JHPR

www.jhpr.birjand.ac.ir

Volume 7, Issue 2, June 2024, E-ISSN: 2588-6169

Journal of Horticulture and Postharvest Research

An International Journal

JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH
2024, VOL. 7(2), 99-114

Journal of Horticulture and Postharvest Research

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

University of Birjand

Maintaining quality of Lisbon lemon (*Citrus limon*) in cold storage using natural elicitors

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ARTICLE INFO

Original Article

Article history:
Received 1 January 2024
Revised 12 April 2024
Accepted 13 April 2024

Keywords:
GABA
Lemon
Melatonin
Methyl jasmonate
Storage

DOI: 10.22077/jhpr.2024.7125.1352
P-ISSN: 2588-4883
E-ISSN: 2588-6169

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ABSTRACT

Purpose: Lemon (*Citrus limon*) is a highly important citrus species worldwide. However, its semi-tropical nature makes it susceptible to chilling, extensive research on postharvest treatments to preserve its quality under low temperatures. **Research Method**: The treatments included 500 µM melatonin (M), 50 µM methyl jasmonate (J), and 5 mM gamma-aminobutyric acid (GABA). Lemons stored at 3 ± 1°C and a relative humidity of 85-95% for 100 days plus 5-day shelf life. **Findings**: The results revealed that most of the experimental treatments, except for the combination of GABA + J and GABA + J + M, significantly reduced fruit weight loss. Notably, the melatonin treatment showed a 22.7% lower weight loss compared to the control fruits. Furthermore, the melatonin treatment exhibited the highest fruit firmness (49 N), while the control treatment had the lowest (36.3 N). Regarding the quality parameters, individual treatments, and the GABA + M treatment resulted in significantly higher total soluble solids (TSS) and a lower TSS/TA compared to the control at the end of the storage period. Except for the M + GABA and M + J treatments, all other treatments showed higher ascorbic acid content compared to the control. Additionally, the melatonin treatment showed significant differences in various color indices compared to the control. **Research limitations**: There was no limitation. **Originality/Value**: Overall, fruits treated with M, J, GABA, and GABA + M demonstrated higher marketability compared to the control and other treatments. Consequently, it is recommended to utilize these treatments individually rather than in combination to maintain the quality of lemon fruits.

INTRODUCTION

Lemon fruit (*Citrus limon* cv. Lisbon) is rich in nutrients and bioactive compounds such as citric acid, ascorbic acid, minerals and rich in natural antioxidant compounds such as phenols and flavonoids and antioxidant properties (Serna-Escolano et al., 2021). The quality of lemon depends on the physiological and biochemical changes that occur during its storage conditions and duration (Mukhim et al., 2015). Cold storage facilities play a crucial role in expediting the desired decrease in temperature, thereby minimizing postharvest losses, and safeguarding the integrity and volume of perishable agricultural produce. By upholding appropriate cold storage conditions, the nutritional and sensory attributes of freshly harvested fruits and vegetables can be effectively preserved, thereby affording consumers with a diverse array of processing prospects (Makule et al., 2022). However, some tropical and semi-tropical products, especially lemons, are very sensitive to low temperature, and their long-term storage in cold storage leads to a decrease in some quality characteristics such as taste, properties affecting fruit juice and bioactive compounds such as ascorbic acid (Rapisarda et al., 2001; Baloyi et al., 2023) and the marketability of the product (Sun et al., 2019). The occurrence of chilling symptoms is due to lipid peroxidation along with a decrease in the activity of antioxidant mechanisms because of excessive production of ROS and because of oxidative stress. In general, all kinds of subtropical fruits, such as lemon, have developed protective mechanisms against oxidative stress. For example, fruits have developed efficient systems (enzymatic antioxidants, liposoluble or membrane-bound antioxidants, and water-soluble antioxidants) to scavenge ROS (Habibi et al., 2020).

In this regard, to increase the tolerance of citrus fruits against chilling during storage, various compounds such as salicylic acid and edible coatings (Rasouli et al., 2019), methyl jasmonate and methyl salicylate (Habibi et al., 2019) and nitric Oxide (Ghorbani et al., 2018) has been studied. These compounds activate new signaling pathways to deal with stress. The studies conducted are mostly on orange and grapefruit fruits and the use of natural compounds such as melatonin, methyl jasmonate and GABA on Lisbon lemon is very limited.

Melatonin is a natural indoleamine and an effective antioxidant that directly destroys ROS. Recently, strong evidence has been obtained that shows that plants produce and accumulate melatonin when exposed to adverse environmental conditions, and the use of exogenous melatonin helps to increase tolerance to stress (Jannatizadeh, 2019). Application of melatonin for 'Guifei' mango effectively delayed the change in ripening parameters including firmness, color, total soluble solids content and titratable acidity (TA) by inhibiting ethylene and ABA biosynthesis (Liu et al., 2020).

Methyl jasmonate, known for its ability to enhance the quality of fresh fruits both pre- and post-harvest, achieves this by promoting the production of secondary metabolites and increasing antioxidant activity. These effects contribute to an overall improvement in fruit quality. Moreover, Methyl jasmonate application can also enhance plant resistance against storage-related problems like chilling injuries and pathogen attacks, effectively preserving the quality of the fruits (Akan et al., 2019). In another study, methyl jasmonate treatment on blood orange delayed the changes in fruit firmness, TA, antioxidant activity and ascorbic acid, and the treated fruit contained higher compounds and showed higher cold resistance (Habibi et al., 2020). MeJA treatments significantly retarded weight loss in blueberries during storage. While the values of L*, chroma, and soluble solids content in MeJA-treated blueberries were lower (Yılmaz, 2024).

GABA is a four-carbon, non-protein amino acid. This compound in plants is known as a metabolite in the field of response to living and non-living stresses that can quickly accumulate in response to stresses and includes the defense system against them (Ramesh et

al., 2015). Research findings have demonstrated that the utilization of GABA on olives led to improved quality during periods of cold storage in comparison to untreated olives. This improvement was attributed to the increased functioning of antioxidant enzymes in the olives that received GABA treatment (Fan et al., 2024).

In recent years, there has been a growing interest in employing environmentally friendly methods to extend the shelf life of agricultural products without compromising human health. The innovation of the article lies in evaluating, for the first time, the effects of melatonin, methyl jasmonate, gibberellic acid, and their combination on the quality of stored lemon fruit in cold storage. Prior to this research, there have been no reports on the use of these compounds and their impact on the quality of lemon fruit during storage. This study aims to explore the potential benefits and effects of these substances on the characteristics of stored Lisbon lemon fruit at a temperature of 3±1°C. By investigating the effects of natural materials, this research aims to contribute to the development of sustainable and safe practices in the agricultural industry.

MATERIALS AND METHODS

Lemon fruits (*Citrus limon* cv. Lisbon) were harvested by hand from a commercial orchard in Juyom city, Fars province, and were immediately transported to the physiology laboratory for treatment and trait evaluation. First, uniform fruits were selected in terms of color, shape, and size, and non-uniform and damaged samples were removed, then they were immersed in 0.05% sodium hypochlorite solution for one minute to remove any contamination and washed immediately. Then the fruits were subjected to immersion treatment (10 minutes) in melatonin 500 µM, methyl jasmonate 50 µM and GABA 5 mM. Control fruits were immersed in distilled water for 10 min. Each treatment included 3 repetitions and each repetition included 3 fruits. After applying the treatment, the fruits were kept at ambient temperature and after drying, they were transferred to the storage (temperature 3±1°C and relative humidity 85-90%). At intervals of 30, 70, and 100 days during the cold storage period, the samples were assessed to measure various parameters (Siboza et al., 2014).

Characteristics under investigation

Weight loss index

To measure the percentage of weight loss (WL), the fruits in each experimental unit were weighed with an accuracy of 0.01 grams as the initial weight after treatment and before storage. Then, at each sampling time, they were re-weighed as the final weight, and finally, according to the amount of initial weight and secondary weight, the percentage of weight loss was calculated according to the following equation:The percentage of weight loss reported with the following equation (1) (Habibi et al., 2020):

$$\text{Weight loss (\%)} = ((\text{Initial weight} - \text{Final weight}) / (\text{Initial weight})) \times 100 \quad (1)$$

Firmness index

The hardness of each fruit was determined with a hardness tester equipped with a probe with a diameter of 3.5 mm. The prop of the device compressed 10% of the equatorial diameter of the fruit and stopped after splitting the skin of the fruit and the data was reported in Newton (N).

Taste index, Total soluble solids, Titratable acidity

Total soluble solids (TSS) in water samples were determined using a digital hand-held refractometer (Atago PAL-1, Tokyo, Japan) and reported as °Brix.

Titratable acidity (TA) was determined by manual titration of lemon juice with 0.1 N NaOH to pH 8.2 as an endpoint with a pH meter (HANA, Romani) as an indicator and expressed as a percentage of citric acid equivalent (2).

$$TA\ (\%\ citric\ acid) = 0.0064 \times titre\ (NaOH)\ mL \times 100 / 10\ mL\ juice \qquad (2)$$

The taste index (TSS/TA) was expressed as the ratio of TSS to TA (Habibi et al., 2020).

Ascorbic acid content

Ascorbic acid (AA) content in fruit juice was measured using 2,6-dichlorophenol indophenol method. 50 µl of lemon juice was added to 5 ml of 1% metaphosphoric acid and vortexed for 20 seconds, and then 500 µl of the vortexed solution was combined with 4.5 ml of indophenol and vortexed again for 20 seconds. Then it was read using a spectrophotometer at a wavelength of 510 nm and reported as mg/100g FW: (AOAC, 2000; Rey et al., 2020).

Fruit color index

The color characteristics of lemon fruit were monitored using a colorimeter (Minolta CR400, Japan), with the CIE L*, a* and b* system proposed by the International Commission on Eclairage (CIE). To measure the skin color of the fruit at each measurement stage, three fruits were selected from each repetition and the readings were taken from three different points on the fruit. The *L** value indicates darkness or lightness, the *a** value is green (-) or red (+), and the *b** value is yellow (+) or blue (-) (Sun et al., 2019).

Marketability

The marketability of lemons was evaluated by 10 evaluators based on the appearance indicators of fruit color, degree of skin freshness, lack of stains from frostbite, etc. using grading and scoring. So that zero has the lowest marketability and 10 has the highest marketability.

Experimental design and data analysis

The experiment was conducted as a factorial in the form of a completely randomized design with three replications (n=3). Statistical analysis of data was done using (9.1) SAS. The mean comparison was analyzed with Duncan's multiple range tests at the 5% probability level. Graphs were also drawn using Excel software.

RESULTS

Weight loss index

The results obtained from the mean comparison showed that the weight loss index increased over time, but this increase was less in the melatonin treatment than in the other treatments and the control. All treatments except GABA + methyl jasmonate and the combination of three treatments melatonin + GABA + methyl jasmonate showed a significant decrease in all three storage times compared to the control and caused a decrease in the percentage of weight loss and juice loss (Fig. 1).

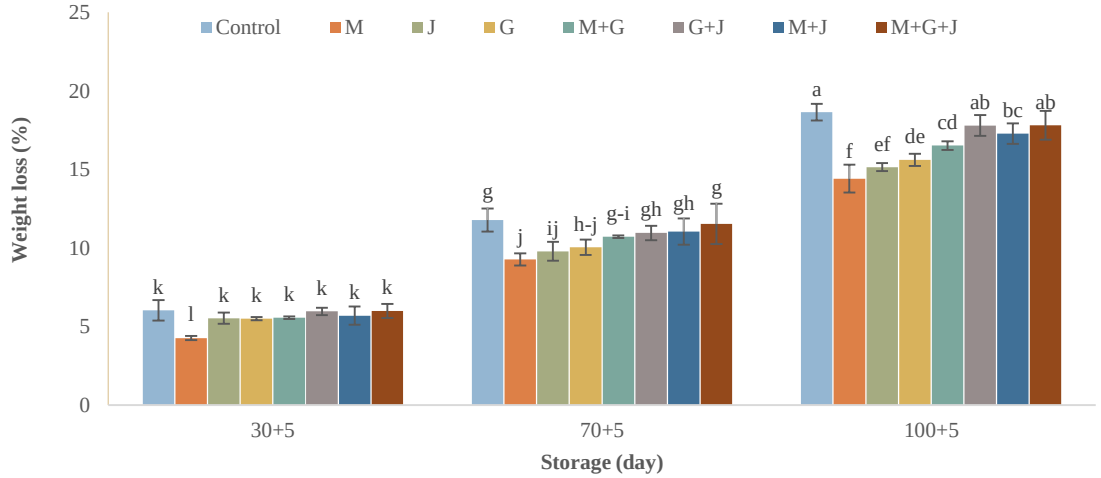

Fig. 1. The effect of melatonin (M), methyl jasmonate (J), GABA (G), melatonin + GABA (M+G), methyl jasmonate + GABA (G+J), melatonin + methyl jasmonate (M+J) and melatonin + methyl jasmonate + GABA (M+J+G) treatments on the weight loss percentage of Lisbon lemons during the cold storage period including 30+5, 70+5, and 100+5 days stored at 3±1°C. Similar letters indicate non-significant difference at the 5% probability level using Duncan's test. The vertical index above the columns represents the standard error.

