of fruits and vegetables which do not require specific skill sets for the operation (Odesola & Onyebuchi, 2009). In addition to these benefits, these passive cooling systems can be constructed using locally available materials (Ambuko et al., 2017).

There are two basic types of non-electric storage technologies with the principle of evaporative cooling, (1) evaporative cooling chambers (ECC) known as zero energy cooling chambers (ZECC), and (2) clay pot coolers, known as zeer pots (Verploegen et al., 2018). Moreover, various small-scale low-cost self-constructible, evaporative-cooling storage units have been developed based on these simple technologies and their modifications.

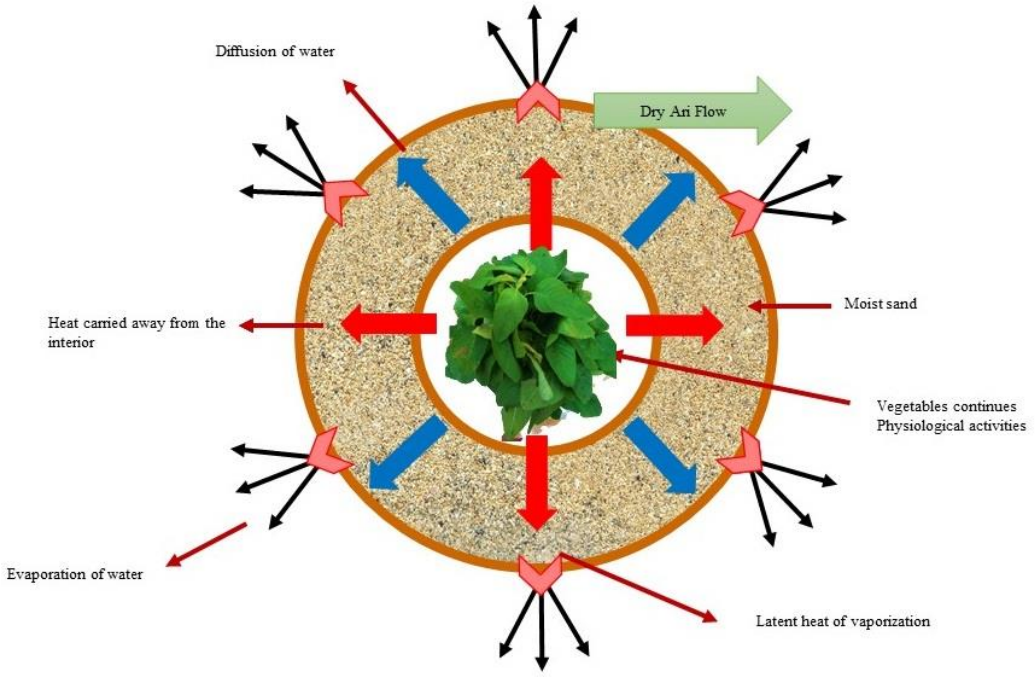

Fig. 1. Principle of evaporative cooling in clay pot cooler. Adapted and modified from Bhaisare et al. (2020).

Evaporative cooling chambers (ECC) are large-scale evaporative cooling chambers used in developed countries by large-scale producers or groups of people which are made of bricks, sand, wood, straw, gunny or burlap sacks, and twine (Verploegen et al., 2018; Rehman et al., 2020). The clay pot cooler consists of two pots with similar shapes and two different sizes. The inner smaller pot acts as the storage pot and the outer larger pot acts as the evaporation surface. The gap between the two pots is filled with sand and moist with water (Verploegen et al., 2018). Evaporative cooling happens with the exchange of heat evaporation which happens due to the latent heat of evaporation. Due to the high latent heat of evaporation in the water, a greater reduction of heat will result. It results in a reduction in the temperature and an increase in the relative humidity in the storage area (Basediya et al., 2013; Ambuko et al., 2017; Fig. 1).

This study was conducted to evaluate the effectiveness of an evaporative clay pot cooler for the preservation of leafy vegetables concerning their quality and shelf-life, including kankun (*Ipomoea aquatica*), gotukola (*Centella asiatica*), lettuce (*Lactuca sativa*), and thampala (*Amaranthus spp.*).

MATERIALS AND METHODS

Experimental site

The study was conducted at the Department of Crop Science, Faculty of Agriculture, University of Peradeniya, Sri Lanka (N 7º 15' 43.92" E 80º 35' 2.76").

Selection of leafy vegetables and sample preparation

As plant materials, four leafy vegetables including, kankun (*Ipomoea aquatica*), gotukola (*Centella asiatica*), lettuce (*Lactuca sativa*), and thampala (*Amaranthus spp.*) were selected. Freshly harvested leafy vegetables were purchased from the market. The samples were cleaned to remove leaf defects and were made into bundles each weighing around 200 g. A total of nine (9) bundles were used per each type of leafy vegetable. Three (3) bundles were

used for the weight loss and leaf color measurements. Six (6) bundles were used for destructive measurements during 7 days of storage.

Fig. 2. The details of the clay pot cooler.

Treatments and experimental design

The experimental design was a completely randomized design (CRD) with three storage methods as treatments, including room temperature storage, refrigerated storage, and clay pot storage. All the experiments were conducted in a single laboratory room.

Description of the clay pot cooler

The clay pot cooler was prepared using the '*Kalenimatta'* clay type with the support of a potter at a pottery in Molagoda, Kegalle, Sri Lanka (N 7.2598°, E 80.3988°). The cost of a clay pot cooler was Rs. 2500.00 (LKR). There were six clay pot coolers in the experiment.

The dimensions of the interior pot were 45.72 cm (18 inches) in height and 30.48 cm (12 inches) in diameter and the exterior pot size was 48.26 cm (19 inches) in height and 38.10 cm (15 inches) in diameter (Fig. 2). The gap between the two pots was filled with sieved river sand and moist with water repeatedly.

Data collection

Data were collected every day with three replicates (n=3, bundles) until samples became unusable. The following parameters were measured to evaluate the postharvest life of selected leafy vegetables.

Relative humidity and temperature

The relative humidity and the temperature of different storage methods were recorded using the digital data loggers (CL 11, Rotronic, Taiwan) during the experimental period.

Evaporative cooling efficiency

Evaporative cooling efficiency (ECE) is calculated using the following equation (1) mentioned in Lertsatitthanakorn et al. (2006).

$$ECE = \frac{Td - Tc}{Td - Tw} \qquad (1)$$

T_d = dry bulb temperature of the ambient condition,

T_c = temperature inside the clay pot cooler,

T_w = wet bulb temperature of the ambient condition.

Weight loss

The weight loss was measured using a digital balance (Model-Kern and Sohn GmbH, D-723336, Germany). The results were expressed as the percentage loss of initial weight using the following equation (2).

$$WL\ (\%) = \frac{(W1 - W2)}{W1} \times 100 \qquad (2)$$

WL: the weight loss percentage,

W1: the initial weight (g),

W2: the weight at sampling date during storage (g)

Soluble solids content (SSC)

Leaf samples from the top, middle, and bottom parts were taken and broken into small pieces. Then 1 g of the sample was weighed and ground using a mortar and pestle. The juice was extracted and the soluble solids content (SSC) of the extracted juice was measured using a handheld refractometer (Digital Refractometer, HI96801, Romania). Calibration was done in °Brix, and SSC was expressed as a percentage.

Chlorophyll content

Chlorophyll content was measured using 80% acetone extraction method. A spectrophotometer (Model; AE-S70-2U, United Kingdom) was used to quantify the wavelength at 645, 663, and 652 nm. Chlorophyll content was calculated using the following equations (3-5).

$$\text{Total Chlorophyll content} = \{20.2(D645) - 8.02(\text{D663})\} \times \frac{V}{(1000 \times w)}$$

$$= \text{D652} \times \frac{1000}{34.5} \times \frac{\text{V}}{1000 \times \text{W}} \qquad (3)$$

$$\text{Chlorophyll a content} = \{12.7\ (\text{D663}) - 2.69(\text{D645})\} \times \frac{V}{(1000 \times w)} \qquad (4)$$

$$\text{Chlorophyll b content} = \{22.9\ (\text{D645}) - 4.68(\text{D663})\} \times \frac{\text{V}}{(1000 \times w)} \qquad (5)$$

V is the volume of the extraction solvent in each sample (1 mL), and W is the fresh tissue weight (1 g) of the sample.

Leaf color

Color values of selected leaves (n=10) from the top, bottom, and middle parts were measured using a colorimeter (CS-10, China) which was calibrated with a white calibration card. L*, a*, and b* values were recorded and a* and b* values were converted into hue angle (H°).

Leaf visual quality evaluation

Visual quality was assessed using a numerical rating scale (hedonic scale) of 1-5 (Kumara & Beneragama, 2020), *i.e.*, 5 - excellent (green and fresh), 4 - good (yellowing and/ or wilting started), 3 - fair (10% leaf yellowing and/or wilting started), 2 - poor (25% leaf yellowing and/or wilting, leaf shedding and decaying started, unmarketable, but usable), 1- unusable (50% leaf yellowing and/or wilting, leaf shedding and decaying continues).

Statistical analysis

The parametric data were subjected to analysis of variance (ANOVA) using statistical analysis software (SAS version 9.0). The significant differences in mean values were evaluated using the least significant difference test (LSD) at the significance level of $P \leq 0.05$. The rank data were analyzed using the Kruskal–Wallis test at the significance level of $P \leq 0.05$. The graphs of the analyzed data were created using Excel (ver. 2013).

RESULTS

Temperature and relative humidity

During the storage period, the temperature in the room temperature storage, clay pot cooler storage, and refrigerator storage varied between 25-30 °C, 20-25 °C, and 5-10 °C, respectively (Fig. 3A). Overall, the temperature fluctuation between the room temperature and the clay pot cooler ranged between 0-3 °C depending on the surrounding environment. Moreover, the clay pot cooler maintained a higher relative humidity (RH, 90-100%) compared to room temperature storage (60-80%) and refrigerated storage (40-60%) (Fig. 3B). Overall, the difference in RH values between the room temperature storage and the clay pot cooler storage ranged between 0% and 33% depending on the environmental conditions.

Cooling efficiency

The changes in temperature, relative humidity, and cooling efficiency in the clay pot cooler for one week are illustrated in Figure 4. The cooling efficiency of the clay pot cooler used in the present study was 66.7% which is more in line with Woldemariam and Abera (2014) who reported a cooling efficiency of 61.6% for the pot-in-pot cooler. Khatun et al. (2019) reported that higher ambient temperature and lower relative humidity will cause higher cooling efficiency, which was similarly reported in the present study with clay pot cooler storage. Compared to other days during the storage period the 2nd, 6th, and 7th-day relative humidity is lower and in those days cooling efficiency is higher.

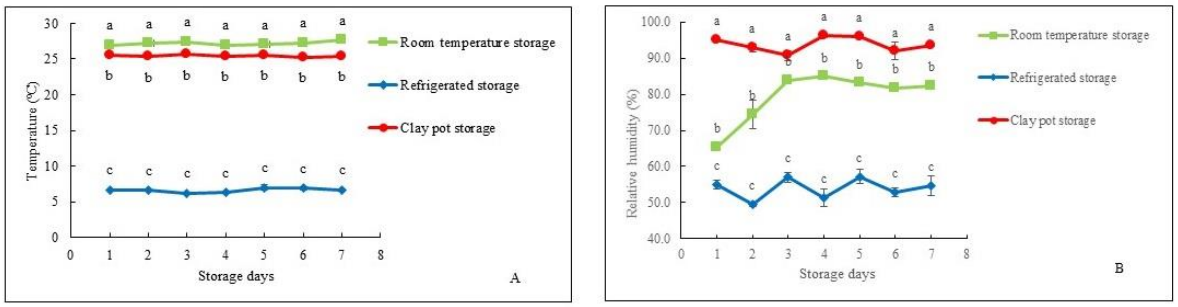

Fig. 3. Changes in temperature (A) and relative humidity (B) in the room temperature storage, refrigerated storage, and clay pot cooler storage.

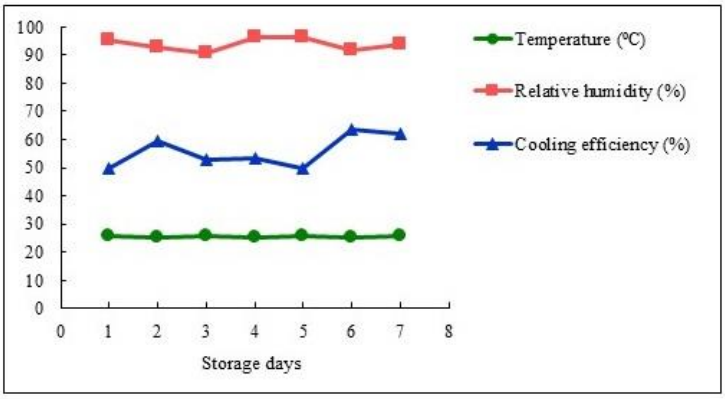

Fig. 4. Changes in temperature, relative humidity, and cooling efficiency in the clay pot cooler.

Weight loss

The changes in weight loss in leafy vegetables during storage are shown in Figure 5. The weight loss of leafy vegetables was significantly (P≤0.05) reduced in clay pot cooler storage compared to the other two storage methods. At the end of the storage, kankun (*Ipomea aquatica*), gotukola (*Centella asiatica*), thampala (*Amaranthus spp*.), and lettuce (*Lactuca sativa*) stored at room temperature showed 20-25%, 0-15%, 12-14%, and 5-6% initial weight loss, respectively. In refrigerator storage, *Ipomea aquatica* showed 10-15% initial weight loss which was greater than clay pot cooler storage, while *Centella asiatica*, *Amaranthus spp.*, and *Lactuca sativa* showed 10-15%, 0-5%, and 0-3% of initial weight loss, respectively. In clay pot cooler storage, *Ipomea aquatica*, *Centella asiatica*, *Amaranthus spp.*, and *Lactuca sativa* showed 0-10%, 0-15%, 0-8%, and 0-4% initial weight loss, respectively.

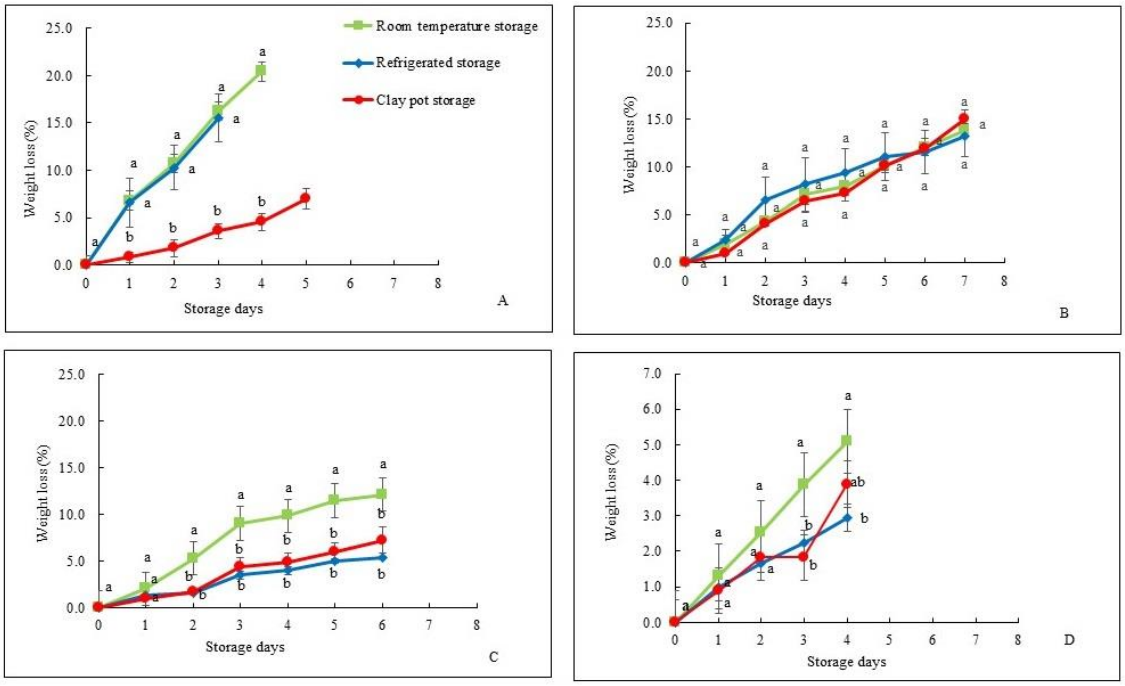

Fig. 5. Changes in weight loss in kankun (*Ipomoea aquatica*, A), gotukola (*Centella asiatica*, B), thampala (*Amaranthus spp*., C), and lettuce (*Lactuca sativa*, D) in different storage methods. Vertical bars indicate standard error (n=3). The same letters at each time point indicate means that do not differ significantly at P ≤0.05 according to the least significant difference test (LSD).

Hue angle

The hue value did not change in kankun (*Ipomoea aquatica*) until the 2nd day of storage (Fig. 6). At the end of the storage, the lower hue values were recorded in the room temperature storage compared to the clay pot cooler storage. In thampala (*Amaranthus spp.*) and gotukola (*Centella asiatica*), the changes in hue values were similar in all three storage methods. In lettuce (*Lactuca sativa*), after the 3rd day of storage hue value was changed, and the room temperature storage reported lower hue values than the other two storage methods.

Soluble solids content (SSC)

Figure 7 shows the changes in SSC of leafy vegetables stored with three different storage methods. During the storage period, irrespective of the storage methods, the SSC of kankun (*Ipomoea aquatica*) was increased which may be due to water loss. However, gotukola (*Centella asiatica*), thampala (*Amaranthus spp.*) and lettuce (*Lactuca sativa*) showed a gradual decrease in SSC during their storage period regardless of storage methods.

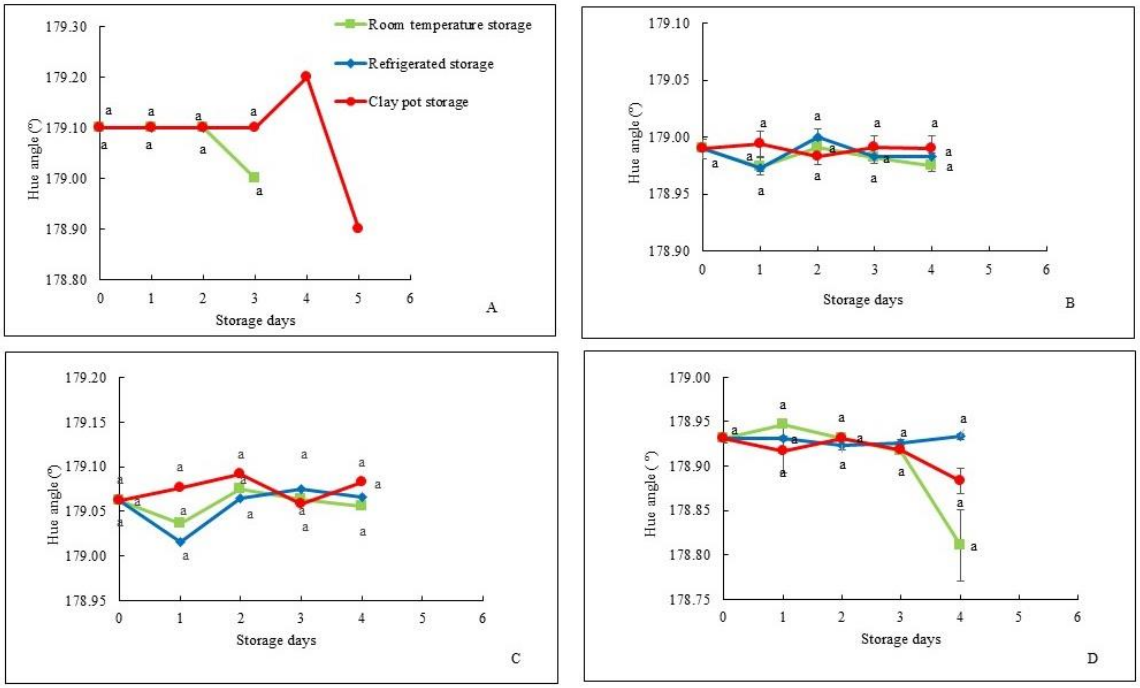

Fig. 6. Changes in hue values in kankun (*Ipomoea aquatica*, A), gotukola (*Centella asiatica*, B), thampala (*Amaranthus spp.*, C), and lettuce (*Lactuca sativa*, D) in different storage methods. Vertical bars indicate standard error (n=3). The same letters at each time point indicate means that do not differ significantly at P ≤0.05 according to the least significant difference test (LSD).

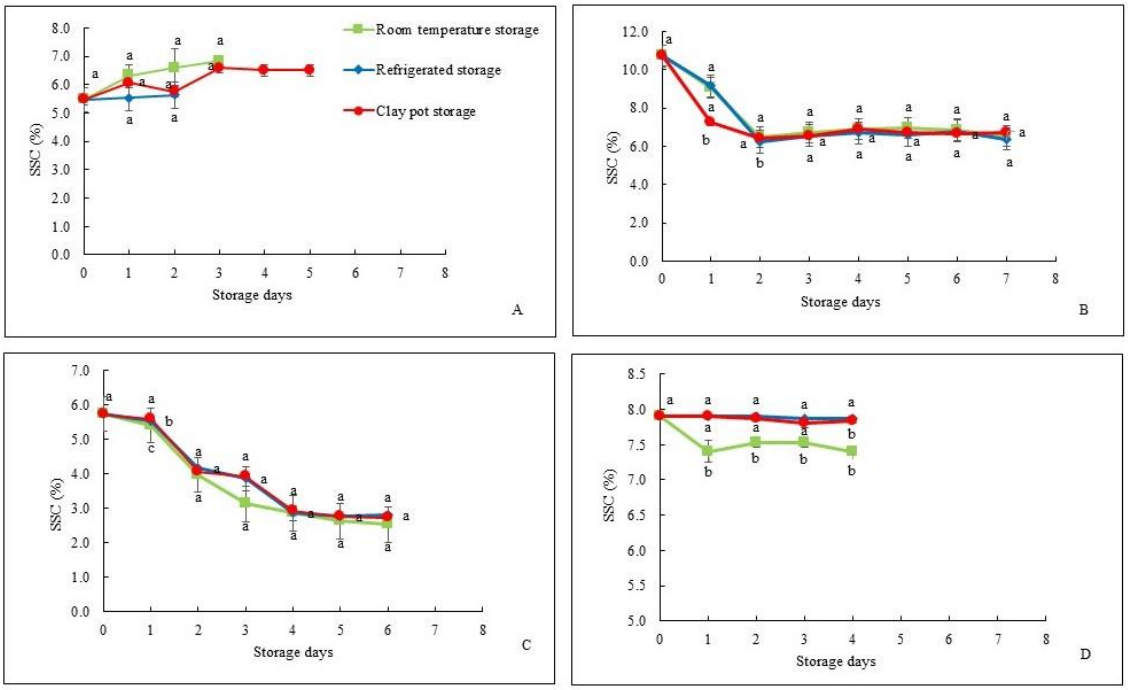

Fig. 7. Changes in soluble solids content (SSC) in kankun (*Ipomoea aquatica*, A), gotukola (*Centella asiatica*, B), thampala (*Amaranthus spp.*, C), and lettuce (*Lactuca sativa*, D) in different storage methods. Vertical bars indicate standard error (n=3). The same letters at each time point indicate means that do not differ significantly at P≤0.05 according to the least significant difference test (LSD).

Chlorophyll content

Figure 8 illustrates the variation in the chlorophyll content during the storage period. The total chlorophyll content was significantly maintained (P≤0.05) in clay pot cooler storage compared to room temperature storage. Reduction in total chlorophyll content in gotukola (*Centella asiatica*), and lettuce (*Lactuca sativa*) was observed after one day of storage while other leafy vegetables showed chlorophyll reduction after 2 days of storage.

Visual quality

In the present study, visual quality was maintained in the clay pot cooler better than in room temperature storage (Fig. 9 and Fig. 10). The quality of kankun (*Ipomoea aquatica*) was preserved for a longer period (5 days) under the clay pot cooler with lower wilting, yellowing and it maintained the usable quality compared to the other two storage methods (3 days in refrigerated storage and 4 days in room temperature storage).

The *gotukola* (*Centella asiatica*) was stored for 7 days, and the rate of reduction in visual quality such as yellowing and wilting was lower in clay pot cooler storage compared to room temperature storage. From the 5th day of the storage, the visual quality was significantly different between the three storage methods. From the 5th day of storage, leafy vegetables showed leaf shedding, wilting, yellowing, and unusable qualities in three storage conditions.

The visual quality changes in thampala (*Amaranthus spp.*) were significantly different from the one day after storage and the changes were continued until the end of storage. The visual quality reduction rate was lower (wilting, yellowing, and leaf shedding) in the clay pot cooler compared to room temperature storage.

A rapid visual quality reduction (wilting, yellowing, and leaf shedding) starting after 2nd day of storage was observed in lettuce (*Lactuca sativa*), and their storage period was limited to four days. The clay pot cooler was effective in visual quality maintenance compared to room temperature storage.