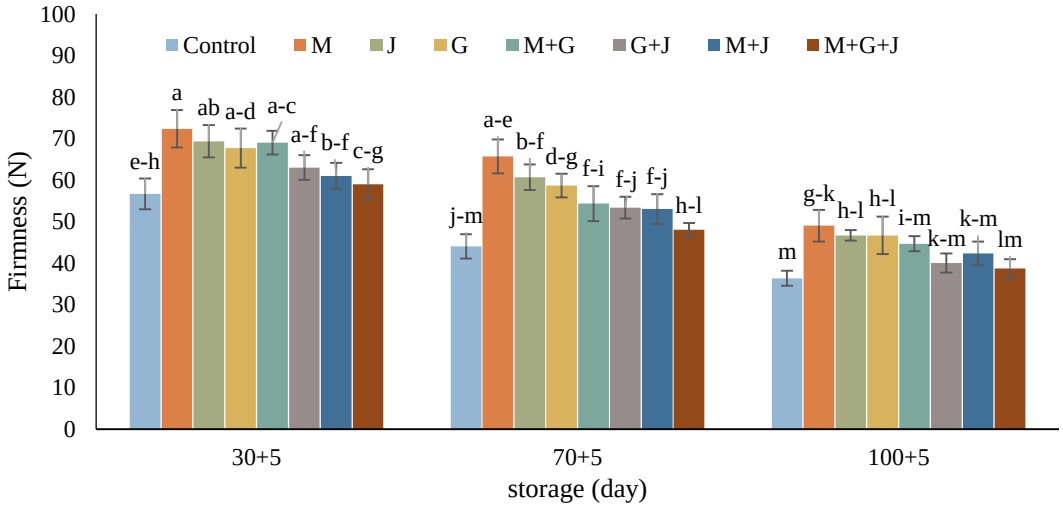

Fig. 2. The effect of melatonin (M), methyl jasmonate (J), GABA (G), melatonin + GABA (M+G), methyl jasmonate + GABA (G+J), melatonin + methyl jasmonate (M+J) and melatonin + methyl jasmonate + GABA (M+J+G) treatments on the firmness of Lisbon lemons during the period of storage in cold storage including 30+5, 70+5 and 100+5 days stored at 3±1°C. Similar letters indicate non-significant difference at the 5% probability level using Duncan's test. The vertical index above the columns represents the standard error.

Firmness index

Comparison of means shows that by as storage period increased, the firmness gradually decreased. According to Figure 2, after 100 days of storage, the fruits treated with melatonin, methyl jasmonate and GABA alone showed the highest level of firmness and the control treatment showed the lowest level of firmness, respectively.

Taste index, Total soluble solids, Titratable acidity

Comparison of means indicates that the soluble solids slightly increased with the progress of storage time. According to Figure 3, this increase was the highest in the control fruits, and on the other hand, the melatonin-treated fruits showed the lowest soluble solids in all three storage times, but no significant difference was observed between the other treatments. In the last stage, melatonin, GABA, methyl jasmonate and the combination of melatonin and GABA showed a significant decrease compared to the control. During the storage period, there was a notable decrease in titratable acidity content in the samples. Specifically, after 70 days of storage, the control samples exhibited a lower acid content compared to the samples treated with melatonin and methyl jasmonate. However, it is worth noting that by the end of the storage period, no statistically significant difference in titratable acidity content was observed between the control samples and the treated samples (Fig. 4).

The comparison of means indicates that the taste index has gradually increased with the progress of the shelf life of the fruits in the cold storage. At the end of the experiment, except GABA + jasmonate, melatonin+ jasmonate and melatonin + GABA + jasmonate treatments, other treatments showed less TSS than the control (Fig. 5).

Ascorbic acid content

The results of means comparison show that as the storage life of fruits increases from 30 to 100 days, the amount of ascorbic acid decreases. This reduction in the control treatments was very noticeable and had a significant difference compared to the rest of the treatments except the combination of melatonin with GABA and melatonin with methyl jasmonate (Fig. 6).

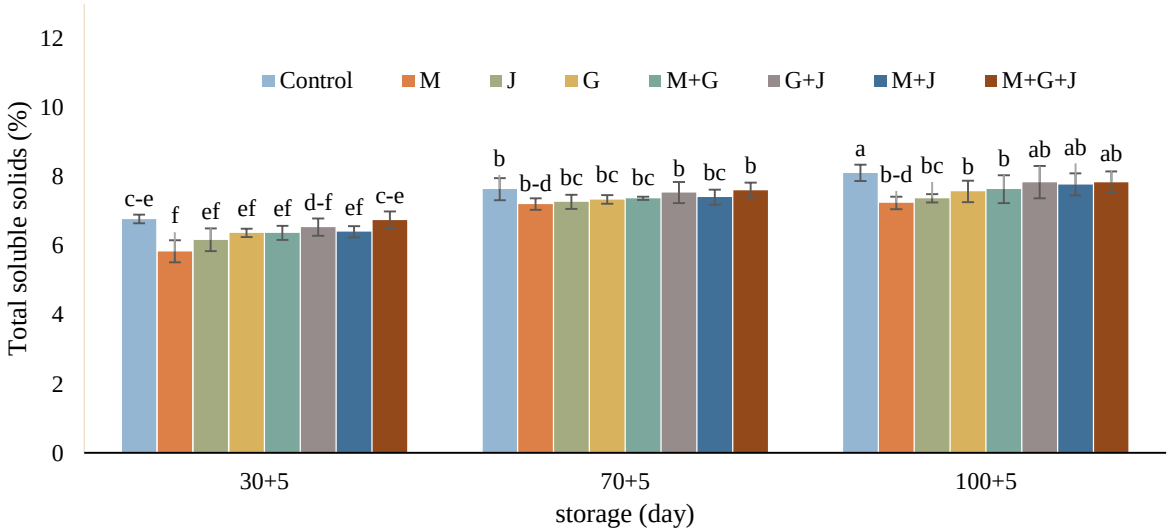

Fig. 3. Effect of melatonin (M), methyl jasmonate (J), GABA (G), melatonin + GABA (M+G), methyl jasmonate + GABA (G+J), melatonin + methyl jasmonate (M+J) and melatonin + methyl jasmonate + GABA (M+J+G) treatments on the soluble solids of the whole Lisbon lemon during the cold storage period including 30+5, 70+5 and 100+5 days stored at 3±1°C. Similar letters indicate non-significant difference at the 5% probability level using Duncan's test. The vertical index above the columns represents the standard error.

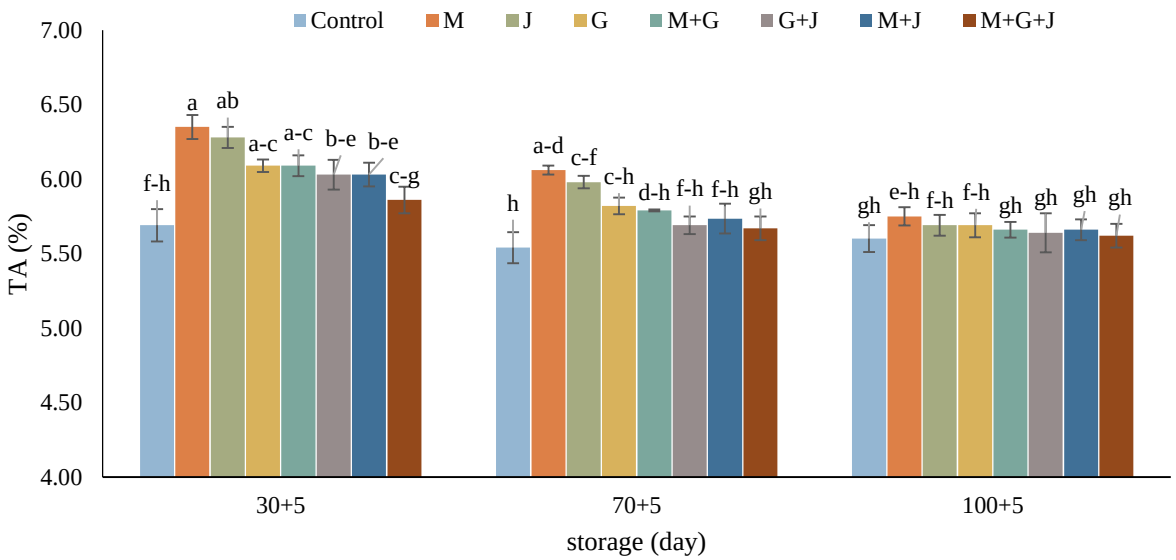

Fig. 4. The effect of melatonin (M), methyl jasmonate (J), GABA (G), melatonin + GABA (M+G), methyl jasmonate + GABA (G+J), melatonin + methyl jasmonate (M+J) and melatonin + methyl jasmonate + GABA (M+J+G) treatments on the percentage of titratable acidity of Lisbon lemon during the period of storage in cold storage including 30+5, 70+5, and 100+5 days at 3±1°C. Similar letters indicate non-significant difference at the 5% probability level using Duncan's test. The vertical index above the columns represents the standard error.

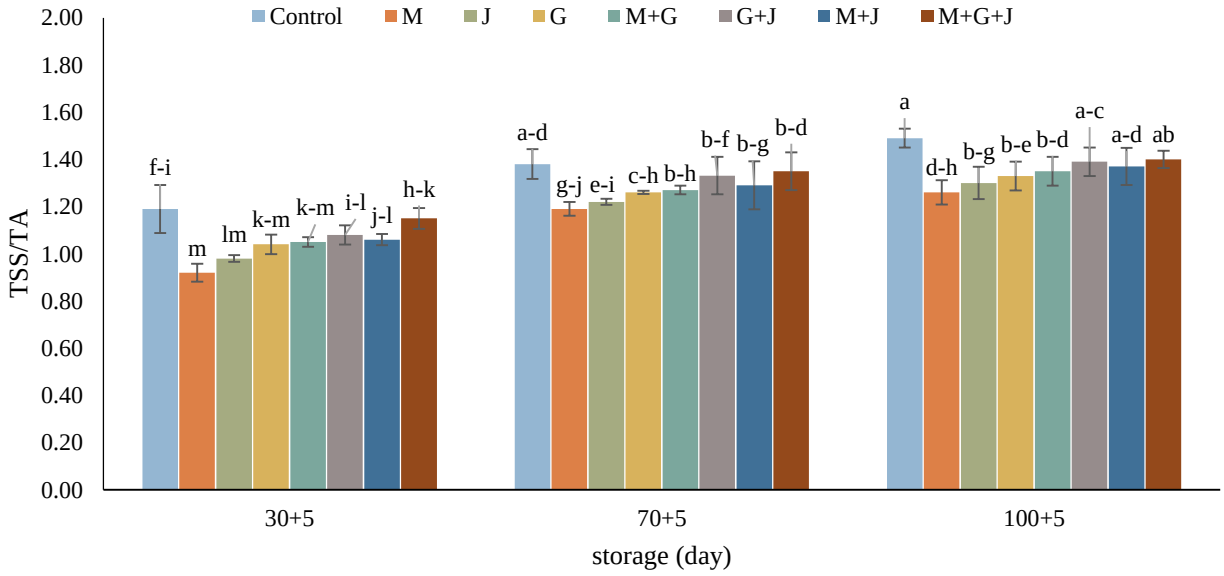

Fig. 5. The effect of melatonin (M), methyl jasmonate (J), GABA (G), melatonin + GABA (M+G), methyl jasmonate + GABA (G+J), melatonin + methyl jasmonate (M+J) and melatonin + methyl jasmonate + GABA (M+J+G) treatments on the taste index of Lisbon lemons during the period of storage in cold storage including 30+5, 70+5 and 100+5 days at a temperature of 3±1°C. Similar letters indicate non-significant difference at the 5% probability level using Duncan's test. The vertical index above the columns represents the standard error.

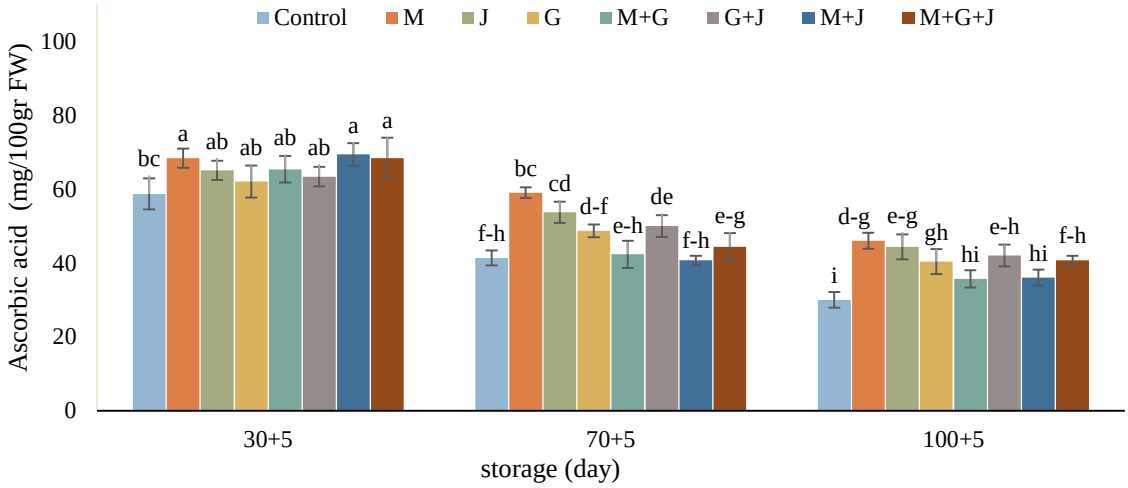

Fig. 6. The effect of melatonin (M), methyl jasmonate (J), GABA (G), melatonin + GABA (M+G), methyl jasmonate + GABA (G+J), melatonin + methyl jasmonate (M+J) and melatonin + methyl jasmonate + GABA (M+J+G) treatments on the amount of ascorbic acid in Lisbon lemons during the period of storage in cold storage including 30+5, 70+5 and 100+5 days at a temperature of 3±1°C. Similar letters indicate non-significant difference at the 5% probability level using Duncan's test. The vertical index above the columns represents the standard error.