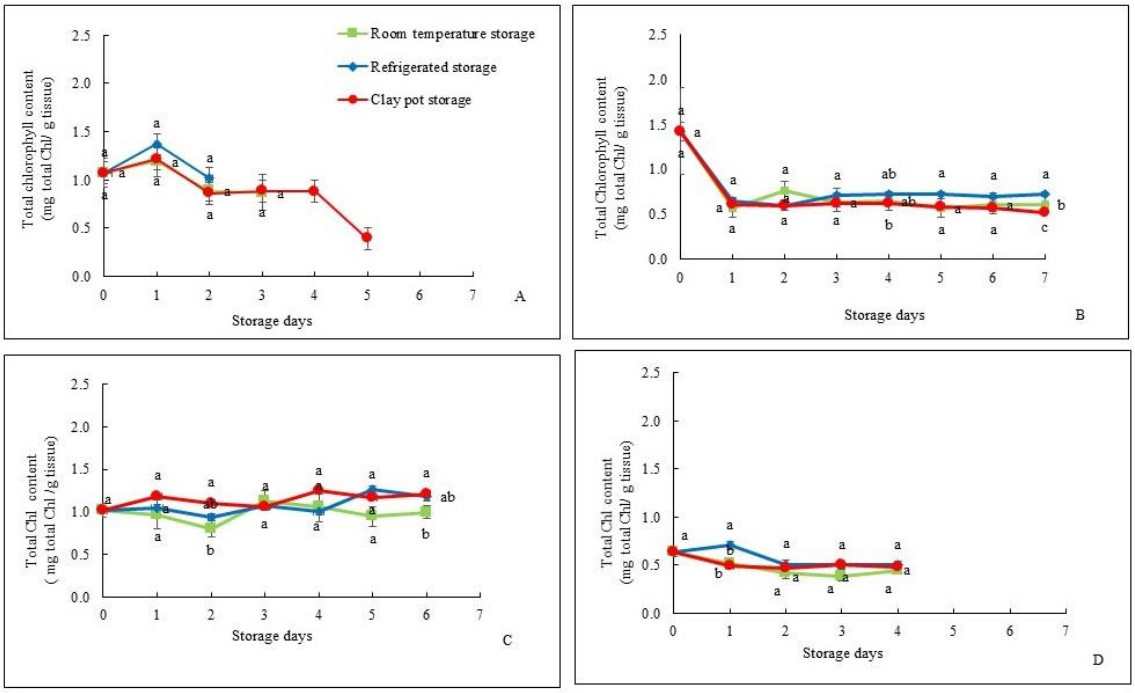

Fig. 8. Changes in total chlorophyll content in kankun (*Ipomoea aquatica*, A), gotukola (*Centella asiatica*, B), thampala (*Amaranthus spp*., C), and lettuce (*Lactuca sativa*, D) in different storage methods. Vertical bars indicate standard error (n=3). The same letters at each time point indicate means that do not differ significantly at P ≤0.05 according to the least significant difference test (LSD).

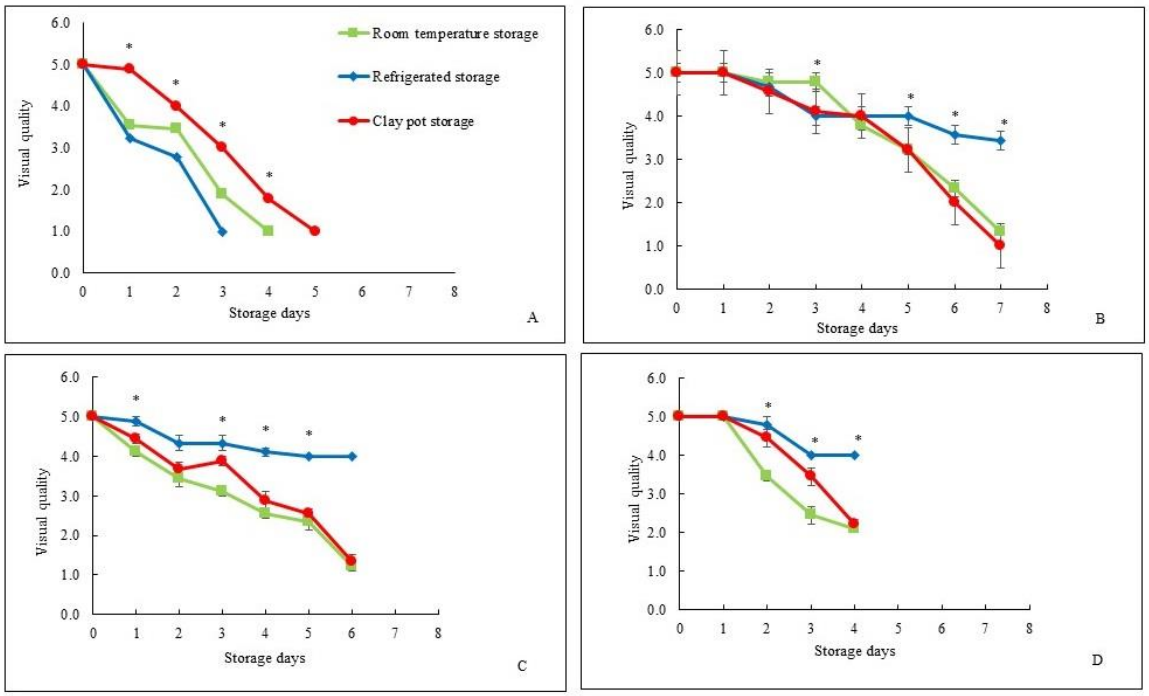

Fig. 9. Changes in visual quality in kankun (*Ipomoea aquatica*, A), gotukola (*Centella asiatica*, B), thampala (*Amaranthus spp.*, C), and lettuce (*Lactuca sativa*, D) in different storage methods. Vertical bars indicate standard error (n=3). *, at each time point describe treatments that differ significantly at P ≤0.05, according to Kruskal–Wallis test

Fig. 10. Changes in visual quality in kankun (*Ipomoea aquatic*, A), gotukola (*Centella asiatica*, B), thampala (*Amaranthus spp*., C), and lettuce (*Lactuca sativa*, D), in different storage methods, room temperature storage (T1), refrigerated storage (T2), and clay pot cooler storage (T3).

DISCUSSION

As leafy vegetables are perishables, they tend to deteriorate quickly after harvesting. The rate of deterioration depends on storage conditions, including storage temperature and relative humidity. High temperature and low humidity storage conditions enhance the deterioration (Ambuko et al., 2017; Ronoh et al., 2018). Cold storage at low temperatures and high relative humidity is the most commonly used storage method to prolong the storage life of fresh produce by reducing their respiration rate. Clay pot cooler storage maintains high humidity around the harvested produce and reduces water loss and weight loss (Ambuko et al., 2017; Oliy, 2020; Ronoh et al., 2020). As clay pot cooler storage contains low temperature and high humidity conditions, it slows down the pathological activity, enzymatic activity, and respiratory activity, and makes the storage environment suitable for safe preservation.

The evaporative cooling efficiency depends on the relative humidity of the surrounding air. As water evaporates between the two pots, the temperature of the inner pot decreases. Meanwhile, the relative humidity of the inner pot increases due to the respiration of the leafy vegetables stored in the inner pot. Generally, greater temperature difference results in greater evaporative cooling efficiency.

Weight loss in fresh produce is due to continuous metabolic activity, including respiration and transpiration. The energy required to maintain the metabolic activity is obtained from respiration, which involves the oxidation of sugars to produce carbon dioxide and water. Fresh produce weight loss includes both moisture loss due to transpiration and carbon loss due to respiration (Kumara & Beneragama, 2020). The rate of weight loss in leafy vegetables was lower in clay pot coolers compared to room temperature storage. Compared to room temperature clay pot coolers have lower temperatures and higher relative humidity conditions. It slows down the pathological activities and suppresses the enzymatic activity and respiratory activity. It makes favorable storage conditions for the preservation of leafy vegetables. It reduces the rate of water loss by maintaining a higher humidity level around the leafy vegetables and the respiration rate which slows or inhibits the growth of spoilage microorganisms and minimizes metabolic activities (Chinenye et al., 2013; Liberty et al., 2013; Ambuko et al., 2017; Ronoh et al., 2020). A higher rate of weight loss was recorded in room temperature storage as it provides optimum conditions for microbial respiration and metabolic activities (Ronoh et al., 2018). Abdul-Rahaman et al. (2015) reported that lowering the temperature and increasing the relative humidity during storage reduced the weight loss of fresh produce. The weight loss for thampala (*Amaranthus spp*.) found in the present study was similar to the findings reported by Ambuko et al. (2017), which reported a lower rate of weight loss in clay pot cooler storage compared to room temperature storage. It implies that the microclimate of clay pot cooler storage minimizes the weight loss in leafy vegetables while reducing the metabolic activities of fresh produce, including respiration and transpiration. The room temperature storage enhances the respiration and transpiration of fresh produce which lead to the structural decay of the perishables. Preservation of perishables is based on lowering the temperature and increasing the relative humidity in storage conditions. It provides not only preservation but also extends the shelf-life while maintaining the quality and ultimately reducing the postharvest losses of perishables (Ronoh et al., 2018).

Soluble solids content is an indicator of the sugar content in the sample, and it depends on the maturity stage. Usually, SSC increases with the progress of the ripening as a result of the breakdown of polysaccharides (Islam et al., 2012). Soluble solids content increases only in kankun (*Ipomoea aquatica*) and the other three leafy vegetables showed a gradual reduction. Kumara and Benaragama (2020) reported a similar trend of reduction in SSC in leafy vegetables.

Many physiological changes happen with continuous respiration after harvesting. Leaf discoloration and leaf wilting are the main visible changes due to chlorophyll degradation during the storage of leafy vegetables. Further, during the storage of green vegetables, carotenoid pigments were increased, and moisture was lost (Kumara & Benaragama., 2020). Due to the chlorophyll degradation, the total chlorophyll contents of the selected leafy vegetables were gradually reduced with storage time.

Color values (L*, a*, and b*) manifest the color variations of leafy vegetables during the storage period. The hue angle shows the changes in green color from dark green (>130°) to light green (<120°) (Ambuko et al., 2017). Chlorophyll reduction and color changes were interrelated in all tested leafy vegetables. These results may be linked with the production of ethylene which affects chlorophyll degradation.

In leafy vegetables, visual quality is one of the major factors that contribute to aesthetic attributes which is directly linked to their salability. Vegetables contain 65%- 95% water. Loss of water from fresh produce causes quality losses such as visual/ appearance changes, wilting/ shriveling, and textural changes (Chinenye et al., 2013). The leafy vegetables remain fresh as long as they retain enough water. Leaf yellowing, leaf shedding, wilting, and

decaying are the signs that consumers look for to determine the acceptability of the leafy vegetable. The lower temperature and high relative humidity in the storage area slow down the deterioration process including wilting and yellowing and loss of salable weight (Ambuko et al., 2017; Bihon et al., 2020; Oliy, 2020). As the clay pot cooler reduced the weight loss compared to room temperature storage, it was able to maintain the visual quality of the leafy vegetables during the storage period.

In refrigerator storage, low temperature and high relative humidity conditions are favorable to prolong the shelf-life of perishable products by reducing pathological activities and suppressing enzymatic activity and respiratory activity (Ambuko et al., 2017; Ronoh et al., 2020). In this experiment refrigerator storage and clay pot cooler showed similar variations in chlorophyll content, soluble solids content, and hue angle in leafy vegetables. Comparatively refrigerator storage showed a lower rate of weight loss and visual quality reduction with room temperature storage and clay pot cooler storage. In conclusion, while comparing the results of all three storage methods tested for leafy vegetables the clay pot cooler storage can be introduced as one of the low-cost and effective storage methods to prolong the postharvest life and preserve the postharvest quality of fresh produce, including leafy vegetables.

CONCLUSION

The present study showed that the leafy vegetables stored in clay pot coolers (25.4 ± 0.1 °C; 93 ± 0.8% RH) had the best quality compared to room temperature storage. The clay pot cooler was significantly effective ($P \leq 0.05$) in maintaining the total chlorophyll content, soluble solids content (SSC), color, and visual quality of leafy vegetables. Therefore, the clay pot cooler is an alternative low-cost storage method to preserve the postharvest quality of leafy vegetables.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgments

The authors are grateful for the research assistance provided by the Department of Agricultural Engineering, Faculty of Agriculture, University of Peradeniya, Sri Lanka. Further, the financial assistance received from the AHEAD/ELTA/ELSE Department Proposal to purchase the required laboratory and field equipment is greatly appreciated.

REFERENCES

Abdul-rahaman, A., Alhassan, N., Charles, T., Charles, A. K., Mohazo, A., & Abdul-shamir, I. K. (2015). Clay cooling pots technologies for storage of Fuzzy Bomb and Bonita Roma varieties of tomato fruits. *Journal of Aridland Agriculture*, *1*(i), 6–12. https://doi.org/10.19071/jaa.2015.v1.2900

Ambuko, J., Wanjiru, F., Chemining'wa, G. N., Owino, W. O., & Mwachoni, E. (2017). Preservation of postharvest quality of leafy Amaranth (*Amaranthus spp*.) vegetables using evaporative cooling. *Journal of Food Quality*, 1–7. https://doi.org/10.1155/2017/5303156

Basediya, A.L., Samuel, D. V. K., & Beera, V. (2013). Evaporative cooling system for storage of fruits and vegetables - A review. *Journal of Food Science and Technology*, *50*(3), 429-442. https://doi.org/10.1007/s13197-011-0311-6

Bhaisare, A., Katekar, V. P., & Deshmukh, S. S. (2020). Novel energy efficient design of water-cooler for hot and dry climate. *Test Engineering and Management*, *82*, 12523-12528.

Bihon, W., Coulibaly, A., Chagomoka, T., Sanogo, O., Cisse, F., Ognakossan, K. E., & Diouf, O. (2020). Extending the shelf-life of vegetables using low-cost evaporative cooling systems in Mali. *Acta Horticulturae*, *1275*, 419–426. https://doi.org/10.17660/ActaHortic.2020.1275.58

Chinenye, N. M., Manuwa, S. I., Olukunle, O. J., & Oluwalana, I. B. (2013). Development of an active evaporative cooling system for short-term storage of fruits and vegetables in a tropical climate. *Agricultural Engineering International: CIGR Journal*, *15*(4), 307–313.

Deoraj, S., Ekwue, E. I., & Birch, R. (2015). An evaporative cooler for the storage of fresh fruits and vegetables. *The West Indian Journal of Engineering*, *38*(1), 86-95.

Islam, M. P., Morimoto, T., & Hatou, K. (2012). Storage behavior of tomato inside a zero energy cool chamber. *Agricultural Engineering International: CIGR Journal*, *14*(4), 209–217.

Khatun, A., Singh, R. P., & Kumar, A. (2019). Development of a low-cost evaporative cooling storage structure for perishable commodities. *Food Science Research Journal*, *10*(2), 221–231. https://doi.org/10.15740/has/fsrj/10.2/221-231

Kumara, B.A.M.S., & Beneragama, C.K. (2020). Modified atmospheric packaging extends the postharvest shelf life of mukunuwenna (*Alternanthera sessilis* L.). *Tropical Agricultural Research*, *31*(2), 87-96. https://doi.org/10.4038/tar.v31i2.8370

Lertsatitthanakorn, C., Rerngwongwitaya, S., & Soponronnarit, S. (2006). Field experiments and economic evaluation of an evaporative cooling system in a silkworm rearing house. *Biosystems Engineering*, *93*(2), 213-219.

Liberty, J. T., Okonkwo, W. I., & Echiegu, E. A. (2013). Evaporative cooling: a postharvest technology for fruits and vegetables preservation. *International Journal of Scientific & Engineering Research*, *4*(8), 2257–2266.

Manyozo, F. N., Ambuko, J., Hutchinson, M. J., & Kamanula, J. F. (2018). Effectiveness of evaporative cooling technologies to preserve the postharvest quality of tomato. *International Journal of Agronomy and Agricultural Research*, *13*(2), 114–127. http://www.innspub.net

Odesola, I. F., & Onyebuchi, O. (2009). A review of porous evaporative cooling for the preservation of fruits and vegetables. *The Pacific Journal of Science and Technology*, *10*(02), 935–941. http://www.akamaiuniversity.us/PJST.htm

Oliy, G. B. (2020). Comparative effect of brick and charcoal made evaporative cooling storage on shelf life of tomato. *International Journal of Scientific and Research Publications*, *10*(5), 729–734. https://doi.org/10.29322/ijsrp.10.05.2020.p10184

Rehman, D., McGarrigle, E., Glicksman, L., & Verploegen, E. (2020). A heat and mass transport model of clay pot evaporative coolers for vegetable storage. *International Journal of Heat and Mass Transfer*, *162*. https://doi.org/10.1016/j.ijheatmasstransfer.2020.120270

Ronoh, E. K., Kanali, C. L., & Ndirangu, S. N. (2020). Effectiveness of an evaporative charcoal cooler for the postharvest preservation of tomatoes and kales. *Research in Agricultural Engineering*, *66*(2), 66–71. https://doi.org/10.17221/52/2019-RAE.

Ronoh, E., Ronoh, E. K., Kanali, C. L., Ndirangu, S. N., Mang, S. M., & John, A. W. (2018). Performance evaluation of an evaporative charcoal cooler and its effects on quality of leafy vegetables. *Journal of Postharvest Technology*, *6*(3), 60–69. http://www.jpht.info

Verploegen, E., Sanogo, O., & Chagomoka, T. (2018). Evaluation of low-cost evaporative cooling technologies for improved vegetable storage in Mali. GHTC 2018 - IEEE Global Humanitarian Technology Conference, Proceedings. https://doi.org/10.1109/GHTC.2018.8601894

Woldemariam, H. W., & Abera, B. D. (2014). Development and evaluation of low-cost evaporative cooling systems to minimize postharvest losses of tomatoes (Roma vf) around Woreta, Ethiopia. *International Journal of Postharvest Technology and Innovation*, *4*(1), 69–80. https://doi.org/10.1504/IJPTI.2014.064165

Yahaya, S. A., & Akande, K. A. (2018). Development and performance evaluation of pot-in-pot cooling device for Ilorin and its environment. *Journal of Research Information in Civil Engineering*, *15*(1), 2045-2059. https://www.researchgate.net/publication/327686854

JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH
2024, VOL. 7(1), 59-68

Journal of Horticulture and Postharvest Research

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

University of Birjand

Effect of farmer's handling practices on postharvest losses of potato (*Solanum tuberosum* L.) at farm gate in Dolakha, Nepal

Rubi Kumari Sah[1,*] and Bidhan Bagale[2]

[1]*Faculty of Agriculture, College of Natural Resource Management, Agriculture and Forestry University, Marin, Sindhuli, Nepal*
[2]*Faculty of Agriculture, Central Campus, Agriculture and Forestry University, Rampur, Chitwan, Nepal*

ARTICLE INFO

Short Communication

Article history:
Received 4 November 2023
Revised 4 February 2024
Accepted 8 February 2024

Keywords:
Farmer handling practices
Fresh potato
Postharvest losses

DOI: 10.22077/jhpr.2024.6913.1342
P-ISSN: 2588-4883
E-ISSN: 2588-6169

***Corresponding author:**
Faculty of Agriculture, College of Natural Resource Management, Agriculture and Forestry University, Marin, Sindhuli, Nepal.

Email: rubisah65@gmail.com

ABSTRACT

Purpose: The main objectives of this study were to find farmers' handling practices on postharvest losses of potatoes, to estimate the post-harvest losses from farm gate to storage level and to know how the post-harvest losses were affected by the socio-demographic factors. **Research method**: The study was conducted in Dolakha, Nepal with 100 respondents selected by simple random sampling method. A semi-structured questionnaire was used to collect the primary data and MS Excel and SPSS were used for data analysis. **Findings:** Out of the total potatoes produced, 89.73% were utilized in various forms while the remaining 10.26% were lost due to poor post-harvest handling practices and lack of adequate cold storage facility. The farmers in the study area relied on practices, such as using spades for harvesting, week-long curing, traditional home storage, sorting and grading to remove the damaged ones, use of jute sacks, and overfilling and dragging of heavy bags. The post-harvest loss was significantly affected by age, gender, land size, and farming experience of farmers whereas the literacy level, ethnicity, and religion had no impact on it. **Limitations:** Transportation was a limitation because motorable roads were unavailable due to which farmers of distant areas couldn't be included in the sampling frame. **Originality/value:** The study reported that only 3% of farmers had access to cold storage facilities and 10.26% of harvested potatoes were lost due to traditional post-harvest practices.

INTRODUCTION

The potato, scientifically known as *Solanum tuberosum* L., has been consumed by humankind for thousands of years (de Haan & Rodriguez, 2016). It is considered the world's leading non-cereal crop feeding more than a billion people daily. Moreover, it is the fourth most worthwhile crop in the world after rice, maize and wheat in terms of area or coverage of production (FAO, 2022). In Nepal, potato holds the topmost position among non-cereal crops, cultivated in 198,256 ha of land with yield of 3,410,829 Mt (MoALD, 2023). The Nepal Agricultural Research Council (NARC) recommended 12 improved varieties of potatoes, along with enhanced production techniques. These improved varieties demonstrated higher yields compared to the local varieties traditionally grown by farmers (Gairhe et al., 2017). Many farmers are not getting the expected returns from their continuous increased potato productivity due to higher postharvest losses encountered.

After harvest, produce remain metabolically active and undergoing all possible physiological processes which must be controlled to prolong the post-harvest quality (Brasil & Siddiqui, 2018). Post-harvest handling practices of any horticultural crops refer to the set of activities employed to slow the degradation process in order to maintain the quality of the produce, specifically fruits and vegetables, after they have been harvested. Immediately after harvest, activities such as trimming, cleaning/washing, sorting, grading, pre-cooling, sometimes curing, packing and packaging are performed as field/packhouse operations (Ait-Oubahou et al., 2019; Gautam et al., 2019 as cited in Gautam, 2020). These practices maintain product quality and extend the shelf life of the harvested crops. Consumers seek produce that is not only visually appealing but also firm to the touch, possesses a pleasing taste, and is nutritionally valuable (Adhikari & Aarati, 2021). The reduction of post-harvest losses has been recognized as a crucial element undertaken by various organizations to combat food insecurity (Kiaya, 2014). To minimize losses, adopting various cultural practices, implementing effective management strategies, and employing appropriate handling techniques are crucial.

Shelf life of vegetables can be enhanced by controlled environment, modified atmosphere with low-temperature settings or use of chemicals that delay senescence and inhibit microbial decay (Colelli & Amodio, 2016). Post-harvest technology is widely acknowledged as a vital component of a comprehensive approach to agriculture in meeting the increasing global demands for food and food security. Hence the need for postharvest loss reduction (Kiaya, 2014).

The yield of potato crop in Nepal is 17.2 Mt ha^{-1} (MoALD, 2023) which is close to the global average of 20.74 Mt ha^{-1} (FAO, 2023). The main objective is to reduce losses and maintain the best possible quality for consumer satisfaction. This can be accomplished by increasing adequate storage capacity for increased potato production coupled with controlling the factors causing post-harvest losses. this study was carried out to (1) find how farmers in the Dolakha district practice the harvesting of potatoes, (2) to estimate the post-harvest losses from farm to storage level to gain an understanding of the real scenario of the post-harvest phase, and (3) to assess the effect of sociodemographic factors on post-harvest losses.

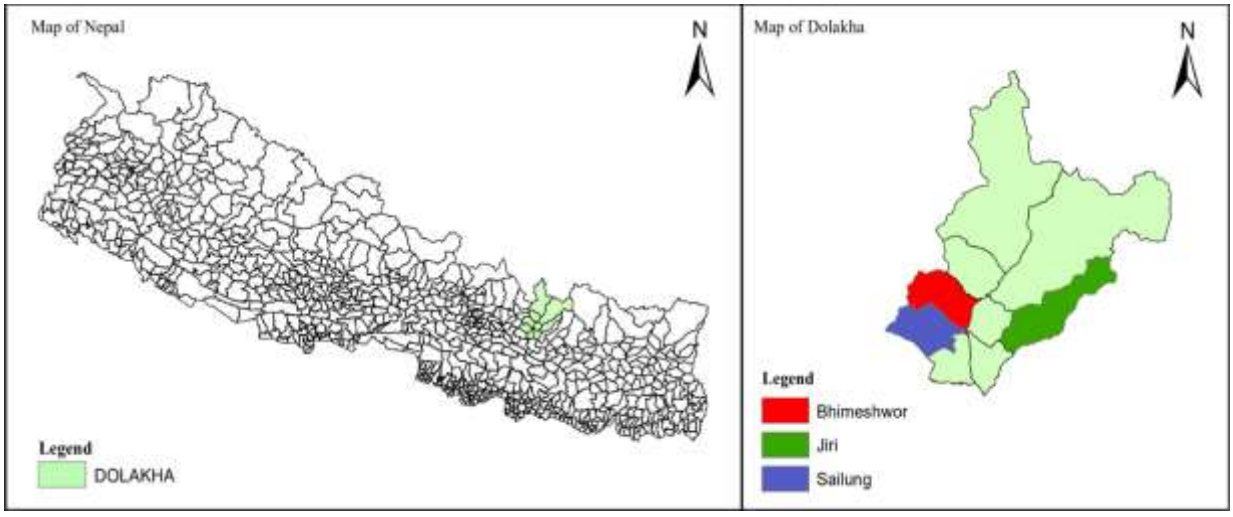

Fig. 1. Maps of Nepal and Dolakha showing study sites.

MATERIALS AND METHODS

Study area

The study area is highlighted in Figure 1. The research was carried out in the Dolakha district of Nepal (geographical coordinates of the site: 27.6693N and 86.0428E) located 1975 meters above sea level. The study area comprised wards 3, 6, and 8 of Sailung Municipality, ward 9 of Bhimeshwor Municipality, and ward 6 of Jiri Municipality. Potato farming is emphasized in these areas by the Government of Nepal through the establishment of the Potato Zone under the Prime Minister Agriculture Modernization Project (PMAMP).

Study design

A preliminary survey was carried out to collect data regarding the feasibility of the research viz. the demographic and socio-cultural characteristics of the study site. Potato growers of Sailung Municipality and Bhimeshwor Municipality were selected purposively as those areas were included in the Potato Zone by PMAMP.

Sampling procedure

A total of 100 potato growers were selected by simple random sampling technique to draw a representative sample.

Data Collection

For this study, a semi-structured questionnaire was used. The questions were written in English and aimed to accomplish the three (3) specific objectives as outlined at the detail end of the introduction in chapter one. Before the final survey, pretesting was done on 20 farmers of Ward 3, Sailung municipality.

Data analysis

Data entry, coding, and analysis were done using Statistical Package for Social Science (SPSS) and Microsoft Excel (MS Excel). Descriptive statistics namely, means, percentages, and frequencies, were used to present the results of the study regarding the three (3) specific objectives. The regression tool was used to determine the factors affecting post-harvest loss and to test the association among various dependent and independent variables.