Fruit color index

According to the results of means comparison, all fruit color traits (L*, a*, b*) were significantly ($P \leq 0.05$) affected by treatments and storage periods in cold storage. According to Figure 7a, L* value increased over time during fruit storage in cold storage. This increase was much higher in control fruits. The fruits stored for 30 days showed significantly the lowest amount of L* compared to the fruits stored for 70 days and 100 days. The amount of a* in cold storage fruits treated with melatonin was significantly lower than the control fruits and the rest of the treatments. These results indicate that melatonin treatment has been able to significantly prevent the color change and chlorophyll degradation of lemons (Fig. 7b).

According to the obtained results, cold storage fruits treated with melatonin significantly had the lowest b* value compared to control fruits. Also, fruits showed a significant increase in b* values from 30 days to 100 days of cold storage. The lowest amount of b* was observed in fruits treated with melatonin after 30, 70 and 100 days of cold storage and the highest amount of b* was observed in control fruits (Fig. 7c).

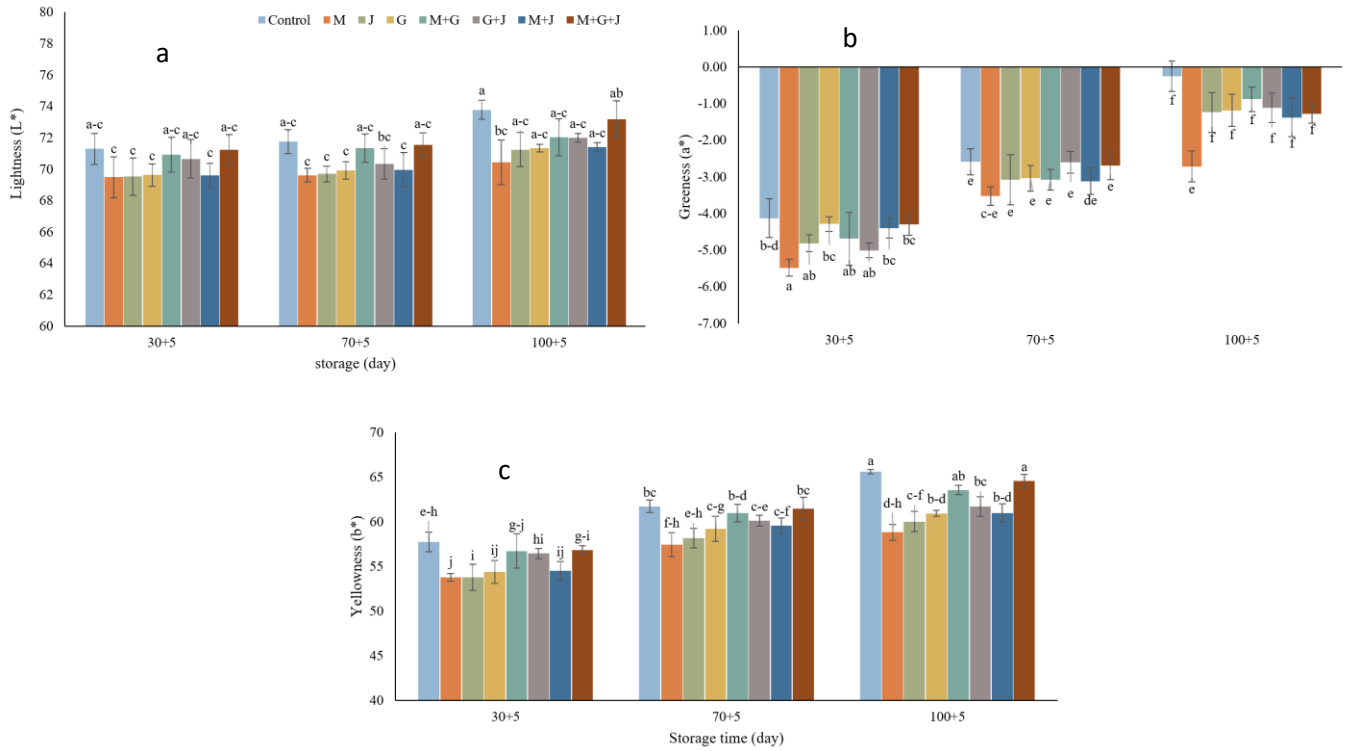

Fig. 7. The effect of melatonin (M), methyl jasmonate (J), GABA (G), melatonin + GABA (M+G), methyl jasmonate + GABA (G+J), melatonin + methyl jasmonate (M+J) and melatonin + methyl jasmonate + GABA (M+J+G) treatments on fruit color properties (A: L*, B: a*, and C: b*) of Lisbon lemon during the cold storage period including 30+5, 70+5 and 100+5 days at a temperature of 3±1°C. Similar letters indicate non-significant difference at the 5% probability level using Duncan's test. The vertical index above the columns represents the standard error.

Marketability

At the end of the storage period, the fruits treated with melatonin, jasmonate and GABA showed the highest level of appearance quality, freshness and marketability compared to the control fruits (Fig. 8).

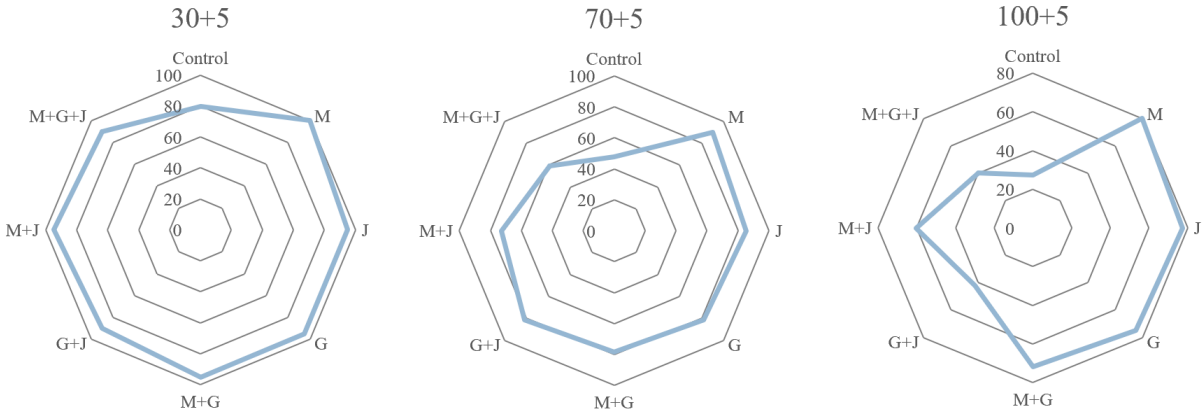

Fig. 8. The effect of melatonin (M), methyljasmonate (J), GABA (G), melatonin + GABA (M+G), methyljasmonate + GABA (G+J), melatonin + methyljasmonate (M+J) and melatonin + methyl jasmonate + GABA (M+J+G) treatments on the marketability of Lisbon lemon fruit during the cold storage period including 30+5, 70+5 and 100+5 days stored at 3±1°C.

DISCUSSION

According to the report, all treatments demonstrated a reduction in weight loss compared to the control fruit during the 100 ± 5 days of storage. After harvesting, the water content of the product gradually decreases due to transpiration and the respiration process (Siboza et al., 2014). This leads to wilting, loss of quality, and other undesirable changes in the product, ultimately reducing its economic value. The decrease in weight loss observed in fruits treated with methyl jasmonate and GABA can be attributed to their effect on maintaining membrane integrity at low temperatures, as reported in previous studies on pomegranate and blood orange (Habibi et al., 2019; Sayyari et al., 2011). However, the melatonin treatment exhibited the lowest percentage of weight loss compared to the other treatments. Melatonin may reduce weight loss by modulating water transport across the cuticle and plasma membrane, as well as by reducing respiration. Other findings suggest that melatonin treatment induces the expression of cuticle formation genes (CER1 and GPAT4/8) while downregulating aquaporins PIP1;4 and PIP2;7 in treated sweet cherry fruits, indicating its potential role in modulating, or reducing water loss. The thickness and specific composition of cuticle waxes and cutin are known to play a role in regulating the permeability of the cuticle against water movement (Lara et al., 2014). Additionally, as noted by Hui et al. (2016), the preservation of membrane integrity and reduced membrane permeability in melatonin-treated peach fruits may be attributed to the higher accumulation of endogenous melatonin, indicating its capacity to scavenge reactive oxygen species (ROS) and indirectly maintain the stability of the cell membrane. Studies have demonstrated that the application of melatonin treatment results in a significant reduction in the rate of weight loss during postharvest storage of 'Newhall' navel orange fruit (Ma et al., 2021). It has been demonstrated that GABA reduces metabolic activity in fruit tissue, thereby preventing water loss and weight loss. This effect is achieved by decreasing the activity of antioxidant enzymes and minimizing stress on the fruit (Asgarian et al., 2022). It has been demonstrated that the application of methyljasmonate treatment resulted in the lowest weight loss (3.2%) compared to the control group (8.4%) during the cold storage period of 'Kinnow' mandarins (Baswal et al., 2020).

Fruit firmness is an essential characteristic that not only affects customers' assessment of the quality of newly harvested lemons but also the determination of their ability to be preserved during postharvest operations. Melatonin inhibits the process of cell wall disintegration through the suppression of enzymatic activities and gene expressions that are associated with cell wall degradation, namely pectin methylesterase (PME), polygalacturonase (PG), and cellulase (Cel). Consequently, this leads to a postponement of fruit softening and senescence (Liao et al., 2024). The investigation conducted by Rastegar et al. (2020) illuminates the beneficial impact of melatonin in maintaining the firmness of mango fruit throughout the storage period. Melatonin enhances the activity of antioxidant enzymes, reduces oxidative damage, and maintains the energy status of postharvest fruit, which contributes to maintaining firmness. Melatonin interacts with reactive oxygen species (ROS) and nitric oxide (NO), which enhances the antioxidant and defense systems in postharvest fruit, leading to the preservation of firmness. The multiple functions of melatonin, including its antioxidant properties and interaction with signaling molecules, play a role in delaying the softening and maintaining the firmness of postharvest fruit (Ze et al., 2021; Liao et al., 2024) .

Research findings have indicated that treating carambola fruit with GABA has a beneficial impact on preserving firmness, even when subjected to chilling and non-chilling stresses. It is widely recognized that the decline in postharvest fruit firmness is primarily linked to the ripening stage's respiration process (Ngaffo Mekontso et al., 2021). Studies have

shown that the application of methyl jasmonate has proven effective in slowing down the activities of pectin methylesterase and cellulase enzymes, which are directly involved in fruit softening. As a result, 'Kinnow' mandarins treated fruit was able to maintain its firmness for a duration of up to 75 days during cold storage (Baswal et al., 2020).

Total soluble solids (TSS) in fruits include sugars and a small percentage of amino acids, organic acids, vitamins, and minerals. TSS is a valuable quality parameter related to consumer acceptance and has a significant impact on fruit taste and is considered one of the chemical indicators (Liu et al., 2020). The soluble solids increase as the fruit ripens. The reason for this gradual increase in Brix is the gradual decrease in fruit juice that occurs with the passage of time and during the storage period, which causes a decrease in solids dissolved in water and as a result, an increase in Brix. In other words, the more water the fruit has, the lower the weight loss, the lower the Brix. As previously reported, postharvest treatments delayed TSS increases during cold storage, possibly due to delays in ripening and aging processes. Melatonin treatment effectively delays the natural increase of TSS during storage. Changes in TSS may be related to changes in the shape of sugars during storage.

Titratable acidity decreased in all treatments during the storage period, although in general, the control fruit had lower titratable acidity values than the control throughout the entire storage period. The titratable acidity is related to the organic acids, which strongly affects the quality of the fruit. Its reduction during cold storage is related to using them as the main respiratory substrates for energy production during storage (Habibi et al., 2019; Liu et al., 2020). The taste index (TSS/TA) or the ratio of soluble solids to titratable acid is directly related to these two factors, and changes in these two factors affect the value of the taste index. This index is one of the important factors in determining fruit ripening and its quality and marketability (Hui et al., 2016) and its increase during cold storage was due to the decrease in organic acids and slight increase in sugars. However, all treatments significantly delayed the increase in flavor index and showed a possible effect on reducing the post-harvest ripening process.

Antioxidant compounds such as ascorbic acid are an important parameter in discussing the quality of fruits and vegetables (Khorshidi et al. 2011). It is worth mentioning that the higher concentration of ascorbic acid in the treated fruit causes higher antioxidant activity and beneficial health effects of lemon fruit consumption (Siboza et al., 2014). Antioxidant systems play an important role in quenching ROS and maintaining cellular redox homeostasis, thus modulating aging processes in plants (Jimenez et al., 2002). The treated fruits had higher ascorbic acid than the control fruit, and the decrease of ascorbic acid in the control samples was probably related to the aging of the fruit after long-term storage. These compounds maintained a higher content of ascorbic acid probably due to the reduction of ascorbic acid oxidase activity and delayed ripening as well as the aging process (Habibi et al., 2020). Postharvest application of methyl jasmonate maintained higher ascorbic acid content in pomegranate (Sayyari et al., 2011). Ascorbic acid is very susceptible to degradation due to oxidation during cold storage. Low temperature can increase the accumulation of free radicals. Ascorbic acid is a major and prominent non-enzymatic antioxidant in citrus fruits that has the potential to eliminate free radicals in cells (Huang et al., 2008). Therefore, the main reason for the reduction of ascorbic acid can be attributed to the inhibition of ROS by the non-enzymatic system as an electron donor to neutralize free radicals during storage at low temperature. In addition, the reduction of ascorbic acid is associated with fruit ripening after long-term storage (Habibi et al., 2019).

The assessment of fruit quality relies heavily on the color of the fruit, as it communicates the visual attractiveness of the fruit and has a significant impact on consumer preferences. Commercial maturity indexes in the citrus industry are usually based on peel coloration,

which is an external characteristic that defines fruit quality (Lado et al., 2018). The alteration of fruit color can occur during the storage process, and this alteration is influenced by various factors such as the duration of storage, the temperature within the cold storage facility, as well as any supplementary treatments applied. Specifically, citrus fruits display modifications in color that can be attributed to the breakdown of chlorophyll pigments and the buildup of carotenoids in flavonoids as they undergo the ripening process (Vidal et al., 2013). Mature citrus fruits are prepared with chromoplasts which possess the capacity to accumulate substantial quantities of carotenoids. These carotenoids, functioning as photoprotective agents, perform a vital function in safeguarding the integrity of the fruit's membranes, thus preventing any potential damage (Serna-Escolano et al., 2021). Research has demonstrated that the utilization of melatonin can impede the deterioration of the* indicator, which signifies the redness aspect of color, leading to elevated values in comparison to the control fruit. Melatonin treatment was found to delay the color changes in yellow-fleshed peach fruit compared to the control group during the storage period. This suggests that melatonin treatment can help maintain the original color of yellow-fleshed peaches for a longer duration, potentially preserving their visual appeal and quality (Wu et al., 2023). Melatonin treatment can have a positive effect on color parameters in postharvest fruit by delaying ripening, senescence, and deterioration, and by enhancing cold resistance (Ze et al., 2021). It has been shown that GABA treatment can effectively preserve the original color of the treated material for a longer period, potentially enhancing its visual appeal and quality. GABA application protects thylakoid membranes, chloroplast envelopes, and reduces swelling, thereby alleviating chlorophyll degradation (Fan et al., 2024). In a study on mango fruit treated with melatonin, it was found that the treatment inhibited pericarp discoloration during storage, possibly due to enhanced membrane integrity (Liu et al., 2020). Furthermore, the application of methyl jasmonate has shown to effectively maintain the color of the fruit. Similar observations have been documented, indicating that this treatment delays the browning of pomegranate skin and ensures the preservation of its overall appearance quality and color (Lorente-Mento, 2023). The treatments exert their effects by delaying metabolic processes, thereby slowing down the color change process.

Freshness has been documented as a primary factor influencing consumer selection of various fruits, such as citrus. Moreover, the visual aspect of fruits significantly contributes to consumers' perception of their freshness (Tarancón et al., 2021). According to the results, the appearance and marketability of the treated fruits were better than the control. The appearance quality and marketability are related to many factors such as the color and water content of the tissue, which damage caused by low temperature and water loss, respectively, by changing the color and drying of the peel during the storage period, can cause a decrease in the appearance quality. In addition, the decrease in appearance quality can be related to the oxidation of antioxidant compounds such as phenols due to the higher activity of polyphenol oxidase and peroxidase and the creation of brown pigment. The reduction of these phenolic compounds causes a change in the brightness of the skin (Habibi et al., 2020). Previous reports have indicated that melatonin treatment enhances the appearance of kiwiberries during storage by increasing the accumulation of total chlorophyll and carotenoids, thereby improving the flesh color of the fruit (Zhang et al., 2023). Several research groups have provided evidence that methyl jasmonate effectively improves color appearance. This positive impact is largely attributed to the ability of methyl jasmonate to enhance the antioxidant capacity of postharvest fruits. Furthermore, the improved color appearance can be attributed to the increased content of anthocyanins, which effectively mitigate skin browning (Wang et al., 2021).

CONCLUSION

In conclusion, this study investigated the effects of various postharvest treatments on lemons stored under low temperatures. The results demonstrated that most of the treatments, except for specific combinations, effectively reduced fruit weight loss. The melatonin treatment showed a significant decrease in weight loss compared to the control. Additionally, the melatonin treatment exhibited the highest fruit firmness. Regarding quality parameters, individual treatments, and the GABA + M treatment resulted in higher total soluble solids and a lower TSS/TA ratio compared to the control. Except for specific combinations, all treatments showed increased ascorbic acid content compared to the control. The melatonin treatment also showed notable differences in color indices. Overall, fruits treated with melatonin, methyl jasmonate, GABA, and the combination of GABA + M demonstrated higher marketability. Therefore, it is recommended to use these treatments individually to maintain the quality of lemon fruits. These findings provide valuable insights for the agricultural industry, suggesting the potential use of these natural compounds to enhance the shelf life and market value of lemons. Future research could explore optimization techniques for the application of these treatments and investigate their efficacy on other fruit varieties.

Conflict of interest

The authors declare that there is no conflict of interest.

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JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH
2024, VOL. 7(2), 115-140

Journal of Horticulture and Postharvest Research

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

University of Birjand

Phenotypic diversity of some Iranian grape cultivars and genotypes (*Vitis vinifera* L.) using morpho-phenological, bunch and berry traits

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ARTICLE INFO

Original Article

Article history:
Received 26 January 2024
Revised 2 April 2024
Accepted 3 April 2024

Keywords:
Berry
Cluster analysis
Correlation coefficient
Grapes
Morphological traits

DOI: 10.22077/jhpr.2024.7165.1355
P-ISSN: 2588-4883
E-ISSN: 2588-6169

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ABSTRACT

Purpose: Grape (*Vitis vinifera* L.) is one of the most important horticultural products that are grown in different parts of Iran and has high nutritional values. In this study, the genetic diversity of cultivars and genotypes of some vineyards of Markazi province were investigated for the preliminary selection of superior cultivars and genotypes in terms of morphological and fruit characteristics for use in grape breeding programs. **Research method:** For this purpose, grouping and comparing 84 grape cultivars and genotypes were carried out using 70 traits including phenological and vegetative traits, trichome and stomata, bunch and berry traits. **Findings:** Based on the results, the "Sahebi Hazaveh" cultivar with 1000.17 g had highest an average bunch weight to compare other cultivars and genotypes. Results showed that, some traits such as bunch weight, bunch shoulders, fresh weight, rachis weight, the ratio of bunch weight to peduncle weight, the ratio of rachis weight to bunch weight, dry weight of bunch shoulders, length of the tail of bunch, berry weight, pedicel weight, seed weight and length of seed had a high coefficient of variation. Factor analysis reduced the evaluated traits to 10 main factors showed that they justified 78.38% of the total variance. Cluster analysis divided cultivars and genotypes into 4 main groups at five Euclidean distances. **Limitations:** No limitations were encountered. **Originality/Value:** This study indicated that grapes germplasm resources in zone are of noticeable diversities and can be promising for the utilization in the breeding programs. Based on the results, cultivars and genotypes of "Khalili Khondab" region, "Yaghoti", "Sahebi", "Fakhri", "Kharvand" and "Kondori" Hazaveh region and "Sahebi" Aghbolagh region in leafing time, late flowering, sugar percentage, bunch and berry characteristics, stomatal density, standing and lying trichome density in leaves were superior to other cultivars and genotypes.

INTRODUCTION

Grapes, scientifically known as *Vitis vinifera* L., belong to the Vitaceae family, also called the Sarmentaceae or Ampelidaceae family (Kellar & Tarara, 2010; Rasouli et al., 2014; Rasouli et al., 2015; Doulti Baneh.,2015; Jahnke et al., 2021; Kupe et al., 2021). This family belongs to the Rhamnales order and is part of the hidden flowering plant group in the Rosids branch. The mentioned family has over 15 genera and approximately 1000 species, with the most important genus, Vitis, having different subgenera with varying chromosome numbers (Rasouli et al., 2015). The Asian group includes 11 species, while the European group consists of only one species. The species found in Europe and the Middle East mainly include *V. vinifera*. American species are highly important due to their resistance to pests, cold weather, and tolerance to calcareous soils (Rasouli et al., 2015; Jalili Marandi et al.,2016; Rasouli & Kalvandi, 2022) Grapes is one of the most important fruits that have been used by humans since ancient times. Some experts believe that grapes were used even before the emergence of cereal. Based on botanical and archaeological studies, the Near East region is considered the primary center of grapes (Kellar & Tarara, 2010; Doulti Baneh., 2015; Jahnke et al., 2021; Kupe et al., 2021). Grapes have a high nutritional value, and according to research by the Food and Agriculture Organization (FAO, 2017) table grapes contain 67 kilocalories per 100 grams, while raisins contain 268 kilocalories per 100 grams (Doulit Baneh., 2015). *Vitis vinifera*, known as the wine grape, is one of the most widely used plant species in horticulture and is favored by farmers. It is the only species extensively used in the food industry and consumption worldwide. Alongside apples, citrus fruits, and bananas, it is one of the most important horticultural plants widely cultivated (Kupe et al., 2021). Climate greatly affects grape diversity and production in a specific location (Akram et al., 2021). Local grape cultivars are essential for preserving crop diversity and can be crucial for food, nutrition, and economic security for many individuals. For smallholder farmers and agricultural communities in rural and marginalized areas, the diversity of local grapes can provide insurance against damage due to reduced yield and supply special ingredients for traditional local dishes and specific dietary needs. In any country where grape cultivation is practiced, there are numerous local cultivars that contribute to global grape diversity (Gago et al., 2009; Antolin et al., 2020) According to experts, grape cultivation has been common in Iran for at least 2000 years before the Common Era. Grapes are an important horticultural product with increasing cultivation area in Iran. Due to its extensive history of grape cultivation and production, Iran is recognized as one of the important centers of grape genetic diversity. With over 255,000 hectares of vineyards (10.2% of the total orchards) and an approximate production of 2.8 million tons (about 12.4% of the total fruit production), Iran is among the most significant production centers.

Table 1. Geographical location of tested vineyards in Markazi province to investigate morphological diversity of grapes.

Number	Country	Province	Location	Above sea level (m)	Latitude	Longitude
1	Iran	Markazi	Marzijaran	1728	34.14346552	49.64018154
2	Iran	Markazi	Hazaveh	1921	34.18479862	49.53418064
3	Iran	Markazi	Khondab	1822	34.38872495	49.15541268
4	Iran	Markazi	Enaj	1765	34.23042971	49.31798172
5	Iran	Markazi	Derman	2012	34.24812195	49.47916174
6	Iran	Markazi	Aghbolagh	1965	34.10024373	49.50501716
7	Iran	Markazi	Anjudan	1972	33.97994426	50.03023696

Grapes have special importance in Iran, and this crop has the highest cultivation area in the horticultural sector after pistachios and the highest production after apples (Papademetriou & Dent, 2001; Rasouli et al., 2015; Elhami et al., 2019; Khan et al., 2020) Markazi province has approximately 57,000 hectares of horticultural products in Iran. The total area of fertile and infertile vineyards in Markazi province (Center of Iran) in 2021 was about 16,000 hectares, with a production of around 148,000 tons grapes in the country according to the latest available information of the statistics of the Ministry of Jihad Agriculture and the Statistics Center of Iran and the statistical yearbooks of different provinces (Organization of Agriculture, 2021; Salehnia & Rafati, 2023). Having precise selection power among plants is necessary for breeding and production of new varieties, which depends on the identification of existing varieties and their diversity. Studying the genetic diversity in plant populations and selecting the appropriate traits for production and introduction of superior genotypes will be helpful. Additionally, studying phenotypic and genotypic diversity is crucial for identifying similar genotypes, evaluating and utilizing genetic reserves, and preserving them. Identifying and differentiating genotypes from each other, as well as studying the diversity of wild, indigenous, or modified germplasm, before starting breeding programs and to respect the intellectual property rights of breeders, is of great importance (Zahedi et al., 2023). Based on the inter- and intraspecific morphological variability, several descriptor lists, manuals and ampelographic studies are available for identification (Bodor-Pesti et al., 2023). Among the organs, leaves have the most traits, while the young shoot, bunch and berry are also important in the characterization of the genotypes. *Vitis* species and cultivars are described by leaf morphological characterization developed in many ways for the identification of genotypes, to clarify synonymies and distinct clones or evaluate the diversity of wild *Vitis* taxa (Bodor-Pesti et al., 2023). The identification of grape genotypes is usually based on the characteristics of the mature plant, which are influenced by environmental conditions. Grape genotypes are typically identified and grouped based on 130 phenological traits, evaluated and identified using phenological methods (Razi et al., 2021). Regarding screening, various studies and experiments have been conducted in Iran and other countries with the aim of finding drought-tolerant or resistant genotypes as the goal of these experiments and studies. In some others, the identification of cultivars and genotypes with superior traits and high yield under these conditions is desired. Identifying resistant and tolerant cultivars and genotypes to abiotic and biotic stresses is one of the most important strategies for coping with these stresses (Razi et al., 2021). By determining appropriate morphological, physiological, and molecular traits for screening, it is possible to select cultivars and genotypes compatible with the climatic conditions of each region (Amiri & Eslamian, 2010). Among the different cultivars, there are some with desirable fruits that have gained the attention of farmers due to their high quality for table grape, raisin production, and processing. Their cultivation area is increasing recently. On the other hand, cultivars without desirable fruits lose their place and receive less attention. However, these cultivars may possess valuable genes such as resistance to pests, diseases, cold, salinity, drought, and the like, which have not been utilized and gradually become extinct due to lack of identification and accurate understanding of their nature (Khadivi-Khub et al., 2014) In a study conducted by Haddadinejad et al. (2013), screening of drought-tolerant genotypes was carried out among 698 genotypes in three stages. Initially, based on the characteristics of trichomes on the vegetative organs, 150 genotypes were selected. In the second stage, screening was done based on trunk diameter, and 44 genotypes with a diameter greater than 4 centimeters, indicating vigorous growth, were identified. In the third stage, several genotypes such as “Kaj Angor Bajnurd”, “Sorkh Ghoochan”, “Siah Zarqhan”, and “Ghalati Shiraz” were introduced as options with traits related to drought tolerance based on 17 morphological markers related to drought stress and Pearson correlation coefficients