RESULTS AND DISCUSSION

Socio-demographic characteristics of the respondents

The socio-demographic characteristics of the respondents are shown in Figure 2. Although a study done by Chauhan et al. (2022) on potato farmers in the Darchula district showed a male-domination of 65%, our study area was determined to be a female-dominated one representing 52%. The age of the respondents ranged from 21 to 79 years with the majority (31%) belonging to the age group of 26 to 35 while the average age was found to be 45.18 years (Table 1). The most respondents belonged to the Brahmin ethnic group (67%) while the least belonged to the Dalit ethnic group (3%). Respondents belonged to two religious groups, Hindu (64%) and Buddhist (36%).

The average years of experience in potato farming of the respondents was determined to be 23.87 years (Table 1). Regarding the education level, 27% of respondents lacked basic reading and writing skills and had no formal education while most of the respondents (30%) were literate with a primary level of education and a small percentage (4%) had a secondary level education. Contrasting to this, the majority of potato farmers in the Achham district, accounting for 32%, had a secondary level of education (Sapkota et al., 2019).

With a range of 0.2 to 3.56 ha, the land area owned varied considerably. The average land size of the respondents was 0.53 ha. The relatively high standard deviation of 0.45 ha indicates a considerable diversity in land ownership within the district (Table 1).

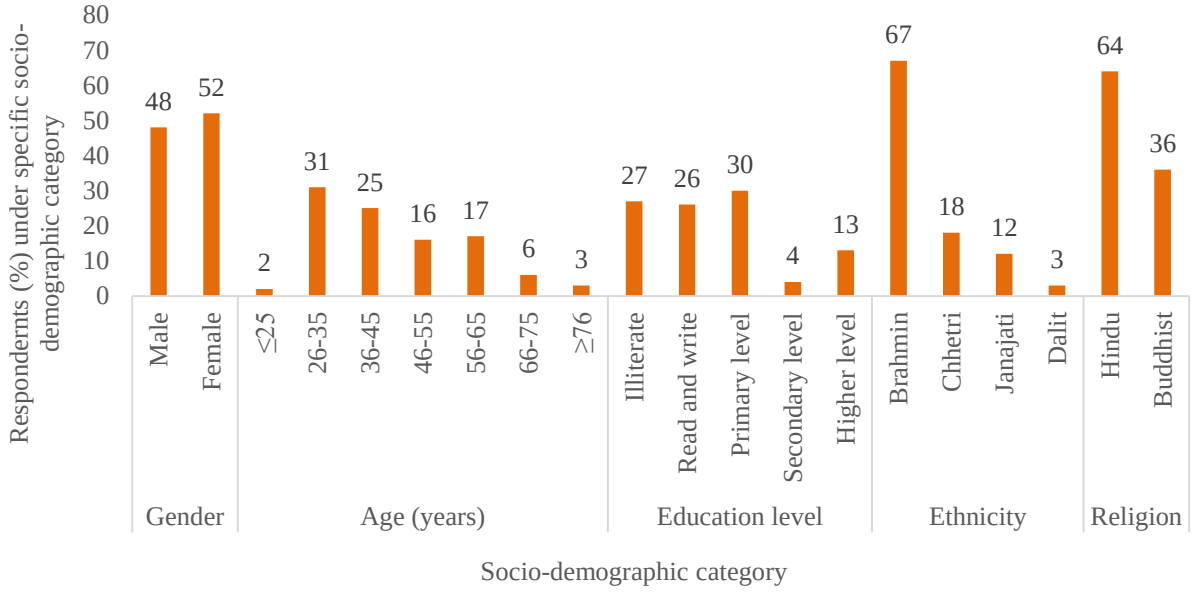

Fig. 2. Socio-demographic characteristics of respondents in the study area.

Table 1. Descriptive statistics of the socio-demographic status of farmers in the study area.

Category	Minimum	Maximum	Mean	SEm±	SD
Age (years)	21	79	45.18	1.40	13.96
Household size	2	10	5.29	0.17	1.74
Experience (years)	10	60	23.87	1.07	10.80
Land size (ha)	0.20	3.56	0.53	0.045	0.45

Note: SEm± = Standard Error of mean, SD = Standard Deviation

Table 2. Average cost of production of potato per hectare in the study area.

Cost item	Average cost (NRs ha^{-1})	Contribution to total cost (%)
Seed Tuber	15,960	25.81
Land	1,573	2.54
Human Labor	25,729	41.60
Irrigation	2,249	3.63
Equipment (Mini-tiller)	11,412	18.46
Fertilizer	4,210	6.80
Insecticides	700	1.13
Herbicides	0	0
Total	61,834	100

Cost of potato production

The cost of production is one of the primary factors that significantly influence the profitability of agricultural production (Subedi et al., 2019). The total average cost of production of potatoes in the study area was calculated to be NRs. 61,834 per hectare (Table 2). The cost of labor was found to be contributing a major portion to the total cost of production followed by seed tuber cost accounting for 41.60% and 25.81% respectively. According to the study on the economics of potato production and marketing in the Darchula district, seed tuber cost occupied the major portion (33.3%) of the cost of production followed by the human labor cost (26.3%) (Subedi et al., 2019). Human labor is required for various cultivation practices like planting, weeding, earthing-up, fertilizer, weedicide, insecticide application, harvesting, and storage. The cost incurred by fertilizer and irrigation were 6.80% and 3.63% of the total cost of production which aligns with the results reported by Subedi et al. (2019) who observed the chemical fertilizers costs for 7% and irrigation costs for 1.7% . The average cost incurred on using mini-tiller was NRs 11,412. Other costs incurred are given in Table 2. It was interesting to note that there was significantly minimal use of chemical fertilizer and insecticides and no use of herbicides at all in the study area, which corresponds with the findings of Bajracharya and Sapkota (2017).

Table 3. Farmers' use pattern of potatoes in the study area.

Particulars	Average of usage pattern (Kg)	SD	Percentage (%)
Seed Tuber	1430	1299.38	15.04
Potato Sold	3828	1733.28	40.29
Family Consumption	2641	3385.50	27.79
To Relatives	628	308.90	6.60
Total	8527	6727.06	89.73
Total Production including loss	9502	4976.74	100

Note: SD = Standard Deviation

Use pattern of potato

Harvested potatoes were utilized in different ways as shown in Table 3. On average, the potato produced was 9,502 kg per hectare out of which, 89.73% was utilized in various ways which includes storing for seed tubers, selling, family consumption and gifting to relatives. The remaining 10.26% of potatoes were lost due to different factors such as suboptimal storage, cut and bruising during harvesting, damage caused by insect pests and transportation. About 27.79% of the potatoes were used by the farming families for their consumption and additionally, 6.60% were given as gifts to other people. A major portion, accounting for 40.29%, was sold during the harvesting period. This finding is in line with the work of Hossain & Miah (2009), who revealed that a significant portion of the total potato produced goes for marketing (62.04%) whereas the lowest percentage of potato produced was expended as gift to relatives (0.52%). The work from the Northern zone of Bangladesh by Hossain et al. (2014) also reported similar results on the marketing of potatoes (69.2%). Another 15.04% of the potatoes were stored traditionally at home or in cold storage as seed tuber for planting in the next season. Hossain and Miah (2009) also found that 12.73% of the potatoes were stored in cold storage, specifically as seed tuber for the next planting season.

Post-harvest handling practices

The distinctive approaches for post-harvest handling practices adopted by the farmers of Dolakha district are shown in Table 4. It was found that almost all the respondents (100%) relied on the manual method for harvesting i.e. use of spades since a mechanical harvester was not available in the study area. Post-harvest curing {essentially a wound-healing operation to replace the damaged periderm (outer-most/cork layer) prior to storage} in shade for a week, was found to be adopted by 59% of the respondents. The practice of cold storage was rare (3%). The reason behind this might be the limited availability and accessibility of cold storage facilities as only one cold storage plant was available in the study area and it too was limited in size. Accessibility to this cold storage was challenging for most farmers due to its considerable distance from their individual farm locations. To remove the damaged potatoes during the storage period, a routine check every 15 days was practiced by 55% of respondents. Prior to packaging, it was found that almost all the respondents performed sorting to sort out the pest-infested and rotten potatoes and then graded them based on shape and size. For marketing, the primary choice of farmers was found to be jute sacks for containment. It was found that all the respondents (100%) practiced overfilling during packing and dragging of jute sacks during loading and unloading. Maremera (2014) reported that inadequate storage facilities and improper handling practices lead to higher post-harvest losses.

Table 4. Farmers' handling practices of potatoes in the study area.

Practices	No. of respondents
Harvesting by Spades	100
Curing for a week	59
Traditional method of storage	97
Cold storage	3
Dragging	100
Overfilling	100
Checking of stored potatoes at 15 day intervals	55
Sorting and grading	100
Use of jute sack for packaging	100

Table 5. Average loss of potato at different stages of post-harvest operation in the study area.

Category	Average loss (kg)	SD	Average loss (%)	Percentage of total production
Rejected During Sorting	321.2	145.53	32.96	3.38
Cut Potato	157.35	73.38	16.15	1.66
Rotten Potato	144.9	82.52	14.87	1.52
Curing Loss	101.55	41.04	10.42	1.07
Pest damage	92	45.07	9.44	0.97
Transportation Loss	79.5	35.7	8.16	0.84
Potato Remained in Soil	77.9	34.52	7.99	0.82
Total	974.4	457.76	100	10.26

Note: SD = Standard Deviation.

Table 6. Effect of socio-demographic factors on post-harvest losses in the study area.

Regression variable	Regression coefficient	p-value	Standard error
Intercept	βo	0.01	243.01
Gender	0.03	0.05	53.27
Age	0.11	0.01	2.29
Education level	-0.03	0.15	25.29
Ethnicity	-0.07	0.17	38.09
Religion	0.06	0.40	57.67
Land size	0.75	0.00	3.40
Experience	-0.16	0.05	2.52
No. of observation		100	
R^2		0.48	
F (100,7)		14.09	

Losses at different post-harvest operations

Basically, physical, biological, and environmental factors which include extreme temperatures, mechanical injuries, pests, and pathogens are responsible for causing post-harvest losses (Clark et al., 2004; Degebasa, 2020). The losses can occur during harvesting, cleaning, sorting, grading, other handling operations including packaging, transportation, storage, distribution and processing (Degebasa, 2020). The post-harvest losses of potatoes at different stages of post-harvest operations in the study area are shown in Table 5. On average, total post-harvest losses incurred comprised 10.26% of total production. Potatoes were manually harvested using spades due to which 1.66% of the harvest showed cuts and bruises. As a result, those potatoes became unfit for marketing. Harvested potatoes were then cured under the shade. The average loss due to curing was found to be 1.07% which was higher than the findings of Hossain and Miah (2009) i.e. 0.88%.

In the conventional approach of storage, farmers regularly monitored stored potatoes at an interval of 10 to 15 days to sort out those that had damaged from pest infestation or decaying of those that had signs of bruising and cutting. However, in this method, farmers' lack of technical knowledge and capability to maintain the proper temperature and humidity results in the loss of potatoes due to disease and pest damage (Hossain & Miah, 2009). Throughout the storage period, the rotten potatoes accounted for 1.52% of the overall harvest whereas those lost due to pest damage accounted for 0.97%. Before transporting them to the market, farmers performed sorting and grading of potatoes based on shape and size, during which a major portion i.e. 3.38% of potatoes were discarded for their unmarketable smaller size, normally not preferred by buyers/consumers. The average loss due to transportation was found to be 0.84% and the potatoes unintentionally left unharvested under the soil at the time of harvesting was estimated to be 0.82% of the overall or total harvest.

Effect of socio-demographic factors on post-harvest loss of potato at different stages of the post-harvest operations

Table 6 represents the results of the linear regression model for determining the effect of socio-demographic factors on post-harvest losses of potatoes in the study area. The influence of sex was found positive and significant at 5% level of significance. This finding indicates that male-dominated households were likely to experience more post-harvest losses as compared to female-dominated households. The post-harvest loss was positively influenced by the age of farmer and was statistically significant at 5% level of significance indicating that younger farmers faced less loss as compared to older ones.

Both farming experience and education level of farmers had a negative effect on post-harvest loss but only the influence of farming experience was significant at 5 % level of significance. Experienced farmers exhibit good knowledge and successful integration of post-harvest handling technologies among other farmers (Tadesse et al., 2018). Regarding the losses, the experience that the farmers had accumulated over the years proved to be more significant and might have compensated for the gap in education.

The effect of religion and ethnicity of respondents on post-harvest loss was found to be non-significant at 5 % level of significance. However, land size for potato production was notably significant at 1% significance level showing a positive correlation with the post-harvest loss. This indicated that for every 1% increase in the size of land, the potato loss would be increased by 7.49%.

CONCLUSION

The post-harvest losses of potatoes occur at different stages of post-harvest operation. Around one-tenth of produced potatoes are lost in the Dolakha district because of improper harvesting and poor post-harvest handling practices. Insect damage, the absence of cold storage and poor handling practices like prolonged curing, overfilling of potato sacks, dragging of heavy bags, and improper sorting and grading lead to a considerable loss in quantity. Farmers mainly cultivate potatoes for commercial purposes followed by family consumption and then for storage as seed tuber for the next season. The post-harvest loss was significantly affected by age, gender, land size, and farming experience of farmers whereas the literacy level, ethnicity, and religion had no impact on it.

Conflict of interest

The authors have no conflict of interest to report.

Acknowledgement

As the corresponding author of this paper, I would like to acknowledge Agriculture and Forestry University (AFU), Nepal and Prime Minister Agriculture Modernization Project (PMAMP), Nepal for providing technical support to carry out the research study.

REFERENCES

Adhikari, B. & Aarati, G.C. (2021). Post-harvest practices of horticultural crops in Nepal: Issues and management. *Archives of Agriculture and Environmental Science, 6(2),* 227-233. https://doi.org/10.26832/24566632.2021.0602015

Ait-Oubahou, A., Brecht, J. K., & Yahia, E. M. (2019). Packing Operations. *Postharvest Technology of Perishable Horticultural Commodities,* 311-351. https://doi.org/10.1016/b978-0-12-813276-0.00009-2

Colelli, G., & Amodio, M. L. (2016). Factors Affecting Quality and Safety of Fresh-Cut Fruits and Vegetables, In *Fresh-Cut Fruits and Vegetables*, 255–282. CRC Press. https://doi.org/10.1201/9781315370132-19

Bajracharya, M., & Sapkota, M. (2017). Profitability and productivity of potato (*Solanum tuberosum*) in Baglung district, Nepal. *Agriculture & Food Security*, *6*(1). https://doi.org/10.1186/s40066-017-0125-5

Brasil, I. M., & Siddiqui, M. W. (2018). Postharvest Quality of Fruits and Vegetables: An Overview, In *Preharvest Modulation of Postharvest Fruit and Vegetable Quality*, 1–40. Academic Press. https://doi.org/10.1016/b978-0-12-809807-3.00001-9

Chauhan, B., Joshi, D., Banjade, D., Bhatta, B. D., Awasthi, P., Paneru, M., Shrestha, M., & Chand, P. B. (2022). Economics of potato (*Solanum tuberosum* L.) production and marketing in Darchula district of Nepal. *Archives of Agriculture and Environmental Science*, *7*(3), 393–401. https://doi.org/10.26832/24566632.2022.0703013

Clark, D., Klee, H., & Dandekar, A. (2004). Despite benefits, commercialization of transgenic horticultural crops lags. *California Agriculture*, *58*(2), 89–98. https://doi.org/10.3733/ca.v058n02p89

Degebasa, A. C. (2020). Prospects and challenges of postharvest losses of potato (*Solanum Tuberosum* L.) in Ethiopia. *Global Journal of Nutrition and Food Science*, *2*(5). 1-10. https://doi.org/10.33552/gjnfs.2020.02.000550

de Haan, S., & Rodriguez, F. (2016). Potato Origin and Production. *Advances in Potato Chemistry and Technology*, 1–32. Second Edition. Academic Press. https://doi.org/10.1016/b978-0-12-800002-1.00001-7

FAO. (2022). Retrieved from: https://www.fao.org/statistics/en

FAO. (2023). FAOSTAT. Retrieved from: http://www.fao.org/faostat/en/. Date of access: October 29, 2023

Gairhe, S., Gauchan, D., & Timsina, K. (2017). Adoption of improved potato varieties in Nepal. *Journal of Nepal Agricultural Research Council*, *3*, 38–44. https://doi.org/10.3126/jnarc.v3i1.17274

Gautam, D. M. (2020). Postharvest Handling and Storage of Vegetables. In *Comprehensive Insights in Vegetables of Nepal* (pp. 1601-1608). Nepal Academy of Science and Technology.

Gautam, D. M., Bhattarai, D. R., & Acharya, U. (2019). *Postharvest management of horticultural crops in Nepal*. Paper presented at 10th National Horticulture Seminar, Kirtipur, Kathmandu, February 7-9.

Hossain, M. A. & Miah, M. A. M. (2009). *Post-Harvest Losses and Technical Efficiency of Potato Storage Systems in Bangladesh.* Bangladesh Agricultural Research Institute (BARI), Farm Machinery and Postharvest Process Engineering Division. Bangladesh: USAID.

Hossain, M. A., Zomo, S. A., Ullah, A., Rahaman, S. M. S., & Sarkar, M. D. (2014). Production and Grower Preference of Potato in Northern Zone of Bangladesh: Scenario from Shibgonj, Bogra and Kalai, Joypurhat. *Journal of Bioscience and Agriculture Research*, *1*(2), 93–101. https://doi.org/10.18801/jbar.010214.11

Maremera, G. (2014). Assessment of vegetable postharvest losses among smallholder farmers in Umbumbulu area of Kwazulu-Natal Province, South Africa. *University of KwaZulu-Natal*.

MoALD. (2023). Statistical Information on Nepalese Agriculture.

Sapkota, S. C., Raj Rokaya, P., Acharya, H., & Uprety, S. (2019). An Economic Analysis of Potato Production in Achham District of Nepal. *International Journal of Horticulture & Agriculture*, *4*(2), 1–9. https://doi.org/10.15226/2572-3154/4/2/00131

Subedi, S., Ghimire, Y. N., Gautam, S., Poudel, H. K., & Shrestha, J. (2019). Economics of potato (Solanum tuberosum L.) production in terai region of Nepal. *Archives of Agriculture and Environmental Science*, *4*(1), 57–62. https://doi.org/10.26832/24566632.2019.040109

Tadesse, B., Bakala, F., & Mariam, L. W. (2018). Assessment of postharvest loss along potato value chain: the case of Sheka Zone, southwest Ethiopia. *Agriculture & Food Security*, *7*(1). https://doi.org/10.1186/s40066-018-0158-4

Kiaya, V. (2014). Post-harvest losses and strategies to reduce them. *Technical Paper on Postharvest Losses, Action Contre la Faim (ACF)*, *25*, 1-25.

JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH
2024, VOL. 7(1), 69-80

Journal of Horticulture and Postharvest Research

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

University of Birjand

Postharvest management practices by roadside orange sellers in Oyo State, Nigeria

Olufemi Bolarin[1], Gbolagade Benjamin Adesiji[1], Sijuade Adebukola Adebayo[1] and Sola Emmanuel Komolafe[1*]

[1]Department of Agricultural Extension and Rural Development, University of Ilorin, Ilorin, Nigeria

ARTICLE INFO

Original Article

Article history:
Received 13 December 2023
Revised 15 February 2024
Accepted 16 February 2024

Keywords:
Orange
Postharvest loss
Postharvest management
Roadside marketing
Weather condition

DOI: 10.22077/jhpr.2024.7041.1348
P-ISSN: 2588-4883
E-ISSN: 2588-6169

***Corresponding author:**

Department of Agricultural Extension and Rural Development, University of Ilorin, Ilorin, Nigeria.

Email: kemmas04@yahoo.com

ABSTRACT

Purpose: This study reported for the first time, the postharvest management practices and estimated lost by roadside orange sellers in Oyo state, Nigeria. **Research Method:** Multistage sampling procedure was employed to select 120 respondents. A structured questionnaire was used to collect data. Data collected for specific objectives were analyzed and presented in frequency, percentages, mean, and standard deviation while the multiple regression analysis was performed to assess hypothesis of the study. **Findings:** Results showed that the roadside orange sellers had low level of education as 25.0% had no formal education while 47.5% had primary education. Under tent/umbrella (60.8%) are the main tent used to display oranges long the roadside. High profitability (81.7%) was the leading factors motiving marketing of oranges along the road side. The marketers (95.8%) moderately used postharvest management practices (PHMPs). The orange sellers incurred high loss of oranges up to 14.2% stored. Unfavourable weather condition ($\bar{x}$=2.12) was the lead constraints to use PHMPs. Multiple linear regression showed that years of experience, average number of orange stocked and age showed significant relationship with the use of PHMPs. **Limitations:** There was no limitation. **Originality/Value**: Roadside orange sellers in Oyo state, Nigeria were moderate users (95.8%) of postharvest management practices and incurred 14.2% lost.

INTRODUCTION

A large portion of fresh fruits are lost worldwide after harvest. The main causes are physiological (wilting, shriveling and chilling injury, etc), pathological (decay due to fungi and bacteria) and physical (mechanical injury) (Adhikari, 2021). These causes, in most instances can be interrelated, that is, mechanical injury can lead to post harvest decay in many cases (Al-Dairi et al., 2022). Losses are estimated at 20 to 40% in developing countries and 10 to 15% in developed countries, depending on the crop and the season (Kahramanoğlu et al., 2021). The problem of postharvest loss is dreadful for fruits like orange. Oranges (*Citrus Sinensis*) is a member of the citrus family besides limes, lemons, tangerine and grapefruits.

Orange is one of affordable fruits in Nigeria, a major source of vitamin C and a choice fruit for roadside merchants and hawkers (Okungbowa et al., 2022). Its major products include orange juice, concentrates, fresh squeezed juice, smoothies and marmalades. Orange, like any other perishable fruits and is susceptible to wastage and losses in Nigeria. Postharvest loss in orange fruit production is due to improper care and use of inappropriate harvesting equipment with high postharvest losses occurring during harvesting, transportation, marketing, storage, display for sale and sometimes glut in the market with poor demand (James et al., 2017). Most of the packaging materials are not suitable for the fruits and this leads to mechanical damage. The use of public transportation for both passengers and commodities, over loading and stacking, high temperature, bad roads with high vibration and collision, lack of vehicle, high cost of transportation, lack of sorting the ripe and unripe fruit, poor storage facilities are factors that lead to most of the post- harvest loss of orange fruit (Aminu et al., 2021; James et al., 2017).

Orange selling is common among the roadside fruit sellers in Afijio Local Government area (LGA), Oyo State. Orange is sold as a major source of cash income for households (Aminu et al., 2021). Post-harvest losses of fruits are considered to be a major problem that affects many fruit sellers in Nigeria (Obayelu et al., 2022). It is based on this that the study sought to understand postharvest management practices of roadside orange sellers in Afijio LGA, Oyo state, Nigeria.

The general objective of this study is to assess the postharvest management practices of roadside orange sellers in Afijio local government area, Oyo state, Nigeria. The specific Objectives are to: (i) examine the socio-economic characteristics of roadside orange sellers, (ii) ascertain factors motivating roadside orange selling, (iii) identify the postharvest management practices used roadside orange sellers, (iv) estimate the quantity of oranges lost by roadside sellers, and (v) identify the constraints that hinder the use of postharvest management practices in Afijio, LGA, Oyo State. The hypothesis (null form) of the study states that there is no significant relationship between the socio-economic characteristics of roadside orange respondents and assessment of postharvest management practices. Even though studies have recently assessed postharvest management practices of orange sellers globally (Dooga et al., 2021; Pérez Romero et al., 2021; Strano et al., 2022), no empirical research has presented the management practices and the quantity lost by roadside orange sellers. Thus, this study is reporting for the first time, the postharvest management practices of roadside orange sellers and the quantity lost in Nigeria.

MATERIALS AND METHODS

This study was carried out at Afijio Local Government Area (LGA) in Oyo State, South-western geopolitical zone, Nigeria. Afiji LGA has an area of 722km^2. The population for the

study comprises of all the roadside orange sellers in Afijio local government area, Oyo state, Nigeria.

Multistage sampling was used to select respondents for the study. The first stage involved the purposive selection of four (4) geographical wards in Afijio LGA. The selection is based on high rate of orange selling activities along the road side. Names of the selected wards were Fiditi, Ilora, Awe and Jobele. The population of orange sellers in the four selected wards was 245 persons distributed across Fiditi (51), Ilora (39), Awe (26), and Jobele (129). The second stage involved a random selection of 50% of the population, Fiditi (25), Ilora (20), Awe (13), and Jobele (65). This gave a total sample size of one hundred and twenty-two (122) sellers of orange along the road side used as respondents.

A structured interview schedule was used to collect data. Content validity was performed by experts in the field of agricultural extension and rural development. The instrument was considered reliable to collect data after a Cronbach alpha coefficient of 0.74 was obtained through test and re-test method. Of the 122 instruments administered, 120 were retrieved, completely filled and analysed. The interview schedule for postharvest management practices were developed by authors from review of similar studies (Aminu et al., 2021; Strano et al., 2022), and can be found in the supplementary file.

Postharvest management practices used by respondents were measured on 4 point Likert-type scales as: always used=3, sometimes used=2, rarely used=1, and never used =0. Constraints to use of postharvest management practices for orange were measured 3 point Likert-type scale as: very severe=2, severe=2, not severe=1. Data collected for specific objectives were analyzed and presented in frequency, percentages, mean, and standard deviation while the multiple regression analysis was performed to assess hypothesis of the study. Ordinary least square regression model was adopted. The model was specified implicitly thus:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 + \ldots + e_i \ldots\ldots (1)$$

Where; Y= postharvest management used by respondents, (X) = Independent variables, X_1 = Age (in years) , X_2 = sex (male=1, female =0), X_3 = Marital status (married=1, otherwise 0), X_4 = Household size (in numbers), X_5 = Education (Formal=1, no formal=0) , X_6 = Membership (yes 1, no 0) , X_7 = Average number of orange stocked (number) , e = Error term

RESULTS AND DISCUSSION

Socio-economic characteristics of respondents

Results presented in Table 1 showed that the average age of the respondents was 43.3 years. This indicates that the marketers were still in their productive and economic age range. This buttressing the findings that orange farming and marketing activities were mostly practiced by middle-aged individuals in Nigeria (Ikwuba et al., 2019; Aminu et al., 2021). Majority (89.2%) were female, indicating that females dominate road side orange marketing in the study area. This is in line with report that similarly found orange marketing was dominated by females (Obayelu et al., 2022).

On their marital status, 19.2% were married 12.5% were single, 10.0% were divorced while most (58.3%) of the respondents were widowed with household size of approximately 5 persons. Regarding education of the respondents, 25.0% had no formal education while 47.5% had primary school education. This implies that the orange marketers had low level of education. This finding is in line with report by Dooga et al. (2021) who found that most orange marketers had primary school education. The average year of orange marketing

experience (years) was 5.8 years. Majority (97.5%) were not member of a marketers' group. The average monthly income was N7158.33 as majority (78.3%) borrowed to market orange and majority (60.8%) also perform the marketing under tent /umbrella.

Table 1. Socio-economic characteristics of respondents.

Variables	Frequency	Percentage	Mean (SD)
Age (years)			
≤ 30	12	10.0	
31 – 40	36	30.0	43.3(9.45)
41 – 50	39	32.5	
51 and above	4	27.5	
Sex			
Male	13	10.0	
Female	107	89.2	
Marital status			
Married	23	19.2	
Single	15	12.5	
Divorced	12	10.0	
Widow	70	58.3	
Level of Education			
No Formal Education	30	25.0	
Primary Education	57	47.5	
Secondary Education	3	2.5	
Tertiary Education	30	25.0	
Household size (persons)			
1 – 5	22	18.3	
6 – 10	70	58.3	4.9(1.94)
11 and above	28	23.3	
Orange marketing experience (years)			
1 – 5	67	55.8	
6 – 10	48	40.0	
11 and above	5	4.2	5.8(2.89)
Membership of any orange sellers association			
Yes	3	2.5	
No	117	97.5	
Average income from orange selling per month (Naira)			
≤ 5000	26	21.7	7158.33(226.06)
5001 – 10,000	94	78.3	
Source of finance			
Owned	26	21.7	
Borrowed	94	78.3	
Others			
Place of sales/ display ripened orange by road side			
Shop	4	3.3	
Under tent/umbrella	73	60.8	
Wheel barrow	43	35.8	

Source: Field survey, 2023.

Table 2. Factors motivating respondents into roadside orange selling.

Motivating Factors	Very True	True	False	Mean	SD	Rank
It is profitable	98(81.7)	13(10.8)	9(7.5)	2.73	0.62	1
It has many health benefits	84(70.0)	34(28.3)	2(1.7)	2.68	0.5	2
To increase their income	72(60.0)	46(38.3)	2(1.7)	2.58	0.53	3
There are no upfront processing cost	72(60.0)	44(36.7)	4(3.3)	2.57	0.56	4
Oranges are in high demand and there are supply for it	60(50.0)	49(40.8)	11(9.2)	2.41	0.65	5
Oranges are food	42(35.0)	69(57.5)	9(7.5)	2.28	0.59	6

Source: Field survey, 2023.

Perceived factors motivating roadside orange selling in Afijio LGA

Factors motivating them into roadside orange selling are presented in Table 2. Findings showed that majority of the respondents strongly agreed orange marketing is profitable (81.7%), It has many health benefits (70.0%), orange marketing increase income with no upfront processing cost (60.0%) while significant percentage also agreed that orange are in high demand and there are supply for it (50.0%) and orange are in the category of food industry (35.%). This finding indicates that profitability, health benefits and income with no upfront processing cost were the main factors motivating the road side orange marketers in the study area.

Postharvest management practices used by orange road side sellers

Postharvest management practices used in orange road side business of the respondents are presented in Table 3. Results shows that all (100.0%) of the respondents clean, sort and keep in fridge/freezer. Also, majority of the respondents always used harvesting at correct stage of maturity (98.3%), use sack to package for transport (86.7%), motor van to transport (76.7%), hanging in open space for fresh air (79.2%), cover with paper materials and woven sheet/sack (66.7%). These findings imply that the prominent postharvest management practices of road side orange marketers in the study area were harvesting/buying orange at correct stage of maturity, cleaning, sorting, packaged by sack, transport by motor van, preserve by hanging in open space for fresh air and keeping in fridge/freezer, and ripened by cover with paper materials and woven sheet/sack. Finding on mode of transportation agrees with report by studies who found that orange markers used pickup/truck van to transport orange (Aminu et al., 2021; Adekalu et al., 2019).

Individual respondents were grouped by score obtained on the postharvest management practices. Table 4 showed that 2.5% were grouped having low usage of orange postharvest management practices, 95.8% were grouped having moderate use of orange postharvest management practices while 1.7% were grouped having high orange postharvest management practices. This indicates that the road side orange marketers in the study area were moderate users of orange postharvest management practices.

Table 3. Postharvest management practices used by respondents.

S/n	Stage/activities of marketers	Postharvest management practices	Frequency of usage				Mean	SD
			Always used	Sometimes used	Rarely used	Never used		
1		Harvesting at correct stage of maturity	118(98.3)	2(1.7)			3.98	0.13
2		Harvesting at correct time of the day	37(30.8)	82(68.3)	1(0.8)		3.30	0.48
3	Cleaning	Cleaning	120(100.0)				4.00	0
4	Sorting	Sorting	120(100.0)				4.00	0
5	Packaging for transport:	Carton	8(6.7)	37(30.8)	74(61.7)	1(0.8)	1.43	0.63
		Wood box	0	2(1.7)	21(17.5)	97(80.8)	1.21	0.45
		Sack	104(86.7)	15(12.5)	1(0.8)		3.86	0.37
		Basket	38(31.7)	75(62.5)	4(3.3)	3(2.5)	3.23	0.63
4.	Transportation	Bicycle/motorbike	15(12.5)	38(31.7)	45(37.5)	22(18.3)	2.38	0.93
		Tricycle	2(1.7)	63(52.5)	30(25.0)	25(20.8)	2.35	0.83
		Motor van	92(76.7)	20(16.7)	5(4.2)	3(2.5)	3.68	0.68
		On head	5(4.2)	6(5.0)	64(53.3)	45(37.5)	1.76	0.73
5	Preservation	Clay pot	2(1.7)	118(98.3)	0	0	1.05	0.39
		Fridge/deep freezer	120(100.0)	0	0	0	1.00	0.00
		Under tree shade	36(30.0)	48(40.0)	10(8.3)	26(21.7)	2.78	1.10
		Hanging in open space	95(79.2)	24(20.0)	1(0.8)	0	3.78	0.43
6	Ripening:	Cover with leafy materials	2(1.7)	0	2(1.7)	116(96.7)	1.58	1.07
		Cover with paper materials	80(66.7)	32(26.7)	5(4.2)	3(2.5)	1.07	0.40
		Cover with woven sheet (sack)	80(66.7)	32(26.7)	5(4.2)	3(2.5)	3.58	0.69

Source: Field survey, 2023.

Table 4. Level of postharvest management practices.

Obtained score range	Level	Frequency	Percentage	Mean
19 – 38	Low	3	2.5	
39 – 57	Moderate	115	95.8	50.02±2.75
58 – 76	High	2	1.7	
Total		120	100.0	

Possible score range 19 –76.

Table 5. Estimate of postharvest loss of orange by respondents.

	Freq.	%	Mean (SD) Min. – Max.	Percent
Number of orange stocked			1,070.7 (1643.03) 3,30 -10,000	
≤ 1000	115	95.8		
Above 1000	5	4.2		
Number of orange sold			9,18.1 (201.38) 3,15 – 1,080	% sold= 85.8
≤ 400	9	7.5		
401 – 800	35	29.2		
801 and above	76	63.3		
Number of orange unsold/spoilt			152,04 (4.88) 10 – 180	% unsold= 14.2
1 – 10	8	6.7		
11 – 20	18	15.0		
21 and above	94	78.3		
Management of unsold/spoilt orange				
Throw-away	110	91.7		
Gift to people	10	8.3		

Source: Field survey, 2023.

Estimated quantity of oranges lost by roadside sellers

Information presented in Table 5 presents the estimate of postharvest loss of orange by respondents. Findings showed that that respondents stocked average of 1,070.7 oranges, sold average of 9,18.1 oranges which accounted for 85.8% sales while the unsold/spoilt oranges was 152,04 oranges which accounted to 14.2% lost. This finding suggests that 14.2% loss is high among the marketers, as the need to adopt more postharvest strategies to reduce the loss incurred. This value is lower than 18.24% postharvest loss of orange found by Okpe et al. (2022) in a study among orange marketers in Benue state. Management of the unsold/spoilt oranges showed that majority (91.7%) throw-away the unsold while few give it out to people. Throw-away practice that is common among the marketers may not be the best way to manage spoilt oranges, thus extension agents may need to train them on conversion practices that can still bring income.

Constraints to using post-harvest management practices

Constraints to use postharvest management practices were presented in Table 6. Findings showed that unfavourable weather condition ($\bar{x}$=2.12) ranked first position, insect pest infestation ($\bar{x}$=1.79) ranked second position, insufficient distribution and lack of ripening techniques ($\bar{x}$=1.65) ranked third position while inadequate supply of electricity ($\bar{x}$=1.29) ranked seventh position as the least constraints indicated by respondents. These findings showed that unfavourable weather condition, insect pest infestation and insufficient distribution and lack of ripening techniques were the main constraints hindering the use of postharvest management practices among roadside orange sellers in the study area.

Table 6. Severity of constraints to use of postharvest management practices.

Constraints	Very severe	Severe	Not severe	Mean	SD	Rank
Unfavourable weather condition	41(34.2)	52(43.3)	27(22.5)	2.12	0.75	1
Insect pest infestation	30(25.0)	35(29.2)	55(45.8)	1.79	0.82	3
Insufficient distribution and lack of ripening techniques	11(9.2)	57(47.5)	52(0.8)	1.65	0.66	4
Improve practices are not easier to use	4(3.3)	60(50.0)	56(46.7)	1.57	0.56	5
Lack of financial incentives from the government	7(5.8)	49(40.8)	64(53.3)	1.53	0.61	6
Inadequate supply of electricity	6(5.0)	23(19.2)	91(75.8)	1.29	0.56	7

Source: Field survey, 2023.

Table 7. Linear regression showing socioeconomic determinants of postharvest management practices used by respondents.

Use of postharvest management practices	Coef. (β)	Std. Err.	t-stat.	Sig (p-value)
Age (years)	-0.017*	0.007	-2.497	0.014
Sex	0.004	0.013	0.289	0.773
Marital status	0.008	0.03	0.253	0.801
Education	0.007	0.029	0.246	0.806
Household size (persons)	-0.031	0.028	-1.093	0.277
Experience (years)	0.041*	0.02	2.039	0.044
Membership of association	0.159	0.263	0.604	0.547
Average number of orange stocked	3.36E-05*	0	2.103	0.038
(Constant)	3.214	0.261	12.309	0.000

R square 0.595, R square Adjusted 0.529, Std. Error of the estimate =0.368.

Relationship between socioeconomic characteristics of respondents and postharvest management practices used by respondents

The results of linear regression showing socioeconomic determinants of postharvest management practices used by respondents in Table 7 showed that years of experience (β = 0.041) and average number of orange stocked (β = 3.36) showed positive significant relationship while age (β = 0.017) indicated negative significant relationship. This implies that increased years of experience in orange marketing and increase quantity of orange stocked when other variable remain constant leads to increase use of postharvest management practices among the road side orange marketers in the study area.

CONCLUSION

This study assessed postharvest management practices by roadside orange sellers in Afijio LGA Oyo State, Nigeria. Based on major findings, the study concluded that the roadside orange marketers in the study area were moderate users of orange postharvest management practices. The prominent postharvest management practices by roadside orange sellers in the study area were harvesting/buying orange at correct stage of maturity, cleaning, sorting, packaged by sack, transport by motor van and preservation by hanging in open space for fresh air and keeping in fridge/freezer, and ripened by cover with paper materials and woven sheet/sack. The orange sellers incurred high loss of oranges up to 14.2% stocked. Unfavourable weather condition, high spoilage and insect pest infestation were the main constraints hindering the use of postharvest management practices among road side orange sellers in the study area.

The following recommendations were drawn based on conclusions made: (i) This finding suggests that 14.2% loss is high among the marketers, thus, there is need for extension agents by the Nigeria Store Product Research Institutes (NSPRI) to disseminate affordable improved technology to control the unfavourable weather condition and insect pest infestations of oranges to the road sellers in order to reduce the high losses incurred. (ii) Throw-away practice that is common management practice for the unsold/spoilt oranges. This is likely no a productive way to manage spoilt oranges, thus extension agents may need to train the road side orange sellers on conversion practices that can still bring income.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgments
The authors highly appreciate the lecturers in the Department of Agricultural Extension and Rural Development, University of Ilorin, Nigeria for supporting this work.

REFERENCES

Adekalu, O. A., Agboola, D. A., Atanda, S. A., & Akande, S. (2019). A survey of post-harvest techniques employed in handling and storage of sweet oranges (*Citrus sinensis* Osberk) in Lagos, Ogun and Osun States, Nigeria. *Asian Journal of Agriculture and Food Sciences, 7*(1), 20-30.

Adhikari, B. (2021). Post-harvest practices of horticultural crops in Nepal: Issues and management. *Archives of Agriculture and Environmental Science, 6*(2), 227-233. http://doi.org/10.26832/224566632.2021.0602015

Al-Dairi, M., Pathare, P. B., Al-Yahyai, R., & Opara, U. L. (2022). Mechanical damage of fresh produce in postharvest transportation: Current status and future prospects. *Trends in Food Science & Technology, 124*, 195–207. https://doi.org/10.1016/j.tifs.2022.04.018

Aminu, F.O., Balogun, E.O.S., & Ojo, O.O. (2021). Analysis of post-harvest losses in orange in Lagos metropolitan markets, Nigeria. *Nigerian Journal of Horticultural Science, 25*(2020), 143-151.

Dooga, M., Agada, P. O., & Ogwuche, I. O. (2021). Ordinal regression Assessment of orange postharvest loss determinants among orange farmers in Konshisha local government area of Benue State. *Nigerian Journals of Pure and Applied Sciences, 4*(1), 220–231. https://doi.org/10.46912/napas.243

Ikwuba, A. A., Wegh, F. S., Agwaza, T. A., & Angera, R. I. (2019). A study on sweet orange production and its post-harvest losses among farmers in northeastern zone of Benue State, Nigeria. *Journal of Biological Sciences and Bioconservation, 11*(3), 64-105.

James, D., Bamishayi, O. M., Shamsuddeen, J., Williams, O., Olukunde, T., & Zainab, M. (2017). Analysis of the level of post- harvest losses in orange marketing case study of Yanlemo orange market in Kano State. *International Journal of Agriculture and Earth Science, 3*(4), 17-24.

Kahramanoğlu, İ., Rengasamy, K. R. R., Usanmaz, S., Alas, T., Helvacı, M., Okatan, V., Aşkın, M. A., & Wan, C. (2021). Improving the safety and security of fruits and vegetables during COVID-19 pandemic with postharvest handling. *Critical Reviews in Food Science and Nutrition,* 1–11. https://doi.org/10.1080/10408398.2021.1935703

Obayelu, O. A., Dairo, D. O., & Olowe, O. O. (2022). What factors explain postharvest losses of orange fruit (*Citrus sinensis*) from farm to fork in the tropics? *Agricultura Scientia, 19*(1), 7–15. https://doi.org/10.18690/agricultura.19.1.7-15.2022

Okpe, P. C., Atagher, M. M., & Iheanacho, A. C. (2022). Effect of socio-economic characteristics on postharvest losses among sweet orange marketers in Benue State, Nigeria. *Journal of Agripreneurship and Sustainable Development (JASD), 5*(2), 130-149.

Okungbowa, A. I., Chukwuemeke-Nwani, P. O., Mwesigwa, L. S., Omoarelojie, F. O., & Adesina-Ojobaro, A. G. (2022). Comparative determination of vitamin C content of fresh fruit juice of selected 'expensive' and 'less expensive' fruits in okha market in Benin city, Edo state, Nigeria. *BIU Journal of Basic and Applied Sciences, 7*(1), 53–61, 2022.

Pérez Romero, L. F., Robles Domínguez, J. K., Pizarro Pariona, L. D., & Casimiro Soriano, E. M. (2021). Evaluation of postharvest loss of oranges (*Citrus sinensis*) in the central jungle of Peru. *Trends in Horticulture, 4*(1), 133. https://doi.org/10.24294/th.v4i1.1808

Strano, M. C., Altieri, G., Allegra, M., Di Renzo, G. C., Paterna, G., Matera, A., & Genovese, F. (2022). Postharvest technologies of fresh citrus fruit: advances and recent developments for the loss reduction during handling and storage. *Horticulturae, 8,* 612. https://doi.org/10.3390/horticulturae8070612

QUESTIONNAIRE

Research Instrument
Survey Identification Information
Questionnaire no: …….
Name of Community: …….

SECTION A: Socio-economic characteristics of the road side orange sellers

1. Age ……. (years)
2. Sex: Male ( ) Female ( )
3. Marital status: Married ( ) Single ( ) Divorced ( ) Widowed ( )
4. Level of Education: Primary ( ) Secondary ( ) Tertiary ( ) No formal education ( )
5. Household size (total number of people): ……..... (persons)
6. Orange marketing experience: …….(years)
7. Membership of any orange sellers association: Yes ( ) No ( )
8. Average income from orange selling per month …… Naira
9. Other sources of income: Farming ( ), Artisan ( ), Others ……..
10. What is your source of finance? Owned ( ) borrowed ( ), Others…….
11. Place of sales/ display ripened orange by road side: Shop ( ), under tent ( ), wheel barrow ( ) others ……

12. Please indicate other fruits available for sales

OTHER FRUITS AVAILABLE FOR SALES	**YES**	**NO**
Apple		
Watermelon		
Citrus		
Banana		
Plantain		
Coconut		
Mango		
Pear		
Avocado		
Others:		

SECTION B: Factors motivating them into roadside Orange selling in Afijio LGA

13. Kindly indicate the extent to which the following constitutes factors motivating them into Roadside orange selling. **T- TRUE, VT- VERY TRUE, F- FALSE**

MOTIVATING FACTORS	VERY TRUE	TRUE	FALSE
It is profitable			
To increase their income			
Orange are in high demand and there are supply for it			
It has many health benefits			
Orange are in the category of food industry			
There are no upfront processing cost			
Others:			

SECTION C: Postharvest Management Practices Used by Orange Road Side Sellers
14. Please indicate Postharvest management practices you use in orange road side business

S/ n	Stage/activities of marketers	Postharvest management practices		Frequency of usage		
			Always used	Sometimes used	Rarely used	Never used
1		Harvesting at correct stage of maturity				

2		Harvesting at correct time of the day				
3	Cleaning	Cleaning				
4	Sorting	Sorting				
5	Packaging for transport:	Carton				
		Wood box				
		Sack				
		Basket				
		Others……				
4.	Transportation	Bicycle/motorbike				
		Tricycle				
		Motor van				
		On head				
5	Preservation	Clay pot				
		Fridge/deep freezer				
		Under tree shade				
		Hanging in open space				
		Others.....				
6	Ripening:	Cover with leafy materials				
		Cover with paper materials				
		Cover with woven sheet (sack)				
		Others…… ……				

SECTION D: Estimating quantity of orange loss among road side sellers in Afijio LGA

16. What quantity of Orange do you often purchase or harvest in stock for sales per month ……..... (Number)
17. What quantity of Orange sold per month: …….... (Number)
18. What quantity of Orange spoilt and unsold per month: …… (Number)
19. Kindly indicate what you often do with spoilt unsold orange:……

SECTION E: Constraints to use of postharvest management practices

20. Please indicate constraints that have hinder you to use postharvest management practices

Constraints	**Very severe**	**Severe**	**Not severe**
Inadequate supply of electricity			
Unfavourable weather condition			
Insufficient distribution and lack of ripening techniques			
Lack of financial incentives from the government			
Improve practices are not easier to use			
Insect and rodent bite			
High spoilage			

JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH
2024, VOL. 7(1), 81-98

Journal of Horticulture and Postharvest Research

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

University of Birjand

The effects of foliar application of some important micronutrient elements on essential oil content and components of *Dracocephalum kotschyi* Boiss.

Mehrab Yadegari[1*]

[1]*Research Center of Nutrition and Organic Products (R.C.N.O.P), Shahrekord Branch, Islamic Azad University, Shahrekord, Iran*

ARTICLE INFO

Original Article

Article history:
Received 23 December 2023
Revised 23 February 2024
Accepted 29 February 2024

Keywords:
Geranial
Iron
Limonene
Myrtenol
Zinc

DOI: 10.22077/jhpr.2024.7079.1351
P-ISSN: 2588-4883
E-ISSN: 2588-6169

***Corresponding author:**

Research Center of Nutrition and Organic Products (R.C.N.O.P), Shahrekord Branch, Islamic Azad University, Shahrekord, Iran.

Email: mehrabyadegari@gmail.com

ABSTRACT

Purpose: *Dracocephalum kotschyi* Boiss. belongs to the Lamiaceae family, is one of the important and endangered endemic species in Iran. The present study was conducted to investigate the effects of micronutrient elements application on the content and composition of essential oil of *D.kotschyi* Boiss. shoots in southwestern Iran (Shahrekord) in 2022 and 2023. **Research Methods:** Four foliar fertilizers including Fe, Cu, Zn and Mn were applied in 0, 20, 40 and 60 $mg.l^{-1}$ in RCBD design by factorial layout and 3 replications. **Findings:** Results obtained from gas chromatography/mass spectrometry (GC-MS) showed 14 essential oil components. According to obtained results, applied micronutrients significantly influenced the essential oil content/ composition of *D.kotschyi.* In both years, the highest content of essential oil (0.98-0.99 %) was obtained in plants treated with 40 $mg.l^{-1}$of micronutrients ($Fe_2Cu_2Zn_2Mn_2$) and the lowest content (0.59-0.66%) made by control plants (0 $mg.l^{-1}$), however the plants treated by 60 $mg.l^{-1}$of micronutrients in most characters were in a same group with the control plants. The most important chemical compounds that determine the quality of *D. kotschyi* essential oil including Neral (9.02-16.31%), Limonene (25.4-35.6%), Geranial (8.6-16.5%), Eucalyptol (3.89-8.01%) and Myrtenol (22.5-32.1%) were identified alcoholic monoterpenes. Limonene belonging to monoterpene hydrocarbons was the predominant constituent of the *D.kotschyi.* **Limitations**: There were no limitations to the report. **Originality/Value**: The foliar application of micronutrients at 40 $mg.l^{-1}$ (Fe, Cu, Zn and Mn) can be a good strategy to improve the essential oil quantity and quality of *D.kotschyi* in cold and semi-arid climates.

INTRODUCTION

Dracocephalum kotschyi Boiss., locally known as Badrandjboie-Dennaie and Zarrin-Giah in Persian, belongs to the Lamiaceae family, is one of the endangered endemic species in Iran (Mozaffarian, 2008). The extreme harvesting, limited distribution, and no commercial farming of *D.kotachyi* have led to the danger of its extinction which is one of the most important challenges. The essential oil and extract from *D. kotachyi* have medicinal properties including anti-hyperlipidemic, immunomodulatory, anti-spasmodic, antinociceptive, cytotoxic (Ashrafi et al., 2017) and antimicrobial (Fallah et al., 2020) effects. Furthermore, the aerial parts and inflorescences of *D.kotachyi* are used for the treatment of pain, fever, headache, inflammation, congestion, stomach, and also seizures. In traditional Iranian medicine, the boiled aerial parts and inflorescences of the herb is used to heal wounds and relieve rheumatic pain (Golparvar et al., 2016; Samadi et al., 2018). This plant is rich in essential oil, flavonoids, monoterpene glycosides, trypanocidal terpenoids, rosmarinic acid, and linolenic acid. The main compounds of the essential oil of *D.kotschyi* are α-pinene, limonene, carvacrol, geranyl acetate, myrtenol, methyl geranate, γ-terpinene, perilla aldehyde, eucalyptol, nerol, and germinal (Taghizadeh et al., 2020; Sonboli et al., 2019), while those found in naturally grown plants were two main compounds: cyclohexylallene (52.63%) and limonene (35.88%) (Ghavam et al., 2021). The environmental factors cause changes in the growth, quantity, and quality of active substances (such as alkaloids, glycosides, steroids and volatile oils) in the medicinal and aromatic plants and by increasing of dry matter, the essential oil percentage increased (Shaykh-Samani et al., 2023a, b; Cham et al., 2022). Aromatic and volatile products of plant secondary metabolism are used in the pharmaceutical, chemical, cosmetic, and food industries. In recent years, there has been an increasing interest in the use of natural substances due to concerns about the safety of some synthetic compounds, which have encouraged more detailed studies on originated substances (Fallah et al., 2020; Pradhan et al., 2017).

When nutrient deficiency cannot be corrected through soil application, foliar nutrition is adopted as an alternate method (Marschner, 1995). It has been shown that micronutrients such as Fe, Mn, Zn and Cu are necessary for plant intensification in much lower amounts for plant intensification than those of the primary nutrients (Bilal et al., 2020).