(quantitative traits) and Spearman (qualitative traits) between traits related to drought tolerance (Haddadinejad et al., 2013).These studies can help identify genotypes with higher tolerance and use them as the basis for commercial cultivars to achieve better water efficiency in crop production (Zahedi et al., 2023). In another study conducted by Rasouli et al. (2014), phenotypic diversity of 32 grape cultivars and genotypes was examined over a period of 3 years for morphological and pomological traits, including phenolic content and the level of the anti-cancer compound resveratrol. The results indicated high diversity among the studied cultivars and genotypes in terms of the measured traits, including bunch, berry, seed and resveratrol content (Rasouli et al., 2014). In an experiment on morphological diversity, 36 grape cultivars and genotypes were evaluated using the international grape descriptor to select superior genotypes. The traits such as bunch weight, dried bunch weight, berry weight, rachis weight, berry weight, seed weight, and skin color showed high diversity among the cultivars and genotypes and had high coefficients of variation (Rasouli et al., 2014; Razi et al., 2021). Significant positive and negative correlations were observed between some traits (Rasouli et al., 2014). Factor analysis revealed that the first and second factors had the highest contributions to the variance. Traits such as bunch weight, dried bunch weight, bunch width, berry length, berry pedicel length, and skin color were included in the first factor (PC1), which accounted for 44.16% of the total variance. Additionally, traits such as diameter, weight, length, and size of the berry were included in the second factor (PC2), which accounted for 15% of the total variance. Based on cluster analysis using the Euclidean distance, the cultivars and genotypes were divided into four groups, with important factors for distinguishing the cultivars including bunch weight, dried bunch weight, fruit sugar content, leaf width, leaf length, and leaf surface area (Rasouli et al., 2014; Razi et al., 2021). Kazemi et al. (2022) evaluated the phenotypic diversity of 60 grapevine cultivars and genotypes available in tropical, subtropical region of Khuzestan province in Iran, by using 105 phenological, morphological, biochemical and pomological traits based on the international descriptor for grapevines. Their results showed, the significant diversity of grapevine cultivars and genotypes existing in vineyards of Khuzestan province showed the superiority of native and local cultivars and genotypes such as 'Soltani' (Sultana), 'Bangi' (Ghermez) and 'Yershi' in some traits compared to other foreign cultivars (Kazemi et al., 2022). The aim of this research was to investigate the phenotypic and morphological diversity of some grape cultivars and genotypes from vineyards in different regions of Markazi province that was located in central of Iran, with a focus on morphological traits affecting drought tolerance, fruit characteristics and yield. Also, to identify and introduce superior genotypes present in native and local populations was another objective of this study.

MATERIALS AND METHODS

The majority of vineyards in Markazi province are located in Hazaveh, Sharra River area, and to some extent in Shazand, Zarandiyeh, and Saveh. The dominant grape cultivars in the grape-growing areas are “Bidaneh Sefid”, “Bidaneh Ghermez”, “Asgari”, “Farahi”, “Yaghoti”, “Lal”, and “Siah” grapes (Organization of Agriculture, 2021). Markazi province, with an area of 29,530 square kilometers, is one of the industrial and agricultural provinces in Iran, located between 33° 30′ to 35° 35′ N and less than 2 percent of the total area of the country. Based on the topography of the region, 75 percent of the province is mountainous and 25 percent is plains.

Table 2. List of grapes cultivars and genotypes tested in Markazi province to investigate morphological diversity.

Row	Cultivar/ Genotype	Location	Row	Cultivar/ Genotype	Location	Row	Cultivar/ Genotype	Location
1	Khalili	Aghbolagh	29	Sahebi	Derman	57	Fakhri	Aghbolagh
2	Khalili	Marzijaran	30	Shirazi	Aghbolagh	58	Fakhri	Marzijaran
3	Khalili	Anjudan	31	Shirazi	Marzijaran	59	Fakhri	Anjudan
4	Khalili	Hazaveh	32	Shirazi	Hazaveh	60	Fakhri	Hazaveh
5	Khalili	Khondab	33	Shirazi	Khondab	61	Fakhri	Khondab
6	Khalili	Enaj	34	Shirazi2	Khondab	62	Fakhri	Enaj
7	Khalili	Derman	35	Shirazi	Enaj	63	Fakhri	Derman
8	Khalili Khani	Marzijaran	36	Shirazi	Derman	64	Fakhri Asgari	Enaj
9	Yaghoti	Aghbolagh	37	Asgari	Anjudan	65	Bidaneh Sefid	Aghbolagh
10	Yaghoti	Marzijaran	38	Asgari	Hazaveh	66	Bidaneh Sefid	Marzijaran
11	Yaghoti	Anjudan	39	Asgari	Khondab	67	Bidaneh Sefid	Anjudan
12	Yaghoti	Hazaveh	40	Asgari	Enaj	68	Bidaneh Sefid	Hazaveh
13	Yaghoti	Khondab	41	Asgari	Derman	69	Bidaneh Sefid	Khondab
14	Yaghoti	Enaj	42	Asgari	Aghbolagh	70	Bidaneh Sefid	Enaj
15	Yaghoti	Derman	43	Asgari bi bazr	Anjudan	71	Bidaneh Sefid	Derman
16	Sahebi	Aghbolagh	44	Asgari Shahrodi	Hazaveh	72	Bidaneh Ghermez	Aghbolagh
17	Sahebi	Marzijaran	45	Asgari gerd	Enaj	73	Bidaneh Ghermez	Marzijaran
18	Sahebi	Anjudan	46	Siah	Marzijaran	74	Bidaneh Ghermez	Hazaveh
19	Sahebi	Hazaveh	47	Siah	Anjudan	75	Bidaneh Ghermez	Khondab
20	Sahebi	Khondab	48	Siah	Hazaveh	76	Bidaneh Ghermez	Enaj
21	Sahebi	Enaj	49	Siah	Khondab	77	Bidaneh Ghermez	Derman
22	Asgari	Marzijaran	50	Siah	Enaj	78	Lal	Aghbolagh
23	Kharvand	Hazaveh	51	Kol Bache	Anjudan	79	Lal	Marzijaran
24	Kharvand	Derman	52	Halvai	Anjudan	80	Lal	Hazaveh
25	Angor Sefid	Aghbolagh	53	Yek Tokhm	Marzijaran	81	Lal	Khondab
26	Kerak	Marzijaran	54	Lorkosh	Hazaveh	82	Lal	Enaj
27	Kole	Aghbolagh	55	Mehdikhani	Hazaveh	83	Lal	Derman
28	Ghazvini	Anjudan	56	Kondori	Hazaveh	84	Moamelan	Derman

Table 3. Some evaluated traits and how to measure them in the investigated grape samples based on the OIV (2007), IPGRI and UPOV (2008) description†.

Row	Trait	Unit	Abbreviation	Measurement method
1	Flowering time	Score	FTI	1= Too early, 2= Very early, 3= Early, 4=Early to medium, 5= Medium, 6= Medium late, 7=Late, 8= Very late, 9= Too late
2	Leafing time	Score	LTIM	1= Early, 3= Medium, 5= Late
3	Growth vigour	Score	BGP	3= Weak, 5= Moderate, 7= Strong
4	Shoot attitude	Score	SATT	1=Erect 3= Semi-erect 5= Horizontal 7= Semi drooping9=Drooping
5	Size of blade	Score	LSI	1= Very small, 3= Small, 5= Medium, 7= Large, 9= Very large
6	Length of teeth	mm	TL	Digital Caliper
7	Petiole length	mm	PL	Digital Caliper
8	Leaf length	mm	LL	Digital Caliper
9	Tendril length	mm	TLE	Digital Caliper
10	Colour of upper surface	Score	CUSL	1=Green yellow 2=Green with bronze spots 3=Yellow 4=Yellow with bronze spots 5=Copper yellow 6=Copper 7=Reddish
11	Number of lobes	Score	NLO	1=Entire leaf (none) 2= Three 3= Five 4=Seven 5=. More than seven
12	Intensity of anthocyanin staining of buds	Score	IASB	0=Absent 1= Very weak 3= Weak 5= Medium 7= Strong 9=Very strong
13	Anthocyanin intensity of young leaves	Score	AIYL	0=Absent 1= Very weak 3= Weak 5= Medium 7= Strong9=Very strong
14	Stomata density in the field of view of forty microscopes	number	SDF40	Counting in the field of view
15	Stomata density in the field of view of Twenty-five microscopes	number	SDF25	Counting in the field of view
16	Internode diameter	mm	ID	Digital Caliper
17	Colour of Ventral Side of Nodes	Score	CVSN	1= Completely green 2= Green and red striped 3= Completely red
18	Colour of Dorsal Side of Nodes	Score	CDSN	1= Completely green 2= Green and red striped 3= Completely red
19	Colour of the ventral side of internodes)	Score	CVSI	1= Completely green 2= Green and red striped 3= Completely red
20	Colour of the dorsal side of internode)	Score	CDSI	1= Completely green 2= Green and red striped 3= Completely red
21	Form of Tip of Young Shoot	Score	FTYS	1=Closed 2= Slightly open 3= Half-open 4= Wide open 5= Fully open
22	Density of erect trichomes on main veins on lower side of blade	Score	DETMB	Absent (0) Very sparse (1) Sparse (3) Medium (5) Dense (7) Very dense (9)
23	Density of erect trichomes on main veins on lower side of blade	Score	DETML	Absent (0) Very sparse (1) Sparse (3) Medium (5) Dense (7) Very dense (9)
24	Density of prostrate trichomes on main veins on lower side of blade	Score	DPTM	Absent (0) Very sparse (1) Sparse (3) Medium (5) Dense (7) Very dense (9)
25	Density of prostrate trichomes between veins	Score	DPTV	Absent (0) Very sparse (1) Sparse (3) Medium (5) Dense (7) Very dense (9)
26	Density of erect trichomes between veins	Score	DETBE	Absent (0) Very sparse (1) Sparse (3) Medium (5) Dense (7) Very dense (9)
27	Density of prostrate trichomes on main veins	Score	DPTM	Absent (0) Very sparse (1) Sparse (3) Medium (5) Dense (7) Very dense (9)
28	Density of erect trichomes on main veins	Score	DETMV	Absent (0) Very sparse (1) Sparse (3) Medium (5) Dense (7) Very dense (9)
29	Density prostrate trichomes of Young Shoot Tip	Score	PTDYS	Absent (0) Very sparse (1) Sparse (3) Medium (5) Dense (7) Very dense (9)
30	Fruit ripening time	Score	FRT	1= Very early, 3= Early, 5= Medium, 7= Late, 9= Very late
31	Bunch size	Score	BZI	3= Small, 5= Medium, 7= Large, 9= Very large

Table 3. (***Continued***). Some evaluated traits and how to measure them in the investigated grape samples based on the OIV (2007), IPGRI and UPOV (2008) description.

Row	Trait	Unit	Abbreviation	Measurement method
32	Bunch density	Score	BDE	3= Open, 5= Medium, 7= Tight, 9= Very tight
33	Density of berry per bunch	Score	DBPB	3 = Open, 5 = Medium, 7 = Compact
34	Bunch number of bush	Count	BNB	Count
35	Brix%	Brix	B	Refractometer
36	Bunch length	mm	BL	Digital Caliper
37	Bunch width	mm	BWII	Caliper
38	The length to width ratio of bunch	mm	LWR	Caliper
39	Bunch weight	g	BWI	Digital scale
40	Bunch shoulder weight	g	BSW	Digital scale
41	Ratio of bunch weight bunch shoulder weight	Ratio	ROBWT	Calculate the ratio of bunch weight to bunch shoulder weight
42	Rachis weight	g	RW	Digital scale
43	Peduncle weight	g	PW	Digital scale
44	Ratio of the Rachis weight to Peduncle weight	Ratio	RRTP	Calculate ratio of the rachis weight to peduncle weight
45	Ratio of bunch weight to Rachis weight	Ratio	RBWR	Calculate ratio of the bunch weight to rachis
46	Ratio of Rachis weight to the bunch weight	Ratio	RBWBS	Calculate ratio of the bunch weight bunch shoulder weight
47	Ratio of the Peduncle weight to the Bunch shoulder weight	Ratio	RRWWB	Calculate ratio of the rachis weight to the weight of bunch shoulder
48	Anthocyanin colouration of fresh	Score	ACF	1=Very slightly coloured 3= Slightly coloured 5= Coloured 7= Strongly coloured 9=Very strongly coloured
49	Skin thickness	Score	STH	3= Thin, 5= Medium, 7= Thick
50	Being juicy	Score	BJ	1= Low water, 2= Slightly watery, 3= Very watery
51	Berry color	Score	BCO	1= Green-yellow, 2= Rose, 3= Red, 4= Red Gray, 5= Dark red-violet, 6= Blue-black
52	Berry hardness	Score	BHA	1=Soft, 2=Slightly hard, 3=Hard
53	Berry shape	Score	BSH	1= Oblong 2= Narrow elliptic 3= Elliptic 4= Round 5= Oblate 6= Ovate 7= Obtuse-ovate 8= Obovate 9= Arched
54	Berry weight	g	BWE	Digital scale
55	Berry length	mm	BLE	Digital caliper
56	Berry width	mm	BWID	Digital caliper
57	The length to width ratio of berry	Calcula te	LWRB	Calculate the ratio length to width of berry
58	Berry diameter	mm	BDI	Digital caliper
59	Berry tail length(mm)	mm	BTLE	Digital caliper
60	Berry weight	g	BWE	Digital scale
61	Berry tail weight(g)	g	BTWE	Digital scale
62	Seed weight	g	SW	Digital scale
63	Seed length	mm	SL	Digital caliper
64	Bunch tail Length	mm	BTL	Digital caliper
65	Existence of seeds	Score	ES	1= None 2= Incomplete growth 3= Complete growth
66	Separating from the pedicel	Score	SFP	1= Hard 2= Fairly easy 3= Very easy
67	Fresh weight of bunch shoulder	g	FWBS	Digital scale
68	Dry weight of bunch shoulder	g	DWBS	Digital scale
69	Ratio the fresh weight to dry weight of bunch shoulder	g	RFWDW	Calculate ratio the fresh weight to dry weight of bunch shoulder

† OVI: International Office of the Vine and Wine (www.oiv.int), IPGRI: International Plant Genetic Resources Institute (www.Bioversityinternational.org), UPOV: International Union for the Protection of new Varieties of Plants (www.upov.int).