Four important micronutrients used in medicinal plants are Fe, Cu, Mn and Cu. Iron (Fe) is one of the essential nutrient elements needed by plants and is a key element in cytochrome structure. In addition, plants treated with this micronutrient produce more yield (Majeed et al., 2020). Copper (Cu) is another essential microelement in higher plants as it occurs as part of the prosthetic groups of several enzymes. Zinc (Zn) is a building block of many proteins and an important chemical element in biological activity. Zn acts on enzymatic activation and cell division, so it has been shown that its deficiency causes cell damage, low protein and carbohydrate synthesis, impaired growth and development, and low crop yields (Alamer et al., 2020; Cakmak et al., 2017; Figueiredo et al., 2017). Manganese (Mn) is involved in many biochemical functions, primarily acting as an activator of enzymes such as dehydrogenases and decarboxylases involved in respiration, amino acid and lignin synthesis, and hormone concentrations (Alejandro et al., 2020). Foliar application of these micronutrients have important effects in morpho-physiological attributes such as chlorophyll, phenol and relative water content that made more essential oil content and composition in *Rosa damascena* (Yadegari, 2023), *Thymus* (Yadegari, 2022), *Satureja* (Bani Taba et al., 2022), *Dracocephalum moldavica* (Yadegari, 2021), *Carthamus tinctorius* (Galavi et al., 2012), *Calendula officinalis* L. *Borago officinalis*, *Alyssum desertorum* and *Thymus vulgaris*

(Yadegari, 2015, 2017a), *Anethum graveolens* (Rostaei et al., 2018), *Matricaria chamomilla* (Nasiri et al., 2010) and *Coriandrum sativum* (Said-Al Ahl & Omer, 2009).

Foliar fertilization is particularly useful to meet the basic needs of plants for one or more micro- or macro- nutrients, especially trace minerals. It also helps correct deficiencies, strengthen weak or damaged crops, and enhance growth (Aziz et al., 2019). The aim of this research was to determine the effects of foliar applications of iron, zinc, copper and manganese on essential oil content and composition in *D.kotschyi* Boiss. To introduce the best combination of these micronutrients for better yield in this multipurpose plant.

MATERIALS AND METHODS

Plant material and fertilizers

Four foliar fertilizers including Librel Fe-Lo, Librel Cu, Librel Zn and Librel Mn were applied and all of them are mineral fertilizers. Librel Fe-Lo contains 13.2% chelated iron, Librel Zn is a foliar fertilizer that contains 14% Zinc in chelated form, Librel Cu has 14% copper in chelated form and Librel Mn contains of 13% Mn chelated with EDTA obtained from the chemical company of Germany (BASF). Theses fertilizers were sprayed at three concentrations (for example Fe_1, Fe_2 and Fe_3 were concentrations of Fe which had 20, 40 and 60 $mg.l^{-1}$of Fe, respectively (Yadegari, 2023, 2017a, b). The concentrations were similar in other micronutrients). The foliar application was done in three stages once every 10 days (before harvest) in the early morning. The control plants had no any micronutrient and distilled water foliar application. For soil analysis, the soil samples were taken from three randomly selected sites in each plot from 0-15 and 15-30 cm of depth. The samples were homogenized, mixed and passed through a 2 mm filter for determination of soil physical and chemical characteristics (Table 1). Soil moisture was measured by a TDR device (PMS-714, Lutron, Taiwan) following the manufacturers' protocol.

Experimental conditions

This investigation was done and repeated in two years from spring (May) 2022 to fall (September) 2023 at the Research Farm of Islamic Azad University, Shahrekord Branch, Iran. Based on the Köppen climate classification, the climate of the area of study is classified as cold and semiarid. The present study was conducted based on a randomized complete block design (RCBD) with three replications and factorial layout. In each year, treatments were applied in stage of plants had 4-8 leaves in 3 steps by 1 week interval. The soil (typic calci xerocrepts) physical and chemical properties and climatic properties of the region are listed in Table 1 and 2 respectively. The top-soil of the experimental plot area was kept moist throughout the growing season when necessary.

Plant material and agronomic practices

Seeds of *D.kotschyi* Boiss. (Lamiaceae) were obtained from Forest and Rangeland Institute, Iran. Firstly, the seeds were sterilized and sown on May 2022 and 2023 under greenhouse condition (25°C, light 12/12 day/night, 65% RH). After about 45-50 days from sowing, when the seedlings had 4-6 true leaves with 8-10 cm tall, were transplanted in the experimental field on 20 July. The dimensions of each experimental plot were 4.0 × 3.0 m and the distance between plants on row and replicates were 20 cm and 2 m respectively. No inorganic fertilizer and systemic pesticide were used during the experiment, and weeds control was done manually. Irrigation (in 75-80% F.C., field capacity) was done every 5 to 7 days.

Table 1. Physico-chemical soil properties of research farm in two years.

Characters	Year	
	2022	2023
N_{total} (%)	0.18	0.25
Organic matter (%)	0.75	0.83
pH	7.82	7.73
P ($mg \cdot kg^{-1}$)	16.44	15.55
K ($mg \cdot kg^{-1}$)	312	308
Ca ($mg \cdot kg^{-1}$)	3.45	3.78
Mn ($mg \cdot kg^{-1}$)	8.44	8.34
Fe ($mg \cdot kg^{-1}$)	1.02	1.91
Cu ($mg \cdot kg^{-1}$)	0.55	0.43
Zn ($mg \cdot kg^{-1}$)	0.82	0.79
EC ($dS \cdot m^{-1}$)	0.62	0.65
Texture	Clay loam	Clay loam

Table 2. Climatic properties of research farm.

Average annual precipitation (mm)	Average of annual temperature (ºC)	Average maximum temperature (ºC)	Average minimum temperature (ºC)	Elevation (m)	Latitude and longitude
331	11.7	23.9	-1.8	2060	32˚19/N-50˚51/E

Preparation of essential oil (EO) extraction

The aerial parts and inflorescences of *D.kotschyi* were hand-harvested at the flowering and then were dried in the shade at room temperature (25±4°C) for two weeks with the moisture content fixed at around 14 to 16%. The samples were ground to fine a powder using a micro hammer cutter mill and passed through a sieve (mesh 20). The essential oil was extracted from 100 g of powdered tissue by hydro-distillation method using the Clevenger-type (made by Glass Fabricating of Ashk-e-Shishe Co., Tehran, Iran) with 500 mL water for 3 h according to the British Pharmacopoeia. Essential oil content was determined by distilling shoots in the Clevenger apparatus. The content of 100 g of deried shoots was placed in 6 L flask capacity Clevenger type distillation apparatus. And distilled for 5 h with 3 L of pure water. The oil content of *D.kotschyi* Boiss. obtained at the end of distillation and measured as mL and % ratios (w/w) then determined by multiplying the oil content with oil density i.e., 0.858. All the essential oil samples were dehydrated over hydrous sodium sulphate and stored at 4°C until analyzed by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) analysis. Ground GC analysis was done on an Agilent Technologies 7890 GC equipped with FID and a HP-5MS 5% capillary column. The carrier gas was helium at a flow of 0.8 mL/min. Initial column temperature was 60°C and programmed to increase at 4°C/min to 280°C. The split ratio was 40:1. The injector temperature was set at 300°C. The purity of helium gas was 99.99% and 0.1 mL of each sample was injected manually in the split mode. GC–MS analyses were carried out on a Thermo Finnigan Trace 2000 GC-MS system equipped with an HP-5MS capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm). The oven temperature was held at 120°C for 5 min and then programmed to reach 280°C at a rate of 10°C/min. The detector temperature was 260°C and the injector temperature was 260°C. The compositions of the essential oil were identified by comparison of their retention indices relative to a series of *n*-alkanes (C7-C24), retention times and mass spectra with those of authentic samples in Wiley library (Adams, 2007).

Data analysis

After Bartlett test, all data were subjected to complex ANOVA and simple Pearson correlation indices using the statistical computer package SAS v.9 and treatment means separated using LSD's multiple range test at P≤0.05 and P≤0.01 levels.

RESULTS AND DISCUSSION

Essential oil content

The results showed that the effects of foliar-spraying on essential oil content were significant ($p \leq 0.01$) (Table 3). The maximum essential oil content was obtained in the foliar-sprayings of 40 mg.l^{-1} (0.98-0.99%), whereas the minimum (0.59-0.66%) was achieved in the control (Tables 4-6). In this investigation, the use of 40 mg.l^{-1} improved the content of *D.kotschyi* essential oil.

Chemical composition of the essential oil

According to the results of the chemical analysis of the essential oils from *D.kotschyi* by GC/MS, the most important chemical compounds that determine the quality of *D.kotschyi* essential oil including neral (9.02-14.11%), limonene (25.4-31.6%), geranial (8.6-15.2%), eucalyptol (3.89-6.32%), myrtenol (22.5-26.95%), α-pinene (1.34-3.08%), α-campholene aldehyde (1.16-2.99%) and geranic acid methyl ester (0.67-1.6%) were identified (Tables 4-6). Limonene belonging to monoterpene hydrocarbons was the predominant constituent of the *D.kotschyi* essential oil (Tables 4-6). The simple effects of the foliar application of each micronutrient were significant on the percentages of some of the major compounds of the essential oil (Table 3). In general, the foliar application of micronutrients in 40 mg.l^{-1} increased the percentage of all compounds compared to the control (Tables 4-6). The interaction effects of micronutrients (Fe, Zn, Cu and Mn) on the concentrations of constituents of essential oil such as neral, geranial, limonene, Myrtenol and eucalyptol ($p \leq 0.01$) was significant (Table 3). The highest levels of neral, limonene, eucalyptol, myrtenol and geranial were obtained under 40 mg.l^{-1} treatments (14.11, 31.6, 15.2, 6.32 and 26.95%, respectively) (Tables 4-6). In this study, the quality of essential oil, which is expressed as a percentage of the chemical compounds, showed a significant increase under concentration of micronutrients such as higher concentration of micronutrients than 40 mg.l^{-1} in most components, had decreasing effects. According to obtained results, application of micronutrients significantly influenced the essential oil content/composition of *D.kotschyi*, however the assessed traits showed slightly variation during the studied years. In most of measured characters, plants treated with 20 and 40 mg.l^{-1} were in the same group. Amounts of some characters in plants treated with 60 mg.l^{-1} were less than control plants. The main constituents of essential oil were neral, limonene, myrtenol, geranial and eucalyptol (alcoholic monoterpenes) that made the most components of essential oil plants. Applied combination of micronutrients showed better effectiveness on essential oil content and composition of *D.kotschyi* and the highest essential oil content was obtained from the treated plants by 20 and 40 mg.l^{-1} of these micronutrients. According to the biennial results of the chemical analysis of the essential oils from *D.kotschyi* by GC/MS, the most important chemical compounds that determine the quality of *D.kotschyi* essential oil including neral, limonene, geranial, eucalyptol and myrtenol were identified. Finally, the application of micronutrients at 40 mg.l^{-1} can be a good strategy to improve morpho-physiological characters and essential oil quantity and quality of *D.kotschyi* in cold and semi-arid climates. The results obtained from GC-MS indicated the presence of 14 components in the essential oil of *D.kotschyi* and in this regard, significant differences in chemical compositions of essential oil were observed between

treatments (Table 3). Although in some main treatments such as foliar application of Cu and Mn, no significant difference in chemical compositions of *D.kotschyi* essential oil was found, however, in combined treatments there were differences in most compositions of essential oil and in this regard, the $Fe_2Cu_2Mn_2Zn_2$ treatment was the best treatment (Tables 4-6). The main components in the essential oil of all plants treated by micronutrients included α-pinene, P-cymene, limonene, eucalyptol, δ-terpinene, myrtenol, neral, geraniol and α.campholene aldehyde. The most amounts in some of constituents such as eucalyptol, p-cymene, carvacrol and geranyl acetate were made by plants that treated by 40 $mg.l^{-1}$ of Fe, Zn and Mn but in another components of essential oil, the most amounts were obtained by 40 $mg.l^{-1}$ of Fe, Zn, Mn and Cu (Tables 4-6). Treatments of 60 $mg.l^{-1}$ of micronutrients in many of constituents had the same group with control plants and the lowest amount of components made by this treatment. In some cases such as the lowest amounts of geranic acid, neral, geraniol, and geranial made by Mn or Cu in 20 $mg.l^{-1}$, but generally the control plants and plants treated with combination of 60 $mg.l^{-1}$of Fe, Mn, Zn and Cu in two cultivation seasons made the lowest levels of essential oil content and compositions (Tables 4-6). By comparing the data of two years, there were positive correlations between essential oil content and main components and a high correlation was observed between essential oil content and α-pinene, p-cymene, limonene, eucalyptol, δ-terpinene, myrtenol, neral, geraniol and α.campholene aldehyde (Table 7). The application of concentration of 20 $mg.l^{-1}$micronutrients improved most of the content and compositions of components but at higher concentrations (i.e. 60 $mg.l^{-1}$), the content and compositions in all treated plants were decreased. The mean content (%) of many chemical compositions in *D.kotschyi* were lower than those of the control treatment when the plants were sprayed with concentrations of 60 $mg.l^{-1}$. It seems that the content and composition of essential oil were more affected by Zn and Fe compared to other micronutrients (Tables 4-6).

Table 3. Complex ANOVA of variation of essential oil content and main compositions in *D.kotschyi* by different micronutrients.

SOV[z]	df[y]	Neral	Limonene	α.Pinene	Carvacrol	Caryophyllene	δ-Terpinene	P-Cymene
Year(Y)	1	14.1**	0.008^{ns}	27.2**	7.7^{ns}	1.4^{ns}	2.72**	15.1**
R/Y	4	0.4	0.009	1.3	8.7	3.1	0.8	0.2
Copper (Cu)	3	0.5^{ns}	0.11**	1.1^{ns}	32.5**	21.9**	24.8**	21.3**
Manganese (Mn)	3	5.2**	0.03^{ns}	14.4**	2.6^{ns}	21.6**	12.8**	31.1**
Iron (Fe)	3	4.9**	0.4**	2.7^{ns}	32.8**	9.8**	22.9**	21.4**
Zinc (Zn)	3	22.2**	0.29**	2.9^{ns}	22.6**	21.5**	12.3**	11.9**
Cu × Mn	9	54.2**	0.9**	14.4**	12.9**	21.1**	23.8**	32.8**
Cu × Fe	9	32.4**	0.7**	8.9**	16.8**	11.4**	23.9**	25.4**
Cu × Zn	9	23.8**	0.21**	8.7**	13.1**	13.3**	17.8**	22.1**
Mn × Fe	9	27.1**	0.51**	9.1**	19.9**	11.9**	27.7**	14.1**
Mn × Zn	9	3.1**	0.3**	10.7**	12.8**	14.9**	23.7**	24.9**
Fe × Zn	9	7.4**	0.4**	7.8**	10.1**	13.9**	23.5**	23.2*
Cu × Mn × Fe	27	2.1**	0.2**	8.91**	12.5**	21.4**	0.94^{ns}	27.8**
Cu × Mn × Zn	27	1.6**	0.8**	14.6**	10.8**	6.1**	1.2^{ns}	21.9**
Cu × Fe × Zn	27	2.22**	0.9**	1.2^{ns}	11.4**	0.9^{ns}	0.2^{ns}	0.7^{ns}
Fe × Zn × Mn	27	2.6**	0.011^{ns}	1.9^{ns}	15.6**	0.7^{ns}	0.1^{ns}	0.8^{ns}
Cu × Zn × Mn × Fe	81	0.32^{ns}	0.01^{ns}	1.7^{ns}	1.9^{ns}	0.1^{ns}	0.8^{ns}	0.9^{ns}
T(Cu,Zn,Mn,Fe) × Y	255	0.18^{ns}	0.021^{ns}	0.5^{ns}	0.42^{ns}	0.22^{ns}	0.3^{ns}	0.28^{ns}
E	1020	0.37	0.02	1.5	1.3	0.88	1.1	1.4
CV[x]		2.2	3.3	7.2	4.7	6.5	4.5	3.1

[z] SOV: source of variation, [y]df: degree of freedom, [x]CV: coefficient of variation, *, ** significant at P=0.05 and P=0.01 levels of probability respectively.

Table 3. (*Continued*).

SOV[z]	df[y]	Myrtenol	Eucalyptol	α.campholene aldehyde	Geranic acid	Geranyl acetate	Geraniol	Geranial	Essential oil
Year(Y)	1	3.3^{ns}	0.006^{ns}	18.2**	24.2**	0.88^{ns}	12.55**	0.99^{ns}	1.7**
R/Y	4	2.2	0.007	1.3	1.2	1.7	1.5	1.1	0.1
Copper (Cu)	3	1.1^{ns}	0.71**	25.1**	17.5**	33.6**	31.5**	0.32^{ns}	4.1**
Manganese (Mn)	3	15.2**	0.65**	14.4**	19.1**	21.8**	12.8**	26.1**	3.8**
Iron (Fe)	3	24.9**	0.25**	21.4**	42.8**	9.9**	18.9**	17.4**	4.5**
Zinc (Zn)	3	33.2**	0.36**	31.1**	31.6**	17.5**	12.9**	20.9**	3.3**
Cu × Mn	9	24.2**	0.44**	11.4**	11.9**	14.1**	31.8**	22.8**	8.9**
Cu × Fe	9	22.4**	0.87**	15.9**	13.8**	9.4**	25.9**	19.4**	14.8**
Cu × Zn	9	14.8**	0.29**	14.7**	15.1**	8.3**	18.8**	27.1**	11.1**
Mn × Fe	9	10.1**	0.8**	12.1**	22.9**	26.9**	17.7**	15.1**	4.7**
Mn × Zn	9	11.1**	0.8**	8.7**	12.8**	22.9**	14.7**	18.9**	8.8**
Fe × Zn	9	27.4**	1.4**	9.8**	14.1**	14.9**	8.5**	29.2**	12.1**
Cu × Mn × Fe	27	32.1**	1.2**	9.9**	9.5**	15.4**	0.31^{ns}	17.8**	4.5**
Cu × Mn × Zn	27	0.6^{ns}	2.8**	24.6**	13.8**	11.1**	0.58^{ns}	14.9**	8.1**
Cu × Fe × Zn	27	0.22^{ns}	1.8**	0.2^{ns}	14.4**	0.14^{ns}	0.42^{ns}	0.88^{ns}	9.4**
Fe × Zn × Mn	27	0.66^{ns}	0.015^{ns}	1.02^{ns}	12.6**	0.8^{ns}	0.21^{ns}	0.55^{ns}	1.4**
Cu × Zn × Mn × Fe	81	0.72^{ns}	0.014^{ns}	0.7^{ns}	0.8^{ns}	0.9^{ns}	0.18^{ns}	0.44^{ns}	0.008^{ns}
T(Cu,Zn,Mn,Fe)×Y	255	0.55^{ns}	0.011^{ns}	0.8^{ns}	0.66^{ns}	0.41^{ns}	0.44^{ns}	0.21^{ns}	0.042^{ns}
E	1020	0.88	0.01	1.1	1.3	1.02	0.88	0.91	0.072
CV[x]		5.8	7.9	4.3	3.7	2.8	4.5	3.9	2.8

[z] SOV: source of variation, [y]df: degree of freedom, [x]CV: coefficient of variation, *, ** significant at P=0.05 and P=0.01 levels of probability respectively.

Table 4. Means of essential oil content and composition (%) in *D.kotschyi* plants affected by micronutrients (20 mg.l^{-1}).

Year	Compound	RI[z]	Fe[y]	Zn	Cu	Mn	Fe×Zn	Cu×Fe	Mn×Fe	Cu×Zn
2022	α-Pinene	940	**1.8±0.01^{d}	1.4±0.02^{e}	1.37±0.03^{e}	1.39±0.02^{e}	1.41±0.01^{e}	1.4±0.02^{e}	2.9±0.02^{a}	2.4±0.02^{c}
	P-Cymene	1026	1.41±0.1^{e}	1.42±0.01^{e}	1.55±0.02^{e}	1.53±0.01^{e}	1.44±0.04^{e}	1.6±0.01^{e}	1.43±0.02^{e}	1.8±0.01^{d}
	Limonene	1033	28.8±0.8^{b}	27.7±0.9^{c}	29.8±0.5^{c}	27.7±0.8^{c}	28.7±0.9^{b}	28.4±0.9^{b}	30.1±0.8^{a}	30.5±1.1^{a}
	Eucalyptol	1035	4.7±0.1^{c}	3.94±0.1^{d}	3.95±0.1^{d}	3.99±0.2^{cd}	4.6±0.08^{c}	4.01±0.1^{c}	5.1±0.1^{b}	4.04±0.1^{c}
	δ-Terpinene	1062	1.57±0.1^{c}	1.59±0.2^{c}	1.61±0.2^{c}	1.63±0.2^{c}	1.64±0.3^{c}	1.59±0.31^{c}	1.9±0.3^{c}	1.59±0.2^{c}
	α campholene	1110	1.18±0.1^{d}	1.21±0.01^{d}	1.22±0.01^{d}	1.25±0.02^{d}	1.77±0.01^{c}	1.9±0.02^{c}	1.22±0.01^{d}	1.7±0.03^{c}
	Myrtenol	1202	22.66±1.1^{d}	22.76±0.9^{d}	23.03±0.8^{cd}	24.1±0.5^{b}	25.1±0.5^{b}	26.2±0.4^{ab}	25.1±0.7^{b}	23.1±0.5^{c}
	Neral	1239	11.7±0.1^{c}	10.7±0.8^{d}	9.02±0.7^{e}	10.44±0.6^{d}	11.66±0.8^{c}	11.1±0.8^{cd}	11.1±0.9^{cd}	10.5±0.5^{d}
	Geraniol	1257	0.8±0.01^{c}	0.77±0.01^{c}	0.66±0.03^{d}	0.7±0.02^{cd}	0.9±0.01^{c}	0.8±0.01^{c}	1.2±0.02^{b}	1.3±0.02^{b}
	Geranial	1270	11.1±0.8^{c}	8.66±0.9^{e}	8.66±0.7^{e}	8.6±0.5^{e}	8.82±0.1^{e}	11.3±0.2^{c}	11.5±0.3^{c}	12.5±0.5^{b}
	Carvacrol	1298	0.8±0.01^{c}	0.8±0.01^{c}	0.7±0.02^{c}	0.2±0.02^{d}	0.7±0.01^{c}	0.52±0.01^{d}	0.9±0.02^{c}	1.1±0.03^{b}
	Geranic acid	1320	0.67±0.1^{c}	0.69±0.01^{c}	0.7±0.02^{c}	0.72±0.01^{c}	0.73±0.02^{c}	1.1±0.01^{b}	0.9±0.02^{b}	1.3±0.03^{ab}
	Geranyl acetate	1385	0.88±0.1^{cd}	0.9±0.02^{c}	0.91±0.01^{c}	0.92±0.01^{c}	0.93±0.04^{c}	0.94±0.03^{c}	0.9±0.01^{c}	0.95±0.1^{c}
	Caryophyllene	1418	1.56±0.1^{b}	1.6±0.01^{b}	1.61±0.02^{b}	1.7±0.01^{b}	1.72±0.02^{b}	1.81±0.01^{b}	1.71±0.01^{b}	1.82±0.1^{b}
	Monoterpene hydrocarbons		33.58±0.9^{c}	32.11±0.8^{c}	34.33±1.2^{c}	32.25±1.3^{c}	33.19±0.9^{c}	32.99±1.1^{c}	36.33±1.2^{b}	36.29±0.9^{b}
	Oxygenated monoterpenes		52.94±1.5^{d}	48.84±1.2^{f}	47.24±1.4^{f}	49.28±1.6^{f}	53.55±1.8^{d}	55.83±1.7^{c}	56.12±1.6^{c}	54.24±1.5^{d}
	Sesquiterpenes		1.56±0.1^{c}	1.6±0.01^{c}	1.61±0.02^{c}	1.7±0.01^{bc}	1.72±0.02^{b}	1.81±0.01^{b}	1.71±0.01^{b}	1.82±0.1^{b}
	Essential oil content (w/w%, g/100g fresh weight basis)		0.69±0.1^{c}	0.71±0.02^{c}	0.73±0.01^{c}	0.7±0.02^{c}	0.68±0.04^{c}	0.81±0.01^{b}	0.83±0.02^{b}	0.8±0.01^{b}

Year	Compound	Mn×Zn	Cu×Mn	Cu×Fe×Zn	Fe×Zn×Mn	Cu×Mn×Fe	Cu×Mn×Zn	Cu×Zn×Mn×Fe	Control
2022	α-Pinene	2.5±0.01^{b}	2.1±0.02^{c}	3.2±0.02^{a}	3.1±0.01^{a}	3.04±0.02^{a}	3.03±0.01^{a}	2.1±0.02^{c}	1.34±0.01^{e}
	P-Cymene	1.9±0.03^{d}	1.5±0.01^{e}	2.5±0.02^{c}	2.5±0.02^{c}	2.4±0.04^{c}	2.8±0.01^{b}	3.1±0.02^{b}	1.41±0.01^{e}
	Limonene	30.2±0.9^{a}	30.5±0.8^{a}	31.6±0.8^{a}	30.6±0.7^{a}	28.8±0.9^{b}	28.8±0.8^{b}	29.7±0.7^{b}	25.5±0.8^{d}
	Eucalyptol	5.4±0.02^{b}	4.4±0.01^{c}	6.6±0.02^{a}	4.5±0.02^{c}	4.9±0.03^{c}	4.7±0.01^{c}	5.7±0.02^{b}	3.93±0.04^{d}
	δ-Terpinene	1.9±0.2^{c}	1.9±0.3^{c}	2.4±0.2^{c}	2.3±0.2^{c}	3.3±0.3^{b}	2.5±0.2^{bc}	2.73±0.2^{b}	1.55±0.2^{cd}
	α campholene	1.8±0.2^{c}	1.3±0.2^{d}	1.6±0.3^{c}	1.2±0.2^{d}	1.8±0.2^{c}	2.7±0.21^{a}	1.81±0.2^{c}	1.16±0.1^{d}
	Myrtenol	23.9±0.6^{bc}	22.7±0.9^{cd}	22.7±0.8^{cd}	23.9±0.7^{c}	24.7±0.8^{b}	23.1±0.9^{c}	24.1±0.8^{b}	22.5±0.7^{d}
	Neral	11.8±0.3^{c}	9.2±0.4^{e}	10.5±0.5^{d}	12.8±0.4^{b}	12.7±0.8^{b}	11.7±0.9^{c}	11.6±0.8^{c}	9.3±0.1^{e}
	Geraniol	0.8±0.01^{c}	1.2±0.02^{b}	1.7±0.02^{a}	1.2±0.01^{b}	0.9±0.02^{c}	0.8±0.01^{c}	0.81±0.02^{c}	0.6±0.01^{d}
	Geranial	11.9±0.7^{c}	13.7±0.8^{a}	12.7±0.6^{b}	12.9±0.5^{b}	13.7±0.7^{a}	13.1±0.7^{b}	12.1±0.6^{bc}	9.9±0.8^{d}
	Carvacrol	0.8±0.03^{c}	0.7±0.02^{c}	0.7±0.04^{c}	0.7±0.02^{c}	0.51±0.01^{c}	1.1±0.01^{b}	1.5±0.01^{ab}	0.23±0.03^{d}
	Geranic acid	0.71±0.01^{c}	0.73±0.03^{c}	0.97±0.02^{bc}	0.9±0.01^{c}	1.1±0.01^{b}	1.6±0.02^{a}	0.11±0.01^{e}	0.68±0.01^{c}
	Geranyl acetate	0.89±0.02^{c}	0.91±0.02^{c}	1.1±0.01^{bc}	0.6±0.01^{d}	0.94±0.02^{c}	0.7±0.01^{d}	0.93±0.01^{c}	0.87±0.02^{cd}
	Caryophyllene	1.86±0.01^{b}	1.89±0.03^{b}	1.11±0.01^{c}	1.93±0.01^{ab}	0.34±0.01^{e}	1.2±0.01^{c}	0.4±0.03^{e}	1.55±0.02^{b}
	Monoterpene hydrocarbons	36.5±0.9^{b}	36±0.81^{b}	39.7±1.1^{a}	38.5±0.85^{a}	39.54±0.76^{a}	37.13±0.9^{b}	37.63±1.1^{b}	29.8±0.65^{e}
	Oxygenated monoterpenes	56.4±1.5^{c}	53.2±1.2^{d}	56.5±1.6^{c}	57.2±1.7^{c}	57.21±1.4^{c}	57.2±1.3^{c}	57.62±1.4^{c}	47.62±0.9^{g}
	Sesquiterpenes	1.86±0.01^{b}	1.89±0.03^{b}	1.11±0.01^{d}	1.93±0.01^{b}	1.34±0.01^{d}	1.2±0.01^{d}	1.4±0.03^{c}	1.55±0.02^{c}
	Essential oil content (w/w%, g/100g fresh weight basis)	0.8±0.03^{b}	0.7±0.01^{b}	0.9±0.02^{a}	0.81±0.01^{b}	0.8±0.01^{b}	0.83±0.01^{b}	0.91±0.01^{a}	0.66±0.01^{cd}

Year	Compound	Fe	Zn	Cu	Mn	Fe×Zn	Cu×Fe	Mn×Fe	Cu×Zn
2023	α-Pinene	2.66±0.02^{b}	2.32±0.1^{c}	2.25±0.1^{c}	2.33±0.08^{c}	2.14±0.01^{c}	2.22±0.1^{c}	2.65±0.1^{b}	2.53±0.02^{b}
	P-Cymene	1.85±0.01^{d}	1.99±0.1^{d}	1.66±0.1^{e}	1.88±0.02^{d}	1.78±0.08^{d}	1.71±0.1^{d}	1.81±0.1^{d}	1.92±0.03^{d}
	Limonene	27.7±0.9^{c}	26.5±0.8^{c}	29.8±0.7^{b}	26.6±0.8^{c}	28.9±0.9^{b}	27.9±0.8^{c}	29.9±0.8^{b}	30.68±0.7^{a}
	Eucalyptol	3.92±0.1^{d}	4.01±0.1^{c}	4.03±0.1^{c}	3.89±0.1^{d}	4.55±0.12^{c}	3.91±0.1^{d}	4.92±0.1^{c}	4.03±0.11^{c}
	δ-Terpinene	2.66±0.3^{b}	2.88±0.2^{b}	2.82±0^{b}	2.78±0.3^{b}	3.02±0.2^{b}	2.98±0.3^{b}	3.14±0.2^{ab}	3.44±0.3^{ab}
	α campholene	1.7±0.2^{c}	1.8±0.2^{c}	1.6±0.3^{c}	1.5±0.2^{cd}	1.99±0.2^{bc}	2.4±0.3^{b}	2.1±0.2^{b}	2.2±0.3^{b}
	Myrtenol	24.15±0.9^{b}	24.6±0.8^{b}	25.6±0.9^{ab}	26.1±0.8^{a}	26.6±0.7^{a}	27.3±0.8^{a}	26.5±0.9^{a}	24.5±0.8^{b}
	Neral	11.7±0.5^{c}	10.7±0.6^{d}	10.6±0.7^{d}	10.1±0.8^{d}	11.66±0.6^{c}	11.1±0.7^{c}	11.1±0.4^{c}	10.5±0.8^{d}

	Geraniol	0.8±0.01^{c}	0.7±0.01^{c}	0.62±0.1^{d}	0.83±0.02^{c}	0.92±0.01^{c}	0.8±0.01^{c}	1.2±0.02^{b}	1.3±0.01^{b}
	Geranial	11.05±0.9^{c}	12.6±0.7^{b}	12.3±0.7^{b}	12.8±0.4^{b}	11.16±0.6^{c}	11.3±0.5^{c}	12.5±0.4^{b}	12.5±0.6^{b}
	Carvacrol	0.93±0.01^{c}	0.94±0.1^{c}	0.95±0.1^{c}	0.97±0.01^{c}	0.99±0.01^{bc}	1.01±0.1^{b}	1.02±0.2^{b}	1.1±0.01^{b}
	Geranic acid	0.9±0.02^{c}	0.92±0.1^{c}	0.94±0.1^{c}	0.96±0.01^{c}	0.97±0.01^{c}	0.98±0.1^{c}	1.01±0.1^{b}	1.03±0.02^{b}
	Geranyl acetate	1.5±0.01^{ab}	1.6±0.01^{a}	1.44±0.01^{b}	0.5±0.01^{d}	1.43±0.03^{b}	1.33±0.1^{b}	0.9±0.02^{c}	1.3±0.03^{b}
	Caryophyllene	0.8±0.02^{d}	0.6±0.01^{d}	1.61±0.01^{b}	0.7±0.01^{d}	0.66±0.01^{d}	0.9±0.02^{d}	0.1±0.01^{e}	0.7±0.02^{d}
	Monoterpene hydrocarbons	34.87±0.9^{c}	33.69±0.8^{c}	36.53±0.9^{b}	33.59±0.8^{b}	35.84±0.76^{b}	34.81±0.9^{b}	37.5±0.82^{b}	38.57±1.1^{a}
	Oxygenated monoterpenes	54.25±1.5^{cd}	55.35±1.4^{c}	55.7±1.2^{c}	56.19±1.3^{c}	57.87±1.2^{bc}	57.82±1.1^{bc}	59.34±1.4^{b}	56.13±0.9^{c}
	Sesquiterpenes	0.8±0.02^{e}	0.6±0.01^{f}	1.61±0.01^{c}	0.7±0.01^{e}	0.66±0.01^{f}	0.9±0.02^{e}	1.1±0.01^{d}	0.7±0.02^{e}
	Essential oil content (w/w%, g/100g fresh weight basis)	0.5±0.01^{e}	0.41±0.01^{f}	0.4±0.02^{f}	0.58±0.01^{d}	0.7±0.04^{c}	0.7±0.01^{c}	0.81±0.02^{b}	0.9±0.02^{a}
Year	Compound	Mn×Zn	Cu×Mn	Cu×Fe×Zn	Fe×Zn×Mn	Cu×Mn×Fe	Cu×Mn×Zn	Cu×Zn×Mn×Fe	Control
2023	α-Pinene	2.47±0.03^{c}	2.25±0.1^{c}	3.08±0.01^{a}	3.06±0.02^{a}	2.11±0.01^{c}	2.55±0.02^{b}	2.99±0.03^{a}	2.01±0.1^{c}
	P-Cymene	1.77±0.1^{d}	1.79±0.2^{d}	1.44±0.03^{e}	2.33±0.1^{c}	2.66±0.1^{c}	1.43±0.02^{e}	1.91±0.01^{d}	1.55±0.01^{e}
	Limonene	29.98±0.8^{ab}	31.1±0.65^{a}	30.65±1.2^{a}	31.01±0.64^{a}	30.8±0.98^{a}	29.9±0.69^{b}	30.09±0.9^{a}	26.01±0.8^{c}
	Eucalyptol	5.22±0.01^{b}	4.66±0.02^{c}	5.54±0.03^{b}	5.45±0.01^{b}	4.76±0.02^{c}	4.81±0.01^{c}	4.44±0.03^{c}	4.49±0.01^{c}
	δ-Terpinene	3.77±0.3^{a}	3.88±0.3^{a}	3.66±0.3^{a}	2.44±0.2^{c}	2.12±0.1^{c}	2.44±0.2^{c}	2.12±0.2^{c}	2.61±0.3^{b}
	α campholene	1.8±0.2^{c}	1.55±0.2^{c}	1.61±0.1^{c}	1.91±0.2^{c}	2.1±0.2^{b}	2.12±0.2^{b}	2.55±0.3^{ab}	1.43±0.2^{d}
	Myrtenol	24.9±0.4^{b}	25.7±0.5^{a}	24.8±0.7^{b}	25.1±0.8^{ab}	25.5±0.7^{ab}	25.1±0.8^{b}	24.1±0.7^{b}	23.1±0.6^{c}
	Neral	11.1±0.8^{c}	11.4±0.5^{c}	11.8±0.8^{c}	10.1±0.5^{d}	10.3±0.8^{d}	10.7±0.6^{d}	10.9±0.9^{d}	10.12±0.9^{d}
	Geraniol	0.96±0.01^{c}	1.2±0.02^{b}	1.6±0.02^{a}	1.85±0.01^{a}	1.83±0.02^{a}	1.77±0.01^{a}	1.79±0.02^{a}	0.99±0.01^{bc}
	Geranial	11.6±0.6	11.9±0.8	12.1±0.7	12.4±0.8	12.3±0.9	12.6±0.8	12.81±0.7	10.99±0.8
	Carvacrol	1.1±0.1^{b}	1.2±0.02^{b}	1.1±0.01^{b}	1.66±0.02^{a}	0.99±0.01^{bc}	1.3±0.01^{a}	1.91±0.01^{a}	0.91±0.02^{c}
	Geranic acid	1.1±0.01^{b}	0.14±0.03^{e}	0.91±0.02^{c}	0.77±0.01^{c}	0.4±0.01^{d}	0.32±0.02^{e}	0.41±0.01^{d}	0.89±0.01^{c}
	Geranyl acetate	1.87±0.02^{a}	0.88±0.02^{c}	0.44±0.01^{e}	0.32±0.01^{e}	0.25±0.02^{e}	0.41±0.01^{e}	0.01±0.01^{f}	1.21±0.02^{b}
	Caryophyllene	0.88±0.01^{d}	1.91±0.03^{b}	0.81±0.01^{d}	0.91±0.01^{d}	0.69±0.01^{d}	0.77±0.01^{d}	0.88±0.03^{d}	0.97±0.02^{d}
	Monoterpene hydrocarbons	37.99±0.9^{ab}	39.02±0.85^{a}	38.83±1.1^{a}	38.84±1.2^{a}	37.69±1.3^{b}	36.32±1.1^{b}	37.11±1.4^{b}	32.18±0.9^{c}
	Oxygenated monoterpenes	56.58±1.4^{c}	57.61±1.2^{bc}	58.55±1.6^{b}	58.38±1.5^{b}	57.78±1.2^{b}	58.4±1.5^{b}	58.5±1.6^{b}	52.03±0.9^{d}
	Sesquiterpenes	1.88±0.01^{b}	1.91±0.03^{b}	0.81±0.01^{e}	0.91±0.01^{e}	0.69±0.01^{f}	0.77±0.01^{e}	0.88±0.03^{e}	0.97±0.02^{e}
	Essential oil content (w/w%, g/100g fresh weight basis)	0.8±0.03^{b}	0.7±0.01^{c}	0.9±0.02^{a}	0.81±0.01^{b}	0.8±0.01^{b}	0.83±0.01^{b}	0.85±0.01^{b}	0.66±0.01^{cd}

[z] RI: Retention Indices, as determined with FID and HP-5MS 5% capillary column using a series of the standards of C7-C30 saturated n-alkanes. [y] Values are means of triplicates ± standard deviation (p <0.05)

**Numbers in each row that have same letter, have same group.

Table 5. Means of essential oil content and composition (%) in *D.kotschyi* plants affected by micronutrients (40 mg.l^{-1}).

Year	Compound	RI [z]	Fe [y]	Zn	Cu	Mn	Fe×Zn	Cu×Fe	Mn×Fe	Cu×Zn
2022	α-Pinene	940	**1.81±0.01^{d}	1.44±0.02^{e}	1.45±0.03^{e}	1.48±0.02^{e}	1.55±0.08^{d}	1.66±0.02^{d}	2.15±0.02^{c}	1.38±0.01^{e}
	P-Cymene	1026	1.9±0.01^{d}	1.71±0.01^{d}	1.82±0.02^{d}	1.94±0.01^{d}	2.1±0.04^{d}	2.41±0.01^{c}	2.38±0.02^{c}	1.91±0.02^{d}
	Limonene	1033	26.2±0.8^{c}	27.9±0.9^{c}	28.82±0.5^{c}	28.99±0.8^{c}	29.9±0.6^{ab}	28.87±0.9^{b}	27.67±0.8^{c}	28.44±0.8^{b}
	Eucalyptol	1035	5.11±0.1^{b}	4.41±0.1^{c}	4.45±0.08^{c}	4.71±0.06^{c}	5.81±0.08^{ab}	5.91±0.02^{a}	5.01±0.02^{b}	5.12±0.02^{b}
	δ-Terpinene	1062	0.9±0.01^{d}	1.1±0.07^{d}	1.12±0.08^{d}	1.13±0.06^{d}	0.91±0.09^{d}	1.14±0.2^{d}	2.11±0.2^{c}	1.41±0.2^{d}
	α campholene	1110	1.88±0.2^{c}	1.91±0.2^{c}	1.77±0.2^{c}	1.67±0.2^{c}	2.99±0.3^{a}	2.99±0.1^{a}	2.51±0.2^{ab}	2.81±0.3^{a}
	Myrtenol	1202	23.1±1.1^{c}	24.91±0.9^{b}	25.9±0.8^{ab}	24.8±0.7^{b}	25.21±0.8^{b}	26.3±0.7^{a}	26.65±0.6^{a}	26.88±0.8^{a}
	Neral	1239	12.17±0.6^{b}	11.1±0.9^{c}	11.45±0.8^{c}	11.21±0.8^{c}	11.8±0.7^{c}	12.1±0.6^{b}	12.21±0.5^{b}	12.32±0.9^{b}
	Geraniol	1257	0.9±0.01^{c}	0.92±0.01^{c}	0.95±0.03^{c}	0.97±0.02^{c}	1.01±0.01^{b}	1.03±0.02^{b}	1.05±0.02^{b}	1.16±0.03^{b}
	Geranial	1270	11.1±0.5^{c}	9.6±0.8^{d}	10.6±0.9^{c}	11.4±0.7^{c}	11.6±0.6^{c}	12.3±0.9^{b}	14.5±0.8^{a}	13.5±0.7^{ab}
	Carvacrol	1298	0.99±0.01^{bc}	0.89±0.01^{c}	0.69±0.02^{c}	0.83±0.02^{c}	0.87±0.01^{c}	0.76±0.01^{c}	0.92±0.02^{c}	0.99±0.01^{c}
	Geranic acid	1320	0.88±0.01^{c}	0.99±0.01^{bc}	0.14±0.02^{e}	0.11±0.01^{e}	0.88±0.03^{c}	0.99±0.01^{c}	0.98±0.02^{c}	0.26±0.03^{e}
	Geranyl acetate	1385	0.96±0.02^{c}	0.99±0.01^{c}	0.96±0.02^{c}	0.99±0.02^{c}	0.81±0.01^{d}	0.1±0.03^{e}	0.45±0.01^{e}	0.78±0.02^{d}
	Caryophyllene	1418	0.97±0.02^{d}	0.77±0.01^{d}	0.79±0.02^{d}	0.65±0.03^{d}	0.89±0.01^{d}	0.77±0.02^{d}	0.51±0.01^{cd}	0.62±0.02^{d}
	Monoterpene hydrocarbons		27.1±0.8^{f}	32.15±0.9^{c}	33.21±0.75^{c}	35.54±0.8^{b}	34.46±1.1^{c}	34.08±0.9^{c}	34.31±0.8^{c}	33.14±0.7^{c}
	Oxygenated monoterpenes		55.25±1.2^{c}	53.74±1.1^{d}	55.81±1.3^{c}	55.59±1.1^{c}	59.29±1.5^{ab}	61.39±1.4^{a}	62.85±1.8^{a}	62.78±1.5^{a}
	Sesquiterpenes		0.97±0.02^{e}	0.77±0.01^{e}	0.79±0.02^{e}	0.65±0.03^{f}	0.89±0.01^{e}	0.77±0.02^{e}	0.51±0.01^{fg}	0.62±0.02^{f}
	Essential oil content (w/w%, g/100g fresh weight basis)		0.77±0.01^{bc}	0.76±0.01^{c}	0.75±0.01^{c}	0.79±0.01^{b}	0.78±0.02^{b}	0.81±0.03^{b}	0.93±0.04^{a}	0.71±0.01^{c}
Year	Compound	RI	Mn×Zn	Cu×Mn	Cu×Fe×Zn	Fe×Zn×Mn	Cu×Mn×Fe	Cu×Mn×Zn	Cu×Zn×Mn×Fe	
2022	α-Pinene	940	2.91±0.01^{a}	1.38±0.02^{e}	2.55±0.03^{b}	2.35±0.02^{c}	2.63±0.03^{b}	2.71±0.02^{b}	0.3±0.02^{g}	
	P-Cymene	1026	2.1±0.01^{d}	2.99±0.01^{b}	2.01±0.02^{d}	2.53±0.09^{c}	2.21±0.04^{c}	2.39±0.1^{c}	1.51±0.1^{e}	
	Limonene	1033	28.87±0.8^{b}	28.1±0.9^{b}	28.11±0.5^{b}	28.14±0.8^{b}	28.88±0.8^{b}	29.1±0.9^{b}	29.12±0.8^{b}	
	Eucalyptol	1035	4.43±0.1^{c}	4.55±0.2^{c}	4.77±0.1^{c}	4.93±0.1^{c}	4.89±0.08^{c}	4.78±0.02^{c}	5.91±0.06^{b}	
	δ-Terpinene	1062	2.78±0.3^{b}	2.99±0.2^{b}	3.04±0.2^{b}	3.15±0.3^{b}	3.14±0.2^{b}	2.36±0.3^{c}	2.01±0.2^{c}	
	α campholene	1110	2.98±0.2^{a}	2.99±0.2^{a}	2.96±0.3^{a}	2.87±0.2^{a}	2.19±0.2^{b}	2.33±0.2^{b}	2.5±0.3^{ab}	
	Myrtenol	1202	25.02±1.1^{b}	25.12±0.9^{b}	25.1±0.6^{b}	25.32±0.8^{b}	25.55±0.9^{b}	25.19±0.8^{b}	26.95±0.9^{a}	
	Neral	1239	12.55±0.7^{b}	12.65±0.8^{b}	12.88±0.9^{b}	12.01±0.8^{b}	12.44±0.9^{b}	12.32±0.6^{b}	13.11±0.6^{a}	
	Geraniol	1257	1.23±0.01^{b}	1.43±0.01^{b}	1.55±0.03^{ab}	1.67±0.02^{a}	1.59±0.01^{a}	1.71±0.01^{a}	1.01±0.02^{b}	
	Geranial	1270	12.3±0.5^{b}	12.41±0.7^{b}	11.02±0.6^{c}	12.3±0.8^{b}	12.91±0.9^{b}	12.88±0.8^{b}	15.2±0.2^{a}	
	Carvacrol	1298	1.87±0.01^{a}	1.78±0.02^{a}	0.91±0.01^{c}	1.02±0.01^{b}	1.17±0.02^{b}	1.21±0.01^{b}	1.34±0.02^{b}	
	Geranic acid	1320	0.89±0.01^{c}	0.91±0.03^{c}	0.99±0.01^{bc}	0.89±0.03^{bc}	0.77±0.02^{c}	0.11±0.05^{e}	0.88±0.02^{c}	
	Geranyl acetate	1385	0.99±0.02^{c}	0.89±0.01^{c}	0.93±0.02^{c}	0.27±0.01^{e}	0.99±0.02^{c}	0.88±0.03^{cd}	0.17±0.01^{e}	
	Caryophyllene	1418	0.89±0.02^{d}	0.91±0.01^{d}	0.77±0.01^{d}	0.88±0.02^{d}	0.93±0.01^{d}	0.3±0.03^{e}	0.77±0.01^{d}	
	Monoterpene hydrocarbons		36.66±0.8^{b}	35.46±0.9^{b}	35.71±0.8^{b}	36.17±1.1^{b}	36.86±1.2^{b}	36.56±11.1^{b}	35.94±1.2^{b}	
	Oxygenated monoterpenes		60.38±1.4^{ab}	60.93±1.2^{a}	59.19±1.3^{b}	59.12±1.7^{b}	60.74±1.8^{a}	60.42±1.4^{a}	62.96±1.6^{a}	
	Sesquiterpenes		0.89±0.02^{e}	0.91±0.01^{e}	0.77±0.01^{e}	0.88±0.02^{e}	0.93±0.01^{e}	0.3±0.03^{g}	0.77±0.01^{e}	
	Essential oil content		0.81±0.03^{b}	0.82±0.01^{b}	0.77±0.01^{bc}	0.81±0.02^{b}	0.91±0.01^{a}	0.88±0.01^{ab}	0.98±0.03^{a}	

Year	Compound	Fe	Zn	Cu	Mn	Fe×Zn	Cu×Fe	Mn×Fe	Cu×Zn
	(w/w%, g/100g fresh weight basis)								
2023	α-Pinene	2.85±0.01^{b}	2.66±0.02^{b}	2.29±0.03^{c}	2.43±0.02^{c}	2.22±0.08^{c}	2.44±0.02^{c}	1.39±0.02^{e}	1.77±0.01^{d}
	P-Cymene	1.88±0.01^{d}	1.82±0.01^{d}	1.88±0.02^{d}	1.99±0.01^{d}	1.04±0.04^{e}	1.33±0.01^{e}	1.41±0.02^{e}	1.87±0.02^{d}
	Limonene	28.83±0.8^{b}	28.12±0.6^{b}	29.01±0.5^{b}	27.14±0.8^{c}	27.88±0.9^{c}	27.73±0.8^{c}	27.88±0.7^{c}	28.05±0.8^{c}
	Eucalyptol	6.02±0.5^{a}	5.57±0.3^{b}	5.39±0.1^{b}	5.88±0.2^{b}	6.88±0.1^{a}	5.02±0.2^{b}	6.12±0.1^{a}	6.32±0.1^{a}
	δ-Terpinene	2.88±0.3^{b}	2.99±0.2^{b}	2.97±0.2^{b}	3.02±0.2^{b}	3.14±0.3^{b}	3.21±0.2^{b}	3.88±0.2^{a}	3.91±0.3^{a}
	α campholene	1.99±0.2^{bc}	1.88±0.2^{c}	1.85±0.3^{c}	1.79±0.2^{c}	0.84±0.1^{e}	1.66±0.3^{c}	0.77±0.08^{e}	1.99±0.3^{bc}
	Myrtenol	24.12±1.1^{b}	25.16±0.9^{b}	26.1±0.8^{a}	25.91±0.7^{ab}	26.99±0.6^{a}	27.98±0.7^{a}	25.78±0.9^{b}	26.98±0.8^{a}
	Neral	13.12±0.5^{a}	14.11±0.6^{a}	13.44±0.8^{a}	12.23±0.6^{b}	12.55±0.7^{b}	12.82±0.8^{b}	12.99±0.6^{ab}	11.41±0.8^{c}
	Geraniol	0.99±0.01^{bc}	0.91±0.02^{c}	0.96±0.04^{c}	0.88±0.02^{c}	0.78±0.01^{c}	0.91±0.01^{c}	1.11±0.02^{b}	1.19±0.02^{b}
	Geranial	12.03±0.5^{b}	12.33±0.8^{b}	11.9±0.9^{c}	12.88±0.7^{b}	12.44±0.3^{b}	12.98±0.5^{b}	12.99±0.6^{b}	11.44±0.7^{c}
	Carvacrol	1.01±0.01^{b}	1.02±0.02^{b}	0.96±0.01^{c}	0.98±0.03^{c}	0.99±0.01^{bc}	0.97±0.02^{c}	1.1±0.04^{b}	1.03±0.01^{b}
	Geranic acid	0.99±0.01^{bc}	0.95±0.01^{c}	0.97±0.02^{c}	0.02±0.01^{f}	0.12±0.03^{e}	0.15±0.01^{e}	0.21±0.02^{e}	0.27±0.03^{e}
	Geranyl acetate	0.99±0.02^{c}	0.89±0.01^{c}	0.75±0.02^{c}	0.55±0.01^{d}	0.75±0.01^{d}	0.63±0.03^{d}	0.63±0.01^{d}	0.93±0.02^{c}
	Caryophyllene	0.83±0.02^{d}	0.73±0.01^{d}	0.88±0.01^{d}	0.82±0.01^{d}	0.71±0.01^{d}	0.88±0.02^{d}	0.77±0.01^{d}	0.91±0.02^{d}
	Monoterpene hydrocarbons	36.44±0.8^{b}	35.59±0.9^{b}	36.15±0.7^{b}	34.58±0.9^{c}	34.28±0.8^{c}	34.71±0.9^{c}	34.56±0.8^{c}	35.6±0.7^{b}
	Oxygenated monoterpenes	59.28±1.2^{ab}	60.98±1.1^{a}	60.6±1.4^{a}	60.55±1.2^{a}	63.47±1.4^{a}	62.34±1.2^{a}	60.86±1.1^{a}	60.36±1.2^{a}
	Sesquiterpenes	0.83±0.02^{e}	0.73±0.01^{e}	0.88±0.01^{e}	0.82±0.01^{e}	0.71±0.01^{e}	0.88±0.02^{e}	0.77±0.01^{e}	0.91±0.02 ^{e}
	Essential oil content (w/w%, g/100g fresh weight basis)	0.77±0.01^{b}	0.62±0.01^{d}	0.63±0.01^{d}	0.66±0.02^{cd}	0.79±0.01^{b}	0.88±0.01^{ab}	0.65±0.03^{cd}	0.82±0.01^{b}