According to the Islamic Republic of Iran Meteorological Organization (IRIMO, 2023), the average rainfall is 311 millimeters, and the climate of the province is classified as semi-arid according to the second De Martons classification system and dry-cold according to the Amberzhe classification (Asakereh et al., 2022; IRIMO, 2023). Some areas of the Markazi province have suitable climate for cultivation of grapevines, and there are old vineyards in some areas. The first phase of this research involved the investigation, evaluation, and screening of some cultivars and genotypes of grapevines in certain vineyards of Markazi province, which started in March 2019 and continued until December 2022. In this study, the morphological diversity of 84 grape cultivars and genotypes, 7 to 10 years old, in the regions (Tables 1 and 2) were evaluated using 69 morphological traits (34 quantitative and 36 qualitative traits) from March 2019 to December 2022 (Table 3). Three mature vines were selected for each variety and genotype to collect data from various growth stages, phenological stages, leaves, bunch, berry and some quantitative and qualitative traits (Table 3) were measured using different and appropriate methods for each trait. Additionally, some of OIV (OIV 2007), IPGRI (IPGRI 2008), and UPOV (UPOV 2008) as presented in Table 3. In the second phase, for the examination of cultivars, quantitative and qualitative traits were evaluated as described in the following table, using the descriptor of OIV (2007), IPGRI (2008), and UPOV (2008), as well as the number and density of tendrils and berries (Table 3). The genetic diversity was assessed based on morphological indices, with emphasis on phenological traits such as leafing time, flowering time, ripening time, and morphological traits (leaf and fruit characteristics). The measurement of quantitative and qualitative traits was conducted using the coding method based on the grape descriptor of OIV, IPGRI, and UPOV (Table 3).

Statistical analysis

Frequency of traits, descriptive statistics, simple correlations between traits, and cluster analysis were performed using SPSS software (Version 21.0). The coefficient of variation was calculated by dividing the standard deviation of each trait by its mean to measure the variation. Pearson's correlation coefficient was used to determine the correlation between traits. Factor rotation technique and maximum variance method were used to extract factors and factor loadings of 0.4 or higher were considered significant. Cluster analysis and grouping of cultivars and genotypes were performed using the Ward's method or the minimum variance method based on the Euclidean distance and standardized data (Rasouli et al., 2014; Zahedi et al., 2023).

RESULTS AND DISCUSSION

Descriptive statistics and frequency distribution of traits

The minimum, maximum, mean, standard deviation, variance, and coefficient of variation for some important measured traits in the grape cultivars and genotypes are presented in Table 3. Also, some important morphological characteristics measured in the examined grape cultivars and genotypes are mentioned in Table 4.

Table 4. Descriptive statistics of quantitative traits in grape cultivars and genotypes studied in Markazi province.

Row	Trait	Min	Max	Ave	Std	Var	CV.%
1	Internode diameter (mm)	7.15	14.56	11.67	1.80	3.24	15.43
2	Leaf length (mm)	71.33	148.36	98.95	16.26	264.34	16.43
3	Petiole Length (mm)	42.23	133.34	77.50	19.80	392.16	25.55
4	Length of teeth (mm)	3.15	9.53	5.32	1.39	1.93	26.11
5	Tendril length (mm)	8.22	221.33	93.88	37.13	1378.28	39.55
6	Stomata density in the field of view of 40 microscopes	2.14	7.22	4.15	1.10	1.20	26.48
7	Stomata density in the field of view of 25 microscopes	35.32	91.51	56.90	12.53	156.88	22.01
8	Bunch weight (g)	102.51	1000.71	354.40	205.97	42424.78	58.12
9	Bunch shoulder weight (g)	9.53	89.93	34.97	16.66	277.52	47.64
10	Ratio of bunch weight to bunch shoulder weight	6.01	16.82	10.13	2.56	6.54	25.26
11	Rachis weight (g)	1.12	24.51	6.13	4.52	20.39	73.62
12	Peduncle weight (g)	0.11	1.78	0.51	0.34	0.11	65.16
13	Ratio of the rachis weight to peduncle weight	3.36	26.48	12.72	4.94	24.38	38.82
14	Ratio of the bunch weight to rachis	10.72	202.46	68.77	33.58	1127.64	48.83
15	Ratio of the bunch weight bunch shoulder weight	0.00	0.09	0.02	0.01	0.00	58.37
16	Ratio of the rachis weight to the of bunch shoulder weight	0.00	0.06	0.02	0.01	0.00	66.00
17	Fresh weight of bunch shoulder (mm)	8.41	196.69	45.88	32.72	1070.79	71.33
18	Dry weight of bunch shoulder (mm)	2.86	58.10	11.96	8.30	68.91	69.39
19	Ratio the fresh weight to dry weight of bunch shoulder	2.11	5.48	3.86	0.68	0.46	17.61
20	Bunch number of bushes	20.50	81.20	47.43	15.55	241.92	32.79
21	Bunch length (mm)	118.24	310.75	187.59	42.08	1770.98	22.43
22	Bunch width (mm)	36.81	126.64	78.87	17.23	297.01	21.85
23	The length to width ratio of bunch	1.38	4.11	2.44	0.59	0.35	24.39
24	Bunch tail length (mm)	10.12	74.94	29.08	12.74	162.19	43.79
25	Berry weight (g)	0.83	6.99	2.79	1.40	1.97	50.29
26	Berry length (mm)	9.54	32.21	18.45	4.47	19.98	24.23
27	Berry width (mm)	9.27	21.12	14.44	2.53	6.42	17.54
28	The length to width ratio of berry	0.98	1.84	1.27	0.17	0.03	13.81
29	Berry diameter (mm)	9.49	20.99	14.33	2.25	5.08	15.73
30	Berry tail length (mm)	2.89	8.61	6.18	1.36	1.86	22.07
31	Berry tail weight (g)	0.01	0.18	0.03	0.02	0.00	85.36
32	Seed weight (g)	0.00	0.30	0.05	0.05	0.00	93.38
33	Seed length (mm)	0.00	10.33	5.04	2.86	8.21	56.84
34	Brix (%)	14.21	26.96	20.14	2.84	8.06	14.09

According to the results, traits such as bunch weight (58.12%), bunch shoulders weight (47.64%), rachis weight (73.62%), peduncle weight (65.15%) , the ratio of bunch weight to peduncle weight (38.82%), bunch shoulders fresh weight (71.33%), dry weight of the bunch shoulders (69.39%), ratio the fresh weight to dry weight of bunch shoulder (17.61%) berry weight (50.29%), seed weight (93.38%), seed length (56.84%) showed high diversity in cultivars and genotypes and have relatively high coefficients of variation (Table 4). The highest bunch weight was observed in the variety “Sahebi Hazaveh” with an average weight of 1000.71 g (Tables 4 and 5). On the other hand, the lowest bunch weight was observed with

102.51g in "Khalili Anjudan" cultivar (Tables 4 and 5). Also, the maximum number of bunches per vine was found in the cultivar "Asgari Hazaveh" with an average of 81.20 bunches, while the minimum number of bunches per vine was observed in the "Kole Bache Anjudan" cultivar with an average of 20.50 bunches (Tables 4 and 5). In this experiment, the longest bunch length (310.75 mm) was attributed to the cultivar "Fakhri Enaj". In the event that the shortest bunch length (118.24 mm) was found in the variety "Khalili Enaj" (Tables 4 and 5). The "Kharvand Hazaveh", cultivar showed widest bunch width (126.64mm), however the smallest width bunch (36.81mm) was found in the cultivar "Khalili Anjudan". Moreover, the highest berry weight (6.99 g) was measured in "Kondori Hazaveh," cultivar, but the lowest weight of berry (0.83 g) measured in "Yaghoti Anjudan". The maximum sugar content (26.96 Brix) was reported from "Bidaneh Ghermez Derman" cultivar, whereas the minimum amount of sugar content (14.21 Brix) was measured in "Shirazi 2 Khandab" cultivar (Tables 4 and 5). Also, the average amount of Brix (sugar level) was 20.14%, which is close to the normal level of grape Brix. In this part, it can be compared that in terms of bunch weight, number of bunches per plant, maximum bunch width and berry weight, Hazaveh cultivars have a higher ratio compared to the rest of the tested regions, and the cultivars of Anjudan region are almost weaker than the other investigated cultivars and genotypes (Tables 4 and 5). The time of berry ripening was delayed in the cultivars "Sahebi Derman," "Shirazi Khondab," "Kol Bache Anjudan," and "Yek Bazr Marzijaran" compared to other cultivars and genotypes. Regarding flowering time, the cultivars "Kol Bache Anjudan," "Kolehe Aghbolagh,","Angur Sefid Aghbolagh," "Lal Derman," "Lal Hazaveh," "Lal Marzijaran," "Lal Aghbolagh," "Keshmishi Ghermez Enaj," "Keshmishi Sefid Enaj," "Fakhri Derman," "Fakhri Hazaveh," "Fakhri Marzijaran," "Shirazi Aghbolagh," "Sahebi Derman," "Sahebi Hazaveh," "Sahabi Anjudan," "Sahabi Marzijaran," and "Sahebi Aghbolagh" (Tables 4 and 5) had later flowering compared to other cultivars and genotypes, indicating that these cultivars may exhibit better tolerance to early spring frost. Therefore, traits with high diversity can be used for a more accurate evaluation of the studied cultivars and genotypes, considering the differences and variations in phenological and morphological traits. Rasouli et al. (2014) reported the average weight of bunch (85.46 g), bunch shoulder (13 g), rachis (2.57 g) and peduncle (0.3 grams), which was consistent with the findings in some cases of this research, so that the average weight of peduncle was obtained (0.51 g) (Tables 4 and 5). The difference in the values of some traits can be due to the genetic diversity, the age of the vines, different growing conditions of the vineyard and the geographical region. In the present study, seed weight varied from 0 in seedless cultivars to 0.3 g with an average of 0.05 g among cultivars and genotypes, which was consistent with the findings of Mouszadeh et al. (2015). Mousazadeh et al. (2015) reported, on the grape cultivars of the Khorasan Razavi Research Center collection, "Samarghandi Lotfabad" cultivar had the highest seed weight and "Dizmari Rezaieh" cultivar had the lowest seed weight, one of the reasons for the increased seed weight can be the genetic potential of this the figures show that this potential causes the rapid growth of the fruit and the increase of its constituents. Also, findings of this investigation, was consistent with the findings of various researchers (Bodor-Pesti et al., 2023) that the efforts of metric characterization of the grapevine leaf with the introduction of the scientific objectives and reviewing the studies showing the innovations in phenotyping during the last years (Bodor-Pesti et al., 2023). Kazemi et al. (2022) reported that there is a significant variation in the evaluated traits of cultivated cultivars and genotypes and its origin from Khuzestan province, southwest of Iran, which was somewhat in line with the results of the present research.

Table 5. Some important morphological characteristics measured of grape cultivars and genotypes studied in Markazi province.