Year	Compound	RI	Mn×Zn	Cu×Mn	Cu×Fe×Zn	Fe×Zn×Mn	Cu×Mn×Fe	Cu×Mn×Zn	Cu×Zn×Mn×Fe
2023	α-Pinene	940	2.12±0.01^{c}	2.54±0.02^{b}	2.44±0.03^{c}	2.56±0.02^{b}	2.54±0.08^{b}	2.39±0.02^{c}	2.55±0.02^{b}
	P-Cymene	1026	2.83±0.01^{b}	3.75±0.01^{a}	2.99±0.02^{a}	3.54±0.01^{a}	3.04±0.04^{b}	2.88±0.01^{b}	2.44±0.02^{c}
	Limonene	1033	29.02±0.8^{b}	30.14±0.5^{a}	30.78±0.5^{a}	30.55±0.8^{a}	29.04±0.9^{b}	30.98±0.8^{a}	31.6±0.7^{a}
	Eucalyptol	1035	5.55±0.1^{b}	5.03±0.1^{b}	5.66±0.06 ^{b}	5.01±0.07 ^{b}	5.82±0.09 ^{b}	5.49±0.08 ^{b}	5.78±0.06 ^{b}
	δ-Terpinene	1062	3.91±0.31^{a}	3.86±0.2^{a}	2.68±0.2^{b}	3.11±0.2^{b}	3.99±0.3^{a}	3.18±0.3^{b}	3.55±0.3^{ab}
	α campholene	1110	1.87±0.2^{c}	0.71±0.08^{e}	1.84±0.2^{c}	1.91±0.2^{c}	1.97±0.2^{c}	2.22±0.3^{b}	2.12±0.2^{b}
	Myrtenol	1202	24.01±1.1^{b}	24.11±0.9^{b}	24.91±0.8^{b}	24.03±0.7^{b}	24.15±0.5^{b}	24.27±0.89^{b}	25.1±0.5^{ab}
	Neral	1239	12.12±0.6^{b}	11.14±0.7^{c}	11.99±0.6^{bc}	12.31±0.8^{b}	12.51±0.9^{b}	12.78±0.7^{b}	11.31±0.8^{c}
	Geraniol	1257	0.82±0.01^{c}	0.99±0.02^{bc}	1.66±0.02^{a}	1.99±0.01^{a}	1.43±0.01^{b}	1.11±0.01^{b}	1.96±0.01^{a}
	Geranial	1270	12.11±0.7^{b}	12.55±0.8^{b}	12.14±0.9^{b}	12.31±0.7^{b}	12.25±0.8^{b}	11.32±0.7^{c}	12.5±0.9^{b}
	Carvacrol	1298	1.04±0.01^{b}	0.12±0.01^{d}	0.99±0.02^{bc}	0.33±0.02^{d}	0.44±0.01^{d}	0.55±0.01^{c}	0.22±0.02^{d}
	Geranic acid	1320	0.09±0.01^{ef}	1.21±0.01^{b}	0.91±0.02^{c}	0.92±0.01^{c}	0.55±0.03^{d}	0.11±0.01^{e}	0.09±0.01^{ef}
	Geranyl acetate	1385	0.81±0.02 ^{d}	1.72±0.01^{a}	0.22±0.02^{e}	0.23±0.01^{e}	0.83±0.01^{d}	0.72±0.03^{d}	0.32±0.01^{e}
	Caryophyllene	1418	0.92±0.02^{d}	1.66±0.01^{b}	0.65±0.01^{d}	0.77±0.01^{d}	0.66±0.01^{d}	0.99±0.02^{cd}	0.44±0.1^{e}
	Monoterpene hydrocarbons		37.88±0.8^{ab}	40.29±1.1^{a}	38.89±0.92^{a}	39.76±1.1^{a}	38.61±1.4^{a}	39.43±1.4^{a}	40.14±1.2^{a}
	Oxygenated monoterpenes		57.52±1.4^{c}	54.65±1.5^{cd}	59.19±1.7^{ab}	56.89±1.6^{c}	58.57±1.5^{b}	57.74±1.4^{bc}	58.99±1.6^{b}
	Sesquiterpenes		0.92±0.02^{e}	1.66±0.01^{c}	0.65±0.01^{f}	0.77±0.01^{e}	0.66±0.01^{f}	0.99±0.02^{de}	0.44±0.1^{g}
	Essential oil content (w/w%, g/100g fresh weight basis)		0.81±0.04^{b}	0.81±0.02^{b}	0.88±0.01^{ab}	0.91±0.04^{a}	0.93±0.01^{a}	0.87±0.02^{ab}	0.99±0.01^{a}

[z] RI: Retention Indices, as determined with FID and HP-5MS 5% capillary column using a series of the standards of C7-C30 saturated n-alkanes. [y] Values are means of triplicates ± standard deviation (p <0.05)

**Numbers in each row that have same letter, have same group.

Table 6. Means of essential oil content and composition (%) in *D.kotschyi* plants affected by micronutrients (60 mg.l^{-1}).

Year	Compound	RI [z]	Fe [y]	Zn	Cu	Mn	Fe×Zn	Cu×Fe	Mn×Fe	Cu×Zn
2022	α-Pinene	940	**1.55±0.01^{d}	1.66±0.02^{d}	1.77±0.03^{d}	1.84±0.02^{d}	1.99±0.08^{d}	1.88±0.02^{d}	2.06±0.02^{c}	2.21±0.01^{c}
	P-Cymene	1026	1.77±0.01^{d}	2.85±0.01^{b}	2.67±0.02^{c}	2.81±0.01^{b}	3.39±0.04^{a}	2.98±0.01^{b}	2.96±0.02^{b}	2.11±0.02^{d}
	Limonene	1033	26.56±0.8^{c}	26.6±0.5^{c}	28.41±0.5^{b}	29.62±0.8^{b}	30.12±0.9^{a}	29.21±0.8^{b}	30.31±0.7^{a}	30.44±0.8^{a}
	Eucalyptol	1035	4.48±0.1^{c}	4.39±0.05^{c}	4.18±0.07^{c}	4.34±0.08^{c}	4.31±0.09^{c}	4.31±0.02^{c}	4.71±0.08^{c}	4.39±0.1^{c}
	δ-Terpinene	1062	2.99±0.01^{b}	3.66±0.01^{a}	2.81±0.02^{b}	2.82±0.02^{b}	3.01±0.01^{b}	2.91±0.09^{b}	3.22±0.01^{b}	2.44±0.01^{c}
	α campholene	1110	2.33±0.2^{b}	2.72±0.2^{a}	2.69±0.3^{a}	2.57±0.2^{a}	2.81±0.3^{a}	2.12±0.2^{b}	2.33±0.2^{b}	2.41±0.3^{b}
	Myrtenol	1202	23.02±1.1^{c}	25.16±0.9^{b}	24.88±0.8^{b}	24.88±0.7^{b}	24.9±0.6^{b}	24.02±0.9 ^{b}	24.31±0.8^{b}	23.77±0.9^{c}
	Neral	1239	10.88±0.8^{d}	10.98±0.5^{cd}	10.33±0.6^{d}	10.08±0.8^{d}	10.88±0.7^{d}	10.82±0.6^{d}	10.84±0.5^{d}	10.06±0.8^{d}
	Geraniol	1257	1.77±0.01^{a}	1.72±0.01^{a}	0.88±0.02^{c}	0.91±0.02^{c}	0.93±0.01^{c}	0.95±0.01^{c}	0.98±0.02^{c}	1.01±0.03^{bc}
	Geranial	1270	12.99±0.7^{b}	12.88±0.5^{b}	12.86±0.5^{b}	12.18±0.7^{b}	11.98±0.8^{c}	11.99±0.9^{c}	10.33±0.8^{d}	11.93±0.7^{b}
	Carvacrol	1298	1.22±0.01^{b}	1.31±0.02^{b}	1.61±0.01^{a}	1.55±0.02^{a}	1.22±0.01^{b}	0.88±0.02^{c}	0.93±0.02^{c}	0.94±0.01^{c}
	Geranic acid	1320	0.99±0.01^{bc}	0.82±0.01^{c}	0.27±0.02^{e}	0.65±0.01^{cd}	0.44±0.03^{d}	0.29±0.01^{e}	0.99±0.02^{bc}	0.01±0.03^{f}
	Geranyl acetate	1385	0.91±0.02^{c}	0.88±0.01^{c}	0.15±0.01^{e}	0.66±0.02^{d}	0.54±0.01^{d}	0.32±0.03^{e}	0.41±0.01^{e}	0.99±0.02^{c}
	Caryophyllene	1418	0.61±0.01^{d}	0.66±0.01^{d}	0.12±0.02^{e}	0.77±0.03^{d}	0.69±0.04^{d}	0.99±0.02^{cd}	0.77±0.04^{d}	0.88±0.02^{d}
	Monoterpene hydrocarbons		31.87±0.8^{d}	34.77±0.9^{c}	35.66±0.6^{b}	37.09±0.8^{b}	38.51±0.7^{a}	36.98±0.8^{b}	37.77±0.7^{ab}	37.2±0.8^{b}
	Oxygenated monoterpenes		57.69±1.4^{bc}	59.16±1.2^{b}	57.43±1.3^{b}	56.51±1.5^{b}	57.03±1.4^{b}	55.09±1.1^{c}	54.43±1.3^{d}	54.51±1.2^{d}
	Sesquiterpenes		0.61±0.01^{f}	0.66±0.01^{f}	0.12±0.02^{g}	0.77±0.03^{f}	0.69±0.04^{f}	0.99±0.02^{e}	0.77±0.04^{e}	0.88±0.02^{e}
	Essential oil content (w/w%, g/100g fresh weight basis)		0.77±0.01^{bc}	0.69±0.02^{c}	0.67±0.01^{c}	0.8±0.02^{b}	0.81±0.03^{b}	0.68±0.01^{c}	0.69±0.02^{c}	0.77±0.01^{bc}

Year	Compound	RI	Mn×Zn	Cu×Mn	Cu×Fe×Zn	Fe×Zn×Mn	Cu×Mn×Fe	Cu×Mn×Zn	Cu×Zn×Mn×Fe
2022	α-Pinene	940	2.55±0.01^{b}	2.1±0.02^{c}	2.01±0.03^{c}	1.55±0.02^{d}	2.21±0.08^{c}	3.08±0.02^{a}	2.21±0.02^{c}
	P-Cymene	1026	2.88±0.01^{b}	2.71±0.01^{b}	1.61±0.02^{e}	1.72±0.01^{d}	1.92±0.04^{d}	1.99±0.01^{d}	1.39±0.02^{e}
	Limonene	1033	29.62±0.7^{b}	29.14±0.5^{b}	28.55±0.5^{b}	27.63±0.8^{c}	26.83±0.9^{c}	26.64±0.8^{c}	25.4±0.8^{d}
	Eucalyptol	1035	4.66±0.1^{c}	5.88±0.2^{b}	5.33±0.06^{b}	5.22±0.07 ^{b}	5.28±0.08 ^{b}	5.37±0.07 ^{b}	3.95±0.09^{d}
	δ-Terpinene	1062	2.44±0.1^{c}	2.57±0.3^{c}	1.93±0.2^{c}	1.57±0.2^{c}	1.88±0.2^{c}	1.99±0.3^{c}	2.21±0.2^{c}

	α campholene	1110	2.61±0.2^{a}	2.56±0.2^{ab}	2.72±0.2^{a}	2.66±0.3^{a}	1.88±0.1^{c}	1.25±0.1^{d}	1.21±0.1^{d}
	Myrtenol	1202	24.91±1.1^{b}	26.11±0.5^{a}	25.88±0.6^{ab}	25.01±0.7^{b}	24.83±0.8^{b}	24.21±0.9^{b}	23.1±0.8^{c}
	Neral	1239	10.88±0.5^{d}	10.99±0.6^{cd}	11.93±0.7^{c}	9.12±0.8^{e}	12.88±0.9^{b}	12.04±0.5^{b}	13.14±0.9^{a}
	Geraniol	1257	0.99±0.01^{bc}	0.95±0.01^{c}	0.91±0.03^{c}	0.85±0.02^{c}	0.77±0.01^{c}	0.67±0.02^{cd}	0.66±0.03^{cd}
	Geranial	1270	10.82±0.5^{c}	10.88±0.6^{c}	9.88±0.7^{d}	8.7±0.8 ^{e}	9.08±0.9^{d}	9.94±0.7^{d}	10.11±0.8^{d}
	Carvacrol	1298	0.55±0.01^{c}	0.61±0.02^{c}	0.71±0.02^{c}	0.24±0.01^{d}	0.52±0.01^{c}	0.44±0.03^{d}	0.34±0.02^{d}
	Geranic acid	1320	0.95±0.01^{c}	0.97±0.02^{c}	0.99±0.01^{c}	0.02±0.02^{f}	0.13±0.04^{e}	0.01±0.01^{f}	0.22±0.02^{f}
	Geranyl acetate	1385	0.99±0.02^{c}	0.05±0.03^{f}	0.12±0.02^{e}	0.44±0.01^{d}	0.44±0.02^{d}	0.91±0.03^{c}	0.85±0.01^{c}
	Caryophyllene	1418	0.66±0.02^{d}	0.75±0.01^{d}	0.81±0.01^{d}	0.73±0.02^{d}	0.71±0.01^{d}	0.99±0.02^{cd}	0.88±0.01^{d}
	Monoterpene hydrocarbons		37.49±0.7^{b}	36.52±0.8^{b}	34.1±0.6^{c}	32.47±0.5^{c}	32.88±0.7^{c}	33.7±0.65^{c}	31.21±0.8^{d}
	Oxygenated monoterpenes		57.42±1.2^{c}	57.98±1.3^{bc}	57.36±1.1^{c}	51.8±0.9^{de}	55.24±1.2^{c}	53.92±1.1^{d}	52.51±1.4^{d}
	Sesquiterpenes		0.66±0.02^{f}	0.75±0.01^{e}	0.81±0.01^{e}	0.73±0.02^{e}	0.71±0.01^{e}	0.99±0.02^{de}	0.88±0.01^{e}
	Essential oil content (w/w%, g/100g fresh weight basis)		0.76±0.03^{c}	0.74±0.02^{c}	0.71±0.01^{c}	0.73±0.01^{c}	0.8±0.02^{b}	0.77±0.01^{bc}	0.69±0.02^{c}
Year	Compound	Fe	Zn	Cu	Mn	Fe×Zn	Cu×Fe	Mn×Fe	Cu×Zn
2023	α-Pinene	2.44±0.02^{c}	2.2±0.01^{c}	2.55±0.1^{b}	2.77±0.1^{b}	1.83±0.09^{b}	3.34±0.1^{a}	2.99±0.1^{a}	2.89±0.09^{b}
	P-Cymene	1.99±0.01^{d}	1.7±0.02^{de}	1.81±0.1^{d}	1.77±0.02^{d}	1.99±0.08^{d}	1.77±0.1^{d}	2.61±0.1^{c}	1.69±0.03^{e}
	Limonene	28.02±0.5^{b}	28.9±0.6^{ab}	28.4±0.7^{b}	28.5±0.8^{b}	27.7±0.9^{c}	26.6±0.9^{c}	28.8±0.8^{b}	28.5±0.7^{b}
	Eucalyptol	3.21±0.1^{d}	3.33±0.1^{d}	3.22±0.2^{cd}	3.99±0.2 ^{d}	3.22±0.12 ^{d}	3.21±0.1 ^{d}	3.33±0.1 ^{d}	3.05±0.11 ^{d}
	δ-Terpinene	3.44±0.2^{ab}	3.7±0.2^{a}	2.66±03^{b}	2.55±0.2^{bc}	4.15±0.2^{a}	3.74±03^{a}	3.85±02^{a}	2.99±0.1^{b}
	α campholene	2.51±0.2^{ab}	2.24±0.2^{b}	2.4±0.3^{a}	1.3±0.1^{d}	1.8±0.1^{c}	1.9±0.2^{c}	1.9±0.2^{c}	1.1±0.1^{d}
	Myrtenol	23.1±0.5^{c}	23.8±0.6^{c}	23.7±0.3^{c}	23.2±0.4^{c}	23.1±0.3^{c}	23.2±0.5^{c}	23.4±0.4^{c}	23.5±0.6^{c}
	Neral	13.3±0.5^{a}	13.9±0.3^{a}	13.8±0.5^{a}	13.87±0.4^{a}	13.69±0.6^{a}	12.8±0.7^{b}	11.8±0.4^{c}	11.9±0.6^{c}
	Geraniol	1.56±0.01^{a}	1.4±0.01^{b}	1.3±0.02^{b}	1.4±0.01^{b}	1.81±0.01^{a}	1.7±0.02^{a}	0.9±0.02^{c}	1.1±0.03^{b}
	Geranial	11.88±0.5^{c}	12.3±0.6^{b}	11.9±0.7^{c}	11.8±0.5^{c}	11.7±0.4^{c}	11.1±0.6^{c}	13.4±0.4^{b}	11.3±0.5^{b}
	Carvacrol	1.33±0.01^{a}	1.44±0.2^{a}	1.88±0.1^{a}	1.33±0.01^{a}	0.99±0.01^{bc}	0.97±0.1^{c}	1.12±0.1^{a}	1.23±0.01^{b}
	Geranic acid	0.99±0.02^{bc}	0.85±0.1^{c}	0.12±0.1^{e}	0.71±0.02^{c}	0.4±0.02^{d}	0.5±0.03^{d}	0.3±0.01^{e}	0.4±0.02^{d}
	Geranyl acetate	0.99±0.01^{c}	0.92±0.1^{c}	0.98±0.01^{c}	0.7±0.01^{d}	0.1±0.01^{e}	0.5±0.01^{d}	0.6±0.02^{d}	0.1±0.03^{e}
	Caryophyllene	1.77±0.02^{b}	1.88±0.1^{b}	2.32±0.01^{a}	1.72±0.02 ^{b}	2.1±0.01^{a}	1.91±0.1^{b}	1.9±0.01^{b}	1.99±0.02^{ab}
	Monoterpene hydrocarbons	35.89±0.8^{b}	36.5±0.9^{b}	35.42±0.7^{b}	35.59±0.6^{b}	35.67±0.8^{b}	35.45±0.7^{b}	38.25±0.6^{a}	36.07±0.5^{b}
	Oxygenated monoterpenes	56.89±1.4^{c}	58.41±1.2^{b}	58.2±1.1^{b}	56.89±1.2^{c}	56.31±1.4^{c}	54.88±1.1^{d}	55.85±1.5^{c}	53.18±1.4^{d}
	Sesquiterpenes	1.77±0.02^{b}	1.88±0.1^{b}	2.32±0.01^{a}	1.72±0.02^{b}	2.1±0.01^{a}	1.91±0.1^{b}	1.9±0.01^{b}	1.99±0.02^{ab}
	Essential oil content (w/w%, g/100g fresh weight basis)	0.77±0.01^{bc}	0.65±0.1^{d}	0.62±0.1^{d}	0.81±0.01^{b}	0.85±0.04^{b}	0.79±0.1^{b}	0.83±0.2^{b}	0.8±0.02^{b}
Year	Compound	RI	Mn×Zn	Cu×Mn	Cu×Fe×Zn	Fe×Zn×Mn	Cu×Mn×Fe	Cu×Mn×Zn	Cu×Zn×Mn×Fe
2023	α-Pinene	940	2.99±0.01^{a}	2.42±0.02^{c}	2.38±0.03^{c}	2.23±0.02^{c}	2.44±0.01^{c}	2.55±0.02^{b}	2.44±0.01^{c}
	P-Cymene	1026	1.77±0.01^{d}	1.71±0.02^{d}	1.6±0.01^{e}	1.55±0.01^{e}	1.47±0.04^{e}	1.44±0.01^{e}	1.39±0.02^{e}
	Limonene	1033	28.5±0.65^{b}	27.88±0.5^{c}	27.5±0.6^{c}	27.4±0.8^{c}	28.7±0.5^{b}	27.6±0.9^{c}	25.98±0.8^{cd}
	Eucalyptol	1035	4.33±0.1^{c}	3.55±0.1 ^{d}	5.12±0.2^{b}	4.88±0.1^{c}	5.15±0.2^{b}	5.01±0.2^{b}	3.93±0.5 ^{d}
	δ-Terpinene	1062	3.88±0.2^{a}	3.77±0.2^{a}	3.55±0.3^{ab}	2.44±0.3^{b}	2.87±0.2^{b}	2.93±0.2^{b}	3.22±0.3^{ab}
	α campholene	1110	1.74±0.2^{c}	1.68±0.2^{c}	1.78±0.2^{c}	1.88±0.2^{c}	1.84±0.2^{c}	1.66±0.1^{c}	1.55±0.1^{cd}
	Myrtenol	120	24.88±1.1^{b}	24.9±0.9^{b}	25.8±0.8^{ab}	24.88±0.6^{b}	23.2±0.7^{c}	23.2±0.6 ^{c}	22.9±0.6^{cd}
	Neral	1239	11.3±0.5^{c}	12.8±0.6^{b}	12.4±0.7^{b}	10.12±0.8^{d}	11.1±0.9^{c}	11.04±0.5^{c}	11.9±0.5^{c}
	Geraniol	1257	1.23±0.01^{b}	1.01±0.02^{c}	0.96±0.1^{c}	0.99±0.02^{c}	0.94±0.01^{c}	0.88±0.01^{c}	0.77±0.02^{c}
	Geranial	1270	11.8±0.5^{c}	10.95±0.6^{c}	10.1±0.7^{d}	9.1±0.8^{d}	10.4±0.9^{d}	10.9±0.6^{c}	10.9±0.6^{c}
	Carvacrol	1298	1.04±0.01^{b}	1.01±0.02^{b}	0.97±0.03^{c}	0.87±0.02^{c}	0.99±0.02^{bc}	0.9±0.02^{c}	0.99±0.02^{bc}
	Geranic acid	1320	0.21±0.01^{e}	0.96±0.01^{c}	0.89±0.02^{c}	0.95±0.01^{c}	0.91±0.03^{c}	0.88±0.01^{c}	0.98±0.02^{c}
	Geranyl acetate	1385	0.66±0.02^{d}	0.99±0.01^{c}	0.98±0.02^{c}	0.99±0.01 ^{c}	0.11±0.01^{e}	0.2±0.03^{e}	0.99±0.01^{c}
	Caryophyllene	1418	1.99±0.02^{ab}	2.01±0.01^{a}	1.88±0.01^{a}	2.12±0.01^{a}	2.22±0.02^{a}	1.82±0.02 ^{b}	1.88±0.01^{b}
	Monoterpene hydrocarbons		37.14±0.65^{b}	35.78±0.8^{b}	35.03±0.5^{b}	33.62±0.7^{c}	35.48±0.8^{b}	34.52±0.7^{bc}	33.03±0.6^{c}
	Oxygenated monoterpenes		56.32±1.2^{c}	55.9±1.1^{c}	57.13±1.2^{c}	52.72±0.9^{c}	53.62±0.9^{c}	53.59±1.1^{d}	52.94±0.9^{d}
	Sesquiterpenes		1.99±0.02^{ab}	2.01±0.01^{a}	1.88±0.01^{b}	2.12±0.01^{a}	2.22±0.02^{a}	1.82±0.02^{b}	1.88±0.01^{b}
	Essential oil content (w/w%, g/100g fresh weight basis)		0.77±0.04^{bc}	0.69±0.02^{c}	0.78±0.01^{b}	0.82±0.01^{b}	0.7±0.01^{c}	0.69±0.02^{c}	0.63±0.01^{d}

[z] RI: Retention Indices, as determined with FID and HP-5MS 5% capillary column using a series of the standards of C7-C30 saturated n-alkanes. [y] Values are means of triplicates ± standard deviation (p <0.05)

**Numbers in each row that have same letter, have same group.

Table 7. Results of simple correlation between essential oil content and main compositions in *D.kotschyi* plants under application of tested micronutrients in two years.

Year	Eucalyptol (10)	α.campholene (9)	Geranic acid (8)	Geranyl acetate(7)	Geraniol (6)	Geranial (5)	Neral (4)	Limonene (3)	Myrtenol (2)	Essential oil (1)	
	-	-	-	-	-		-	-	-	1	1
	-	-	-	-	-		-	-	1	0.95**	2
	-	-	-	-	-		-	1	0.75**	0.64*	3
	-	-	-	-	-		1	0.8**	0.52**	0.5**	4
2022	-	-	-	-	-	1	0.66**	0.71**	0.55**	0.9**	5
	-	-	-	-	1	0.54**	0.95**	0.47**	0.64**	0.68*	6
	-	-	-	1	0.95**	0.78**	0.66**	0.82**	0.6**	0.4*	7
	-	-	1	0.69**	0.48**	0.48**	0.86**	0.67**	0.72**	0.88**	8
	-	1	0.86**	0.53**	0.81**	0.81**	0.68**	0.79**	0.65**	0.75**	9
	1	0.81**	0.95**	0.9**	0.92**	0.95**	0.77**	0.95**	0.65**	0.85**	10
	-	-	-	-	-	-	-	-	-	1	1
	-	-	-	-	-	-	-	-	1	0.84**	2
	-	-	-	-	-	-	-	1	0.9**	0.66**	3
	-	-	-	-	-	-	1	0.62**	0.59**	0.51**	4
2023	-	-	-	-	-	1	0.3	0.75**	0.55**	0.82**	5
	-	-	-	-	1	0.79**	0.42**	0.45**	0.71**	0.5**	6
	-	-	-	1	0.68**	0.95**	0.76**	0.73**	0.62**	0.4**	7
	-	-	1	0.77**	0.95**	0.77**	0.66**	0.63**	0.7**	0.8**	8
	-	1	0.67**	0.72**	0.88**	0.81**	0.88**	0.65**	0.66**	0.45**	9
	1	0.81**	0.86**	0.53**	0.81**	0.86**	0.66**	0.79**	0.65**	0.45**	10

*, ** significant at P=0.05 and P=0.01 levels of probability respectively.