Row	Cultivar/Genotype	Berry colour	Fruit ripening time	Density of erect trichome between veins	Density of prostrate trichome between veins	Leaf timing	Brix	Berry width	Berry length	Bunch shoulder weight	Bunch weight	Stomata density	Internode diameter
		Score	Score	Score	Score	Score	%	(mm)	(mm)	(g)	(g)	count	(mm)
1	Khalili Aghbolagh	1	1	5	3	5	16.32	14.11	20.11	17.81	190.55	3.85	10.91
2	Khalili Marzijaran	1	1	9	1	1	19.11	13.92	19.65	23.18	219.81	3.42	13.21
3	Khalili Anjudan	1	3	9	1	3	17.13	11.12	13.92	11.23	102.51	4.16	12.25
4	Khalili Hazaveh	1	1	9	5	5	16.77	13.71	19.34	22.58	208.08	3.15	10.81
5	Khalili Khondab	1	5	9	1	5	19.82	15.12	16.52	54.07	541.72	3.66	10.11
6	Khalili Enaj	1	1	9	3	5	16.83	13.91	21.61	13.23	220.11	3.11	11.54
7	Khalili Derman	1	1	7	5	3	18.22	13.45	19.32	23.18	215.81	4.17	7.89
8	Khalili Khani Marzijaran	1	3	9	1	1	18.37	13.95	19.91	36.12	244.32	2.52	13.41
9	Yaghoti Aghbolagh	3	1	9	7	5	17.75	10.11	11.99	25.61	211.09	3.81	10.47
10	Yaghoti Marzijaran	5	3	7	1	1	17.35	9.79	10.91	26.52	261.31	4.33	10.11
11	Yaghoti Anjudan	5	3	3	1	3	17.87	9.27	9.54	9.53	145.12	3.45	9.91
12	Yaghoti Hazaveh	6	1	7	3	5	23.78	10.42	11.23	57.34	688.05	2.14	11.53
13	Yaghoti Khondab	6	3	1	1	5	19.64	9.83	12.61	20.13	245.84	2.52	10.68
14	Yaghoti Enaj	5	1	9	3	5	17.35	10.12	12.42	17.18	227.57	3.16	11.67
15	Yaghoti Derman	6	1	7	5	3	22.12	10.61	11.23	37.42	487.01	3.48	7.15
16	Sahebi Aghbolagh	5	5	7	3	5	21.84	16.89	26.12	42.41	597.06	2.75	13.32
17	Sahebi Marzijaran	5	5	3	0	5	19.51	14.11	15.98	18.61	167.78	5.71	10.71
18	Sahebi Anjudan	5	5	7	1	5	20.83	16.92	21.14	36.55	298.32	3.54	10.48
19	Sahebi Hazaveh	6	5	9	3	5	21.44	20.11	25.81	86.57	1000.71	2.92	11.11
20	Sahebi Khondab	3	5	1	1	5	16.39	15.57	21.41	40.88	367.66	3.32	12.22
21	Sahebi Enaj	5	7	3	0	3	19.47	21.12	24.62	39.12	310.53	5.12	14.12
22	Sahebi Derman	6	9	7	3	3	19.18	18.31	23.99	32.31	389.31	3.51	8.77
23	Shirazi Aghbolagh	1	5	9	1	5	15.72	16.02	22.98	20.91	176.64	5.75	13.64
24	Shirazi Marzijaran	1	7	7	1	5	22.34	12.79	20.01	20.95	194.31	4.51	13.31
25	Shirazi Hazaveh	1	7	9	1	5	17.45	19.23	32.21	36.02	283.82	4.16	12.49
26	Shirazi Khondab	1	3	5	0	5	17.46	15.52	26.22	29.11	175.21	5.16	12.68
27	Shirazi-2 Khondab	1	9	7	1	5	14.21	17.22	22.35	21.42	173.89	6.32	12.42
28	Shirazi Enaj	1	5	7	1	3	16.72	17.51	28.22	41.54	351.49	3.31	13.99
29	Shirazi Derman	1	9	9	1	3	19.86	17.72	27.11	28.52	226.76	4.55	7.56
30	Fakhri Aghbolagh	1	5	3	1	5	24.73	14.96	19.18	45.47	401.02	5.76	12.56
31	Fakhri Marzijaran	1	5	0	1	5	23.52	14.95	20.71	89.93	915.45	4.66	13.59
32	Fakhri Anjudan	1	5	1	1	5	26.10	13.51	22.20	39.07	389.14	4.83	11.15
33	Fakhri Hazaveh	1	5	5	1	5	23.44	16.23	22.52	62.32	901.55	3.32	12.23
34	Fakhri Khondab	1	5	0	0	5	22.11	15.24	21.31	33.12	402.75	5.33	11.48
35	Fakhri Enaj	1	7	1	1	3	16.18	15.22	21.62	42.63	476.72	3.11	13.41
36	Fakhri Derman	1	7	3	1	3	25.17	16.42	21.12	16.37	212.67	4.11	8.82
37	Fakhri Asgari enaj	1	7	1	0	3	21.85	13.55	16.83	34.18	253.32	3.82	12.71
38	Bidaneh Sefid Aghbolagh	1	5	1	1	5	23.23	10.99	12.24	25.01	215.62	2.76	13.71
39	Bidaneh Sefid Marzijaran	1	7	1	1	5	21.27	11.99	14.99	33.14	368.93	3.25	13.12
40	Bidaneh Sefid Anjudan	1	5	3	1	1	18.81	13.42	15.13	20.11	160.13	3.65	9.11
41	Bidaneh Sefid Hazaveh	1	7	3	1	5	23.98	12.25	14.86	38.72	570.55	4.66	13.13
42	Bidaneh Sefid Khondab	1	7	1	0	5	21.76	13.16	16.11	53.87	905.22	6.83	12.11
43	Bidaneh Sefid Enaj	1	7	1	0	5	21.77	11.58	13.57	34.17	364.11	5.15	14.16

Table 5. (*Continued*). Some important morphological characteristics measured of grape cultivars and genotypes studied in Markazi province.

Row	Cultivar/Genotype	Berry colour	Fruit ripening time	Density of erect trichome between veins	Density of prostrate trichome between veins	Leaf timing	Brix	Berry width	Berry length	Bunch shoulder weight	Bunch weight	Stomata density	Internode diameter
		Score	Score	Score	Score	Score	%	(mm)	(mm)	(g)	(g)	count	(mm)
44	Bidaneh Sefid Derman	1	7	1	1	1	24.38	15.32	17.12	20.72	313.09	2.66	9.75
45	Bidaneh Ghermez Aghbolagh	5	5	7	5	5	24.94	12.06	13.98	20.23	227.49	2.5	13.58
46	Bidaneh Ghermez Marzijaran	5	7	1	1	5	20.23	11.96	13.97	25.42	203.11	2.66	12.26
47	Bidaneh Ghermez Hazaveh	3	5	0	5	5	23.15	11.83	14.83	46.33	498.81	4.32	13.52
48	Bidaneh Ghermez Khondab	4	7	1	0	5	24.89	12.83	14.63	48.31	732.65	5.52	12.85
49	Bidaneh Ghermez Enaj	3	7	1	0	5	23.67	12.15	14.24	41.66	340.58	4.66	14.56
50	Bidaneh Ghermez Derman	3	7	1	3	1	26.96	13.95	17.94	25.28	288.66	4.51	8.49
51	Lal Aghbolagh	1	3	7	3	5	18.64	16.15	18.46	42.15	305.32	3.75	10.34
52	Lal Marzijaran	1	5	3	1	5	17.93	16.97	18.89	38.65	289.86	5.31	12.12
53	Lal Hazaveh	1	7	3	1	5	18.27	17.25	22.84	86.64	810.22	4.32	11.25
54	Lal Khondab	3	5	9	1	5	18.52	15.58	20.11	36.42	409.22	5.32	10.99
55	Lal Enaj	1	7	1	5	3	18.16	15.58	20.67	38.66	429.71	4.53	13.22
56	Lal Derman	1	7	9	5	5	21.37	18.21	24.21	42.35	392.39	4.16	8.89
57	Asgari Aghbolagh	1	3	7	0	1	21.15	14.87	17.52	23.51	213.25	4.37	11.22
58	Asgari Marzijaran	1	5	5	0	3	19.14	11.11	14.98	15.96	165.87	5.14	14.11
59	Asgari Anjudan	1	5	1	0	3	21.79	15.42	18.16	37.58	352.51	5.11	10.97
60	Asgari Hazaveh	1	5	7	1	3	18.48	13.66	17.53	58.71	570.28	4.33	13.41
61	Asgari khondab	1	5	1	1	1	18.33	14.71	17.16	32.86	314.19	3.62	13.32
62	Asgari Enaj	1	7	1	0	3	19.97	14.22	17.41	46.11	290.23	6.52	14.15
63	Asgari Derman	1	5	1	1	1	23.26	14.21	15.97	19.48	209.26	4.12	8.43
64	Asgari bi bazr Anjudan	1	5	1	1	3	18.37	12.33	15.57	24.09	210.04	3.42	12.12
65	Asgari Shahrodi Hazaveh	1	5	1	1	3	18.96	13.33	19.54	33.59	381.92	3.13	12.57
66	Asgari gerd Enaj	1	7	1	5	3	18.74	13.21	14.12	42.28	262.12	3.75	12.92
67	Siah Marzijaran	6	7	3	0	5	18.46	14.86	16.12	35.72	245.22	2.42	12.91
68	Siah Anjudan	6	7	9	1	3	21.27	15.43	17.81	32.05	229.76	3.11	12.21
69	Siah Hazaveh	6	5	1	0	3	24.13	16.53	19.42	50.44	721.03	5.85	11.48
70	Siah Khondab	5	5	3	0	5	17.84	14.13	14.94	31.53	239.15	5.23	10.99
71	Siah Enaj	6	5	1	0	3	17.88	14.82	17.11	57.33	382.65	5.83	13.81
72	Kharvand Hazaveh	1	7	5	1	5	16.35	16.44	17.32	73.71	897.51	2.95	10.21
73	Kharvand Derman	1	5	7	5	3	17.37	18.92	19.89	17.62	215.48	3.32	7.81
74	Angor Sefid Aghbolagh	1	7	9	3	5	22.25	14.08	18.13	27.96	251.13	5.14	9.98
75	Kerak Marzijaran	1	5	3	0	5	19.64	13.12	14.88	32.99	430.76	4.75	12.42
76	Kole Aghbolagh	1	5	0	1	3	23.43	14.88	17.26	16.97	220.74	7.22	10.74
77	Ghazvini Anjudan	5	5	3	0	3	24.96	13.62	16.23	22.41	275.22	3.35	13.13
78	Kol Bache Anjudan	1	9	1	1	5	16.34	12.62	12.43	18.96	163.04	3.11	9.98
79	Halvai Anjudan	1	5	5	1	5	19.72	17.28	24.11	20.39	157.71	4.55	12.11
80	Yek bazr Marzijaran	1	9	5	1	5	18.34	11.99	17.95	33.92	283.31	4.28	13.25
81	Lorkosh Hazaveh	1	7	7	1	5	19.35	18.75	20.23	48.96	344.82	4.15	12.45
82	Mehdikhani Hazaveh	1	3	9	1	5	23.24	13.66	25.23	32.33	200.65	3.33	11.15
83	Kondori Hazaveh	6	5	7	1	3	17.25	19.25	25.77	63.23	652.11	5.32	12.81
84	Moamelan Derman	1	5	5	3	3	20.14	14.12	13.98	15.58	188.92	3.81	7.99

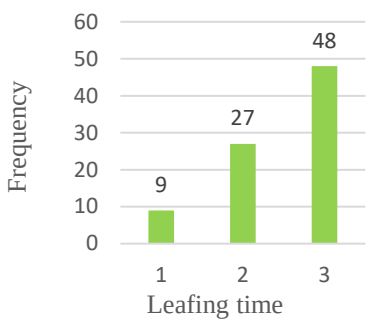

Fig. 1. The frequency of leafing time in different studied grapes varieties and genotypes (1- Early, 3- Medium, 5- Late).

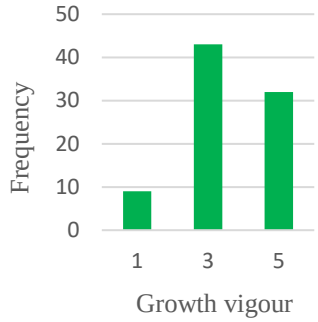

Fig. 2. The frequency of growth vigour in different studied grapes varieties and genotypes (3= Weak, 5= Moderate, 7= Strong)

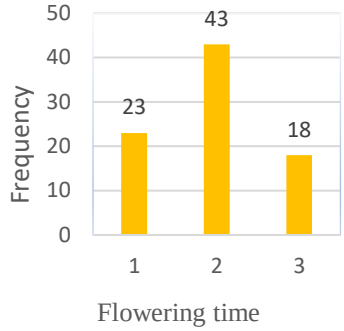

Fig. 3. The frequency of flowering time in different cultivars and genotypes of studied grapes (1- Too early, 2- Very early, 3- Early, 4- Early to medium, 5- Medium, 6- Medium to late, 7- Late, 8- Very late, 9- Too late).

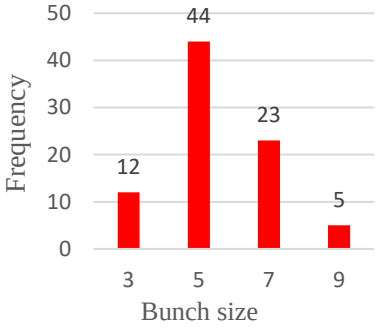

Fig. 4. The frequency of the bunch size in the different investigated cultivars and genotypes of studied grapes (3- Small, 5- Medium, 7- Large, 9- Very large).

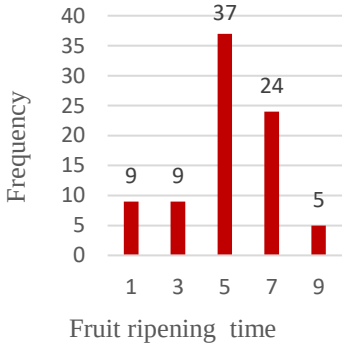

Fig. 5. The frequency of fruit ripening time in different cultivars and genotypes of studied grapes (1= Very early, 3= Early, 5= Medium, 7= Late, 9= Very late).

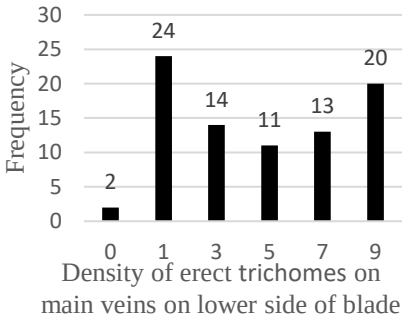

Fig. 6. The frequency of density of erect trichome on main veins on lower side of blade in different cultivars and genotypes of studied grapes (Absent (0), Very sparse (1), Sparse (3), Medium (5), Dense (7), Very dense (9)).

In the study of grape cultivars and genotypes of Khuzestan province, it showed that the most descriptive statistics in the most important quantitative traits are related to fresh weight of bunch (2174.24 g), bunch length (279.68 mm), bunch width (157.03 mm), number of berries per bunch (1088.83 berry), berry fresh weight (6.85 mg), berry diameter (18.60 mm), berry length (30.89 mm) and berry width (22.79 mm) (Kazemi et al., 2022). The frequency distribution of traits such as leafing time, growth power, flowering time, bunch size, fruit ripening time, density of erect trichome on main veins on lower side of blade, density of prostrate trichome on main veins are shown in Figures 1 to 6. In terms of leafing time, most of the genotypes in the studied growth conditions had late leafing, although there were early leafing cultivars such as “Khalili”, “Khalili Khani”, “Yaghoti Marzijaran”, “Bidaneh Sefid Anjudan”, “Bidaneh Sefid Derman”, “Bidaneh Ghermez Derman”, “Asgari Aghbolagh”, “Asgari Khondab”, and “Asgari Derman” (Fig. 1). Among these cultivars (“Khalili Khani Marzijaran” (2.52), “Yaghoti Hazaveh” (2.14) and “Yaghoti Khondab” (2.52), “Sahebi Aghbolagh” (2.75) and “Sahebi Hazaveh” (2.92), “Bidaneh Sefid” (2.76) and “Bidaneh

Ghermez" (2.5) Aghbolagh, "Bidaneh Sefid Derman" (2.66), "Bidaneh Ghermez Marzijaran" (2.66), "Siyahe Marzijaran" (2.42), and "Kharvand Hazaveh" (2.95) had fewer open stomata in the field of view under a microscope at a magnification of 40. Additionally, the field evaluation for selecting drought-tolerant cultivars in this experiment showed that the cultivars ("Khalili Marzijaran", "Khalili Anjudan", "Khalili Hazaveh", "Khalili Khondab", "Khalili Derman", "Khalili Khani Marzijaran", "Yaghoti Marzijaran", "Yaghoti Hazaveh", "Yaghoti Derman", "Sahabi Anjudan", "Sahabi Hazaveh", "Shirazi Aghbolagh", "Shirazi Hazaveh", "Shirazi Derman", "Lal Derman", "Siyah Anjudan", "Kharvand Derman", "Angor Sefid Aghbolagh", "Lorkosh Hazaveh", " Mahdikhani Hazaveh") had the highest volume of standing trichome between the main leaf veins on the lower surface of the leaf (Tables 4 and 5) (Fig. 8 and Fig. 9). Moreover, there was a relatively high diversity among different cultivars and genotypes in terms of growth power, length, width, weight, shape, and color of the berry. Some important characteristics and average values of the important traits evaluated are mentioned in Table 5. The findings obtained were consistent with the results reported by (Alizadeh, 2004) and (Nejatian, 2006), who reported a wide diversity among the studied cultivars in terms of various traits related to vegetative and fruit parts.