In the current study it was found that the essential oil content is in a yield ranging from 0.59 to 0.99% (w/w) made by control plants and plants treated with 40 $mg.l^{-1}$ of micronutrients ($Fe_2Zn_2Mn_2Cu_2$) respictively (Tables 4-6). The main reasons for this enhancement in the essential oil content by applying micronutrients can be related to the balance between absorption of the essential elements in the root environment, increasing the rate of photosynthesis, stimulating the vital enzymes, activating plant growth regulators (PGR) production as inducing signal for terpenes biosynthesis (Pradhan et al., 2017). In this research use of 40 $mg.l^{-1}$ of Fe, Zn, Mn and Cu increased essential oil content from 0.59% to 99% with an increase of 67%. It has been shown those micronutrients of Fe, Cu, Zn and Mn help to increase better and more absorption of nutrients by influencing enzyme activities (Marschner, 1995; Pradhan et al., 2017). Copper deficiency limits the activity of many plant enzymes, including ascorbate oxidase, phenolase, cytochrome oxidase, diamine oxidase, plastocyanin, and superoxide dismutase. Oxidation–reduction cycling between Cu(I) and Cu(II) oxidation states is required during single electron transfer reactions in copper-containing enzymes and proteins (Barker & Pilbeam, 2007). Iron is limited largely by diffusion in the soil solution, and thus the absorption is highly dependent on root activity and growth. In terms of fertilizers for terrestrial plants, iron deficiency usually comes about because of alkaline pH in the soil, and supply of iron salts to the soil would have no effect. Foliar application of iron-cheletes can be effective. Therefore, the usual way in which lime-induced chlorosis is alleviated is by supply of iron chelates to the foliage (Barker & Pilbeam, 2007; Pradhan et al., 2017). Manganese involved in many biochemical functions, primarily acting as an activator of enzymes such as dehydrogenases, transferases, hydroxylases, and decarboxylases involved in respiration, amino acid and lignin synthesis, and hormone concentrations, but in some cases it may be replaced by other metal ions (Marschner, 1995). Zinc is an integral component of enzyme structures and coordinated to four ligands in enzymes with catalytic functions. Three of them are amino acids, with histidine being the most frequent, followed by glutamine and asparagine (Marschner, 1995; Pradhan et al., 2017). As a result, these positive impacts of the micronutrients could lead to the improvement of photosynthetic rate, biomass production and yield of aerial parts of medicinal plants (Hamedi et al., 2020; Yadegari, 2023). In the current study it was found that the EOs content is in a

yield ranging from 0.37 to 0.68% (w/w) made by control plants and plants treated with 40 mg.l-1 of micronutrients (Fe2Zn2Mn2Cu2) respectively. The main reasons for this enhancement in the EOs content by applying micronutrients can be related to the balance between absorption of the essential elements in the root environment, increasing the rate of photosynthesis, stimulating the vital enzymes, activating plant growth regulators (PGR) production as inducing signal for terpenes biosynthesis (Pradhan et al., 2017).

The findings of this research showed that by increasing essential oil content, the main compositions such as limonene, geranial, neral, myrtenol and eucalyptol in *D.kotschyi* plants treated with micronutrients in two years, increased (Table 7). Also, in the current study, 14 chemical components of the essential oils of *D.kotschyi* were recognized. It has been shown that the percentage of the main compounds of essential oil is the main factor determining the quality of the essential oil. It has been reported that among these compounds, monoterpene alcohols such as limonene, geranial, eucalyptol and myrtenol improve the quality of the essential oil of *D.kotschyi* (Shaykh-Samani et al., 2023a, b; Ashrafi et al., 2017, Golparvar et al., 2016). Maintaining the balance between nutrients and soil fertility is critical in sustainable soil management. Organic, biological and chemical fertilizers return nutrients consumed by plants to the soil. According to the results of the present study, the availability of optimum amounts of micronutrients provided the necessary nutrients for producing higher content and composition of essential oil of *D.kotschyi*. The essential oil composition of *D.kotschyi* varies depending on the variety, climatic conditions, and nutritional status of the plant and soil. According to reported literature myrtenol, limonene, beta-pinene, neral, geranial, geraniol, geranyl acetate, geranic acid, alpha campholene, eucalyptol and alpha pinene, were the identified constituents in the extracted essential oils from aerial parts of *D.kotschyi* (Shaykh-Samani et al., 2023; Cham et al., 2022; Ghavam et al., 2021; Fallah et al., 2020). A combination of the four micronutrients had a greater effect than a single micronutrient. Thus, the foliar application of 40 $mg.l^{-1}$ Fe, Cu, Mn and Zn was the most effective treatment compared to other treatments. The amount (percentage) of the main components in plants treated with 40 $mg.l^{-1}$ of micronutrients was produced twice more than those of the control plants (Tables 4-6).

Exogenous micronutrients affect respiration, photosynthesis, carbohydrate assimilation, and amino acids biosynthesis. These processes usually are simultaneous with the changes in the content of intermediate compounds and the activity of involved enzymes in the primary and secondary metabolism of plants. Therefore, induced variations in the plant's physiological behavior by micronutrients determine the quality of produced secondary metabolites. Essential oils belong to the group of terpenes and glucose is an essential precursor in the synthesis of terpenoids, especially monoterpenes. Therefore photosynthesis and photosynthetic products directly determine the biosynthesis of essential oils. A sufficient supply of nutrient elements in plant in response to exogenous micronutrients affects the biosynthesis of involved substrates and enzymes in terpenoids biosynthesis (Aghaei et al., 2021; Bohlman & Keeling, 2008; Pradhan et al., 2017). For instance, providing a sufficient amount of magnesium may affect the activity of geranyl diphosphate synthase, which requires this element for its activity (Chiyaneh et al., 2022). Essential oil content is directly correlated with the main compounds such as myrtenol and limonene. In the present study based on GC and GC-MS results, monoterpene hydrocarbons represented by alpha-pinene, p-cymene, limonene and gamma-terpinene, made more 35% of compounds of essential oil and oxygenated monoterpenes represented by eucalyptol, alpha-campholene, myrtenol, neral, geraniol, geranial and carvacrol made more than 52% of compounds of essential oil of treated plants. In all of treated plants, the most compound belongs of oxygenated monoterpenes and monoterpene hydrocarbons, were myrtenol and limonene respectively. The main of

compounds of essential oil of treated plants by 40- mg.l^{-1} of micronutrients are more than other treated plants, especially oxygenated monoterpenes in compounds were more than other categories and otherwise the sesquiterpenes in plants treated by 60 mg.l^{-1} of micronutrients increased. Treatment of $Fe_2Cu_2Zn_2Mn_2$ in this research made the most myrtenol such as oxygenated monoterpenes and limonene belongs to the category of monoterpene hydrocarbons. Also treatment of $Fe_2Cu_2Zn_2Mn_1$ and $Fe_2Cu_1Zn_2Mn_2$ in most of main compounds were in the same group with $Fe_2Cu_2Zn_2Mn_2$. It seems that the role of iron and zinc are more important than other micronutrients that reported in pervious researches (Yadegari, 2023; Bilal et al., 2020; Hamedi et al., 2020). The results of previous investigation by Shaabani et al. (2020) showed that the essential oil of *D.kotschyi* mainly consisted of β-caryophellene, α-pinene, and limonene. The results of another study indicated that the major components of volatile oil from *D.kotschyi* were limonene, carvacrol, γ-terpinene, and α-pinene. Numerous studies have reported that the chemical compounds of *D.kotschyi* have antibacterial and antiseptic properties. Terpenoids, such as geranial, lower blood triglycerides and cholesterol, and ultimately reduce cardiovascular disease (Ashrafi et al., 2017; Golparvar et al., 2016; Samadi et al., 2018). Compounds such as geranial and neral cause the antioxidant activity of *D.kotschyi*. The upper range of micronutrients (i.e. 60 mg.l^{-1}) decreased the content of the essential oil of *D.kotschyi* but the composition of the essential oil was similar in all treatments. Control plants made better amounts of many essential oil components of *D.kotschyi* than those treated plants with 60- mg.l^{-1} concentrations of Fe, Cu, Mn and Zn. In most treatments, the combination of $Fe_2Cu_2Mn_2Zn_2$, $Fe_2Cu_3Mn_3Zn_2$ and $Fe_2Cu_2Mn_2Zn_2$ made the maximum amount of essential oil. However, $Fe_2Cu_2Mn_2Zn_2$ was the best combination. The increase in the essential oil content in the plants treated with 20 and 40 mg.l^{-1} may be due to its role in enhancing the overall growth of aerial parts. In addition, active substances such as volatile oils are made by plants due to the plant's adaptation to biological and abiotic stresses, and the signals of these stresses act as elicitors for the plant cell (Sonboli et al., 2019; Ashrafi et al., 2017).

In some essential oil compositions, control plants were similar to plants treated with $Fe_3Cu_3Mn_3Zn_3$, $Fe_3Cu_3Mn_2Zn_3$ and $Fe_2Cu_2Mn_3Zn_3$. It seems for toxicities of upper concentrations of Fe, Cu, Mn and Zn (i.e. 60 mg.l^{-1}), the content of components obtained at higher concentrations of micronutrients was similar to control plants. It was clear from the presented data that the highest levels of the four foliar fertilizers were more effective than the lower levels, and Librel Zn and Fe fertilizers were superior over other micronutrients. However, the highest essential oil percentage was found with $Fe_2Cu_2Mn_2Zn_2$ (Tables 4-6). Similar to the results obtained in this study regarding *D.kotschyi*, the beneficial effect of micronutrients (Fe, Zn, Cu and Mn) and production of higher essential oil content were reported by other researchers in *Rosa damascena* (Yadegari, 2023), *Thymus* (Yadegari, 2022), *Satureja* (Bani Taba et al., 2022), *Dracocephalum moldavica* (Yadegari, 2021), Safflower (*Carthamus tinctorius*) (Galavi et al., 2012), marigold (*Calendula officinalis* L.), borage (*Borago officinalis*), alyssum (*Alyssum desertorum*) and thyme (*Thymus vulgaris*) (Yadegari, 2015, 2017a), lemon balm (*Melissa officinalis* L.) (Yadegari, 2017b), dill (*Anethum graveolens*) (Rostaei et al., 2018), *Matricaria chamomilla* (Nasiri et al., 2010) and *Coriandrum sativum* (Said-Al Ahl & Omer, 2009).

Results of this research indicated that foliar application of micronutrients resulted in higher essential oil content in the shoots of *D.kotschyi* plants than the control plants. In the present study, the effect of micronutrients on the essential oil content and composition was determined over two consecutive years. The essential oil yield increased with Fe, Cu, Zn and Mn applications because of a significant rise in dry matter and the number of flowers (data not published). It was revealed that Fe, Cu, Zn and Mn are beneficial for *D.kotschyi* plants

with concentrations of 40 mg.l^{-1}or lower, and can result in more content of essential oil up to 40%. Fe, Cu, Zn and Mn have immediate impacts on the growth and development of plants. There are still many unanswered questions about the mechanism of Fe, Cu, Zn and Mn in enhancing yield and its components for *D.kotschyi* plants. One possibility is that the foliar applied Fe, Cu, Zn and Mn can affect absorbtion of other minerals and then increase shoot dry matter and finally the essential oil yield in plants increased (Alamer et al., 2020; Alejandro et al., 2020; Aziz et al., 2019). It was determined in this study that control plants with no foliar treatment experienced better growth than the plants with a higher concentration of the micronutrients. Combinations of micronutrients with an optimum concentration (i.e. 40 mg.l^{-1}) had the best effect. However, combinations of micronutrients of a concentration higher than 40 mg.l^{-1} (i.e. 60 mg.l^{-1}) had more diminishing effects than single micronutrients. These results reflect the role of the simultaneous application of an optimum concentration of the four foliar fertilizers in improving the total essential oils in medicinal plants. Micronutrients of higher than 40 mg.l^{-1} concentration especially in three micronutrient- or in four micronutrient-combinations reduced the content and composition of the essential oil (Tables 4-6). Generally, the production of the secondary metabolites and the chemical compositions of the plant essential oils is influenced by genetic factors, ecological, soil conditions, management (sowing to harvesting and post-harvesting processes) and their interactions (Golparvar et al., 2016, Ashrafi et al., 2017, Shaykh-Samani et al., 2023a, b).

CONCLUSION

D.kotschyi plants treated with 40 mg.l^{-1} of iron, zinc, manganese and copper in the chelate formula, produced higher content and composition of the essential oil. It could be concluded from the results that iron, zinc, manganese and copper fertilization had significant effects on the measured characters as well as the chemical composition of the essential oil of *D.kotschyi* plants. Also, the combined application of micronutrient fertilizers had a more pronounced effect in comparison with the individual use of the micronutrients. This study provides some useful information about the impact of foliar application of micronutrients where soils have undesirable characteristics and chemical properties in particular. The main constituents of the volatile oils of *D.kotschyi* were limonene, neral, geranial, myrtenol and eucalyptol (45-65%) were identified in the essential oil. In this study, the highest content of essential oil and the percentages of neral, geranial, myrtenol, eucalyptol and limonene in the essential oil were observed in the herbs under treatments of 40 mg.l^{-1} of micronutrients however the combination of 40 mg.l^{-1} of iron, zinc and copper was in the same goup. Finally, the use of 40 mg.l^{-1} of micronutrients (Fe, Zn, Cu and Mn) is recommended for stabilizing the quantitative and qualitative yield of *D.kotschyi* in same climates.

Funding
The author did not receive support from any organization for the submitted work.

Conflict of Interest
The author declares that he has no conflict of interest.

REFERENCES

Adams, R. P. (2007). Quadrupole mass spectra of compounds listed in order of their retention time on db-5. In identification of essential oil components by gas chromatography/mass spectroscopy, 4th ed. IL, USA: Allured Publishing Corp, Carol Stream.

Aghaei, K., Ghasemi Pirbalouti, A., Mousavi, A., Naghdi Badi, H.A., & Mehnatkesh, A.M. (2021). Effects of different fertilizers and the foliar application of L-phenylalanine on mineral contents of hyssop [*Hyssopus officinalis* L. subsp. *angustifolius* (Bieb.)]. *Journal of Horticultural Plants Nutrition, 4*(2), 13-28. https://doi.org/10.22070/hpn.2021.4639.1038

Alamer, K., Ali, E., Althubaiti, M., & Alghamdi, M. (2020). Zinc nutrition and its activated roles on growth, inflorescences attributes and some physiological parameters of *Tagetes erecta* L. *Plants Pakistan Journal of Biological Science, 23*(1), 35-44. https://doi.org/10.3923/pjbs.2020.35.44.

Alejandro, S., Höller, S., Meier, B., & Peiter, E. (2020). Manganese in plants: From acquisition to subcellular allocation. *Frontiers in Plant Science, 11*, 1–23. https://doi.org/10.3389/fpls.2020.00300.

Ashrafi, B., Ramak, P., Ezatpour, B., & Talei, G.R. (2017). Investigation on chemical composition, antamicrobial, antioxidant, and cytotoxic properties of essential oil from *Dracocephalum kotschyi* Bois. *African Journal of Traditional Complementary and Alternative Medicines, 14*, 209-217. https://doi.org/10.21010/ajtcam.v14i3.23.

Aziz, M. Z., Yaseen, M., Abbas, T., Naveed, M., Mustafa, A., Hamid, Y., Saeed, Q., & Xu, M. (2019). Foliar application of micronutrients enhances crop stand, yield and the biofortification essential for human health of different wheat cultivars. *Journal of Integrated Agriculture, 18*(6), 1369–1378. https://doi.org/10.1016/S2095-3119(18)62095-7.

Bani Taba, Yadegari, M., & Naderi Darfaghshahi, M.R. (2022). Effect of irrigation regimes and foliar application of manganese and copper on morphophysiological traits of three species of *Saturjea* sp. in Isfahan and Golpayegan regions. *Plant Process and Function, 11*(48), 129-147. http://dorl.net/dor/20.1001.1.*23222727*.1401.11.48.9.0

Barker, A.V., & Pilbeam, D.J. (2007). Hand book of plant nutrition. Taylor and Francis Group, Library of Congress Cataloging‐in‐Publication Data. QK867.B29 2006 631.8‐‐dc22, New York. 662 pp.

Bilal, H. M., Tahir, R., Adnan, M., Ali, S. M., Islam, H., Umer, M. S., Mir, F.A… & Iftikhar, M. (2020). Does foliar application of macro and micronutrients have any impact on roses production? A review. *Annals Review Research, 6*(1), 1-5. http://dx.doi.org/10.19080/ARR.2020.06.555677

Bohlman, J., & Keeling, C.I. (2008). Terpenoid biomaterials. *Plant Journal, 54*, 656-669. https://doi.org/10.1111/j.1365-313X.2008.03449.x

Cakmak, I., Mclaughlin, M. J., & White, P. (2017). Zinc for better crop production and human health. *Plant and Soil, 411*, 1–4. https://doi.org/10.1007/s11104-016-3166-9.

Cham, R., Abtahi, S.A., Jafarinia, M., & Yasrebi, J. (2022). Physiological responses of *Dracocephalum kotschyi* Boiss. to drought stress and bio-fertilizers. *South African Journal of Botany, 148*, 180-189. https://doi.org/10.1016/j.sajb.2022.04.008

Chiyaneh, S.F., Rezaei-Chiyaneh, E., Amirnia, R., Keshavarz Afshar, R., & Siddique, K.H.M. (2022). Changes in the essential oil, fixed oil constituents, and phenolic compounds of ajowan and fenugreek in intercropping with pea affected by fertilizer sources. *Industrial Crops Products, 178*, 114587. https://doi.org/10.1016/j.indcrop.2022.114587.

Fallah, S., Mouguee, S., Rostaei, M., & Adavi, Z. (2020). Chemical compositions and antioxidant activity of essential oil of wild and cultivated *Dracocephalum kotschyi* grown indifferent ecosystems: A comparative study. *Industrial Crops and Products, 143*, 11885. http://dx.doi.org/10.1016/j.indcrop.2019.111885

Figueiredo, M., Boldrin, P. F., Hart, J. J., Andrade, M. J. B., Guilherme, L. R. G., Glahn, R.P., & Li, L. (2017). Zinc and selenium accumulation and their effect on iron bioavailability in common bean seeds. *Plant Physiology and Biochemistry, 111*, 193–202. https://doi.org/10.1016/j.plaphy.2016.11.019..

Galavi, M., Ramroudi, M., & Tavassoli, A. (2012). Effect of micronutrients foliar application on yield and seed oil content of safflower (*Carthamus tinctorius*). *African Journal of Agricultural Research, 7*, 482-486. https://doi.org/10.5897/AJAR11.1323.

Ghavam, M., Manconi, M., Manca, M.L., & Baccheta, G. (2021). Extraction of essential oil from *Dracocephalum kotschyi* Boiss. (Lamiaceae), identification of two active compounds and evaluation of the antimicrobial properties. *Journal of Ethnopharmacology, 267*, 113513.

https://doi.org/10.1016/j.jep.2020.113513
Golparvar, A.R., Hadipanah, A., Gheisari, M.M., & Khaliliazar, R. (2016). Chemical constituents of essential oil of *Dracocephalum moldavica* L. and *Dracocephalum kotschyi* Boiss. from Iran. *Acta Agricultural Slov, 107*(1), 25-31. http://dx.doi.org/10.14720/aas.2016.107.1.03
Hamedi. B., Ghasemi Pirbaloutib, A., & Rajabzadeh, F. (2020). Manures, vermicompost, and chemical fertilizer impacts on the yield and volatile compounds of the damask rose (*Rosa damascena* Mill.) flower petals. *Industrial Crops and Products, 187*, 115470. https://doi.org/10.1016/j.indcrop.2022.115470.
Li, L., Zhang, K., Gill, R. A., Islam, F., Farooq, M. A., Wang, J., & Zhou, W. (2018). Ecotoxicological and interactive effects of copper and chromium on physiochemical, ultrastructural, and molecular profiling in *Brassica napus* L. *Bio-Medical Research International, 92*(10), 1-17. https://doi.org/10.1155/2018/9248123.
Majeed, A., Minhas, W.A., Mehboob, N., Farooq, S., Hussain, M., Alam, S., & Rizwan, M. S. (2020). Iron application improves yield, economic returns and grain-Fe concentration of mungbean. *PLoS One, 15*, e0230720. https://doi.org/10.1371/ journal.pone.0230720.
Marschner, H. (1995). *Mineral nutrition of higher plants*. Second ed. London: Academic Prees.
Mozaffarian, V. (2008). A Pictorial Dictionary of Botanical Taxonomy Latin-English-French-Germany-Persian. Germany: Koeltz Scientific Books.
Nasiri, Y., Zehtab-Salmasi, S., Nasrullahzadeh, S., Najafi, N., & Ghassemi-Golezani, K. (2010). Effects of foliar application of micronutrients (Fe and B) on flower yield and essential oil of chamomile (*Matricaria chamomilla* L.). *Journal of Medicinal Plants Research, 4*(17), 1733-1737. https://doi.org/10.5897/JMPR10.083.
Pradhan, J., Sahoo, S.K., Lalotra, S., & Sarma, R.S. (2017). Positive impact of abiotic stress on medicinal and aromatic plants. *International Journal of Plant Science, 12*(2), 309-313. https://doi.org/10.15740/HAS/IJPS/12.2/309-313.
Rostaei, M., Fallah, S., Lorigooini, Z., & Surki, A. A. (2018). The effect of organic manure and chemical fertilizer on essential oil, chemical compositions and antioxidant activity of dill (*Anethum graveolens*) in sole and intercropped with soybean (*Glycine max*). *Journal of Cleaning Production, 199*, 18–26. https://doi.org/10.1016/j.jclepro.2018.07.141. S.
Said-Al Ahl, H. A. H., & Omer, E. A. (2009). Effect of spraying with boron and / or iron on growth and chemical composition of coriander (*Coriandrum sativum* L.) harvested at three stages of development. *Journal of Medicinal Food Plants, 1*(2), 30-46. https://www.jmedfoodplants.com. 1947-3710.
Samadi, L., Larijani, K., Naghdi Badi, H., & Mehrafarin, A. (2018). Qualitative and quantitative variations of the essential oils of *Dracocephalum kotschyi* Boiss. as affected by different drying methods. *Journal of Food Processing Preservation, 13816*, 1-12. https://doi.org/10.1111/jfpp.13816
Shaykh-Samani, A., Ghasemi Pirbalouti, A., Yadegari, M., & and Rajabzadeh, F. (2023a). Foliar application of salicylic acid improved the yield and quality of the essential oil from *Dracocephalum kotschyi* Boiss. under water deficit Stress. *Journal of Essential Oil Bearing Plants, 26*(3), 769-779. https://doi.org/10.1080/0972060X.2023.2236647
Shaykh-Samani, A., Ghasemi Pirbalouti, A., Yadegari, M., & and Rajabzadeh, F. (2023b). Effects of foliar application of salicylic acid and proline on the growth traits and the essential oil content of *Dracocephalum kotschyi* Boiss. under water deficit conditions. *Journal of Plant Environmental Physiology, 18*(69), 1-25. https://doi.org/10.30495/iper.2022.1952014.1771
Shaabani, M., Mousavi, S.H., Azizi, M., & Jafari, A.A. (2020). Cytotoxic and apoptogenic effects of *Dracocephalum kotschyi* Boiss., extracts against human glioblastoma U87 cells. *Avicenna Journal Phytomedation, 10*(6), 594-603. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7711299
Sonboli, A., Mirzania, F., & Gholipour, A. (2019). Essential oil composition of *Dracocephalum kotschyi* Boiss. from Iran. *Natural Products Research, 14*, 2095-2098. https://doi.org/10.1080/14786419.2018.1482550
Taghizadeh, M., Nasibi, F., Manouchehri Kalantari, Kh., and Benakashani, F. (2020). Callogenesis optimization and cell suspension culture establishment of *Dracocephalum polychaetum* Bornm.

and *Dracocephalum kotschyi* Boiss.: An *in vitro* approach for secondary metabolite production. *South African Journal of Botany*, *132*, 79-86. https://doi.org/10.1016/j.sajb.2020.04.015

Yadegari, M. (2023). The effects of foliar application of some micronutrient elements on the content and composition of the essential oil of Damask rose (*Rosa damascena* Mill.). *Iran Agricultural Research*, *41*(2), 35-49. https://doi.org/10.22099/iar.2022.42152.1467

Yadegari, M. (2022). Effects of NPK complete fertilizer, botamisol, and humic acid on morphophysiological characteristics and essential oil in three *Thymus* species under drought stress conditions. *Iranian Journal of Medicinal and Aromatic Plants Research*, *38*(2), 301-321. https://doi.org/10.22092/ijmapr.2022.357303.3119

Yadegari, M. (2021). The effect of organic and chemical fertilizers on morpho-physiological traits and essential oil in *Dracocephalum moldavica* L. *Plant Process and Function*, *10*(45), 27-41. http://dorl.net/dor/20.1001.1.23222727.1400.10.45.8.6

Yadegari, M. (2017a). Irrigation periods and Fe, Zn foliar application on agronomic characters of *Borago officinalis*, *Calendula officinalis*, *Thymus vulgaris* and *Alyssum desertorum*. *Communication of Soil Science and Plant Analysis*, *48*(3), 307–315. https://doi.org/10.1080/00103624.2016.1269796.

Yadegari, M. (2017b). Effects of Zn, Fe, Mn and Cu foliar application on essential oils and morpho-physiological traits of Lemon balm (*Melissa officinalis* L.). *Journal of Essential Oil Bearing Plants*, *20*(2), 485-495. https://doi.org/10.1080/0972060X.2017.1325010.

Yadegari, M. (2015). Foliar application of micronutrients on essential oils of borago, thyme and marigold. *Journal of Soil Science and Plant Nutrition*, *15*(4), 949-964. https://doi.org/10.4067/S0718-95162015005000066.