Simple correlation coefficients of traits

Significant correlations existed among variables related to vegetative growth, fruit, and bunch traits in this experiment. The results showed a positive and significant correlation between bunch weight and leaf length (R= 0.31). Bunch shoulders weight also had a positive and significant correlation with bunch weight (R = 0.88). The rachis weight had a positive and significant correlation with bunch weight (R=0.68). But, the ratio of rachis weight to bunch weight had a significant negative correlation with the ratio of bunch weight to rachis weight (R =-0.67). Also, the ratio of rachis weight to bunch weight showed a positive and significant correlation with the ratio of rachis weight to bunch shoulders weight (R=0.71). The bunch shoulders dry weight had a positive and significant correlation with bunch shoulders fresh weight (R=0.96). Although, the number of bunches per vine had a positive and significant correlation with leaf length (R=0.25), bunch weight (R=0.24), and the ratio of bunch weight to rachis weight (R=0.33), its correlation value was not high. The traits of bunch length had a positive and significant correlation with bunch weight (R=0.67), bunch shoulders weight (R=0.60), and rachis weight (R= 0.44). Also, the bunch width had a positive and significant correlation with bunch weight (R=0.84), bunch shoulders weight (R=0.77), and bunch length (R=0.44). Moreover, berry width had a positive and significant correlation with berry weight (R=0.80) and berry length (R=0.82). Also, berry diameter had a positive and significant correlation with berry weight (R= 0.87), berry length (R= 0.79), and berry width (R=0.90). Furthermore, seed length had a positive and significant correlation with berry weight (R=0.62), berry length (R =0.60), and berry width (R=0.60). In general, based on the results of simple correlation of traits in this research, significant correlations existed among some variables related to vegetative growth and fruit traits. These findings are consistent with the findings of Ekhvaia et al. (2009) who reported associations and correlations among various grape vegetative and fruit traits. The consistent with the results of traits correlations mentioned in Table 6 of this research, Leão and Oliveira (2023) reported that most of the phenotypic correlations between morpho-agronomic variables were significant ($p<0.05$), indicating that yield per vine was positively correlated with number of bunches, bunch length, soluble solids content and titratable acidity. Only berry length had a significant negative correlation with yield per vine. The significant negative correlation between berry length and yield per vine can be explained by the fact that in vines whose bunches had longer berries, the number of bunches per vine was reduced (R= -0.537), as well as the bunch length (R= -

0.466). On the other hand, these last two variables have a positive and significant correlation with the yield per vine. Also, Leão and Oliveira (2023) Shown phenotypic correlations showed that the trait number of bunches per vine is highly correlated with yield; however, berry weight, length and diameter were negatively correlated with soluble solids content, titratable acidity and SS/TA ratio. Furthermore, Cargnin (2019) in the study of "Cabernet Sauvignon" cultivar showed that fruit yield (weight) has a positive and significant phenotypic correlation with bunch weight (R=0.98) and berry weight (R=0.98), and selecting a plant with higher bunch and berry weight increases fruit yield, which was somewhat consistent with the present findings. There was also a positive and significant correlation between number of bunches (R=0.78) and pH (R=0.89). The phenotypic correlation between number of bunches with bunch weight (R= -0.83) and berry weight (R= -0.82) was negative and significant. The more bunches per plant, the lower the bunch weight and the lower the berry weight, resulting in lower fruit yield (Cargnin, 2019). Also, Cargnin (2019) obtained similar results in a study of "Cabernet Sauvignon" and showed that fruit yield (weight) had a positive and significant phenotypic correlation with bunch weight (R=0.91) and number of berries per bunch (R =0.88), and these traits indicated high fruit yield potential in the plant. There was a negative and significant correlation between pH and fruit yield traits (R = -0.95), bunch weight (R = -0.99) and number of berries per bunch (R = -0.98).

As fruit yield, bunch weight and number of berries per bunch increase, pH decreases. The results obtained from this research are consistent with the results of other researchers and show that increasing yield components such as number of berries per bunch, berry weight and number of berries per bunch leads to an increase in fruit yield. Some results showed a negative correlation between the number of berries per bunch and berry weight. According to Silva et al. (2009), negative correlations between yield components probably occur mainly due to competition between them (sinks-sources) during plant development in each crop cycle. Positive or negative correlations occur due to genetic and environmental variations in the plant.

Table 6. Eigenvalues, percentage of variance, and percentage of cumulative variance of the 10 main components in this research.

Factor	Eigenvalues	Eigenvalues to percent variance	Percentage of variance cumulative
1	6.142	18.065	18.065
2	4.804	14.128	32.193
3	3.872	11.387	43.580
4	2.618	7.701	51.281
5	2.186	6.430	57.711
6	1.628	4.787	62.499
7	1.508	4.434	66.933
8	1.462	4.299	71.232
9	1.350	3.971	75.202
10	1.080	3.177	78.379

Table 7. Coefficients related first to 10 main components of grapes cultivars and genotypes.

Trait	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Internode diameter	.250	.014	-.255	-.152	.296	-.650	.131	-.031	.004	.211
Leaf length	.263	.318	-.284	.289	-.013	-.180	-.153	.294	.602	.005
Petiole length	-.050	.143	-.299	.585	-.166	-.042	-.252	-.083	.277	.068
Length of teeth	-.079	-.052	.066	.650	-.107	-.036	-.469	-.043	.038	-.004
Tendril length	.079	.260	.155	.185	-.385	.193	.036	.345	.112	-.253
Stomata density in the field of view of 40 microscopes	.274	-.068	-.020	-.003	.702	.276	.001	-.276	.328	-.035
Stomata density in the field of view of 25 microscopes	.251	-.076	.003	.043	.678	.353	.119	-.333	.311	-.130
Bunch weight	.464	.793	-.194	.019	-.077	.001	.027	-.214	-.094	.072
Bunch shoulder weight	.602	.666	-.006	-.047	-.031	-.209	-.047	-.211	-.156	-.080
Ratio of bunch weight bunch shoulder weight	-.185	.475	-.320	.121	-.123	.433	.161	-.059	.083	.333
Rachis weight	.150	.861	.337	.004	.025	.099	.007	.047	-.034	.055
Peduncle weight	.058	.685	.602	-.046	-.028	-.170	.017	-.147	.064	-.076
Ratio of the rachis weight to peduncle weight	.035	.336	-.433	-.050	.179	.511	.001	.265	-.250	.264
Ratio of bunch weight to Rachis weight	.362	-.317	-.569	.118	-.226	.026	.084	-.201	-.020	-.155
Ratio of rachis weight to the bunch weight	-.308	.352	.626	.047	.155	.184	.122	.369	.110	.010
Ratio of the peduncle weight to the Bunch shoulder weight	-.445	.251	.699	.052	-.047	.035	.172	.054	.244	.020
Fresh weight of bunch shoulder weight	.417	.077	-.172	-.742	.069	-.051	-.137	.301	.222	-.036
Dry weight of bunch shoulder weight	.341	.169	-.124	-.747	.084	-.071	-.276	.293	.209	-.009
Fresh to dry weight ratio of bunch shoulder	.511	-.371	-.198	.006	.026	.113	.516	-.023	.010	-.096
Bunch number of bushes	-.185	.372	-.400	.052	-.166	-.110	.532	.094	.213	-.050
Bunch length	.384	.582	-.227	.291	.322	-.170	.122	.209	-.269	.062
Bunch width	.389	.738	-.176	-.137	-.262	.019	.041	-.290	-.021	-.108
Length to width ratio of bunch	.011	-.103	-.016	.441	.556	-.176	.079	.474	-.267	.164
Bunch tail length	.139	.244	-.029	.380	.370	-.277	-.097	-.006	.115	-.323
Berry weight	.886	-.177	.158	.125	-.105	.029	.044	-.005	-.149	.002
Berry length	.855	-.254	.062	.158	-.204	.116	.102	.179	.063	-.027
Berry width	.810	-.062	.258	.185	-.040	.228	.043	.148	-.086	-.160
Length to width of berry	.162	.232	.154	.105	-.090	-.286	.081	-.178	-345	.476
Berry diameter	-.002	-.121	.068	-.022	.056	.025	.099	.260	-.080	.882
Berry tail length	.626	.247	-.134	-.057	-.058	-.080	.090	.013	-.127	.407
Berry tail weight	.312	.039	-.198	.178	-.076	-.066	-.024	.536	.075	-.055
Seed weight	.130	-.159	-.119	-.262	-.071	.085	-.092	.519	-.161	.439
Seed length	.073	.119	.000	-.142	.147	-.099	-.017	.541	-.285	.590
Brix%	-.002	-.117	.011	-.511	.286	.054	-.076	-.477	.269	-.056

F: Factor.

Factor analysis

Factor analysis was performed to determine the variations of each trait with each factor and ultimately the total (factor-extracted) and specific (residual) variances (Tables 6 and 7). The relative variance of each factor indicates the importance of that factor in explaining the total variance of the traits and is expressed as a percentage. In the factor analysis, a total of 10 independent and principal factors with eigenvalues greater than 1 were able to account for 78.37% of the total variance (Table 6). Table 8 presents the results of the factor analysis, indicating the placement of some important examined traits in different factors with their

positive and negative factor loadings (due to the high volume of data, only significant traits with factor loadings are mentioned in the table). According to Tables 7 and 8, cultivars and genotypes were grouped in the first factor (PC1) for traits such as weight, width and diameter of berry and seed length, which accounted for 18.06% of the variance (Table 6). Therefore, this factor can be named the "berry factor." In the second factor (PC2), cultivars were grouped based on traits such as bunch weight, bunch shoulder weight, rachis weight, bunch length and bunch width, which accounted for 14.12% of the variance. This factor can be referred to as the "bunch size factor." The factors PC1 and PC2, where most fruit-related traits were placed, had the most significant role in differentiating cultivars and genotypes from each other, accounting for a total of 32.19% of the total variance (Table 6). Traits such as seed weight, pedicel weight, the ratio of peduncle weight to bunch shoulder weight and the ratio of bunch shoulder weight to bunch weight were placed in the third factor, accounting for 11.38% of the total variance. Traits such as petiole length, Length of teeth, bunch shoulder fresh weight, and bunch shoulder dry weight were placed in the fourth factor, explaining 7.70% of the variance (Table 6). The fifth factor included traits such as the bunch length-to-width ratio and stomata density in the field of view at 25 and 40, which accounted for 6.43% of the total variance (Table 6). In the sixth factor, the internode diameter and the ratio of rachis weight to peduncle weight justified 4.78% of the variance (Table 6). The seventh factor justified 4.43% of the variance and included traits such as the ratio of bunch shoulder fresh weight to bunch shoulder dry weight and the number of bunches per vine (Table 6). The eighth factor, with a variance of 4.29%, included the trait of tendril length (Table 6). The ninth factor accounted for 3.97% of the variance and consisted of the leaf length trait (Table 6). The tenth factor included the trait of seed length and accounted for 3.17% of the variance (Table 6). In a study on the genetic diversity of 20 grape cultivars, morphological traits were analyzed using factor analysis (Hashemzehi., 2010). The results showed that the first three factors accounted for 79.34% of the existing variations among the traits. The first factor explained 31.86% of the variance between traits and played a significant role in justifying variables such as seed length, seed weight, and kernel length. Also, Haddadinejad et al. (2013), for the initial screening, 698 grape genotypes were analyzed based on drought tolerance using factor analysis. In this analysis, seven primary and independent factors with eigenvalues greater than one were able to account for 78.96% of the total variance. Some of their findings were consistent with the results obtained from this study. In the comparison of this research with other similar researches, it was shown that the first factor and the second factor in most of the conducted researches were related to berry and bunch factors (Haddadinejad et al., 2013; Rasouli et al., 2015; Razi et al., 2021; Rasouli & Kalvandi, 2022; Kazemi et al., 2022). Rasouli et al. (2015) results showed that the factor analysis justified 74.22% of the total variance. The investigated factors such as bunch size, bunch density, skin thickness, shape, size, weight, length and width of the berry, seed length were place on first factor. The first factor includes 20.74% of the variance and the berry factor is placed in this first factor. The bunch size factor with 11.79% was also the second factor of this research (Rasouli et al., 2015). Also, Hashemzehi et al. (2010) studied diversity of grape cultivars and they reported factor analysis justified 79.34% of total variance. The results of first and second factors analysis of Hashemzehi et al. (2010) were in line with the results of this research in the berry and bunch factors. Furthermore, Rafiei et al. (2016) reported the percentage of variance showed that the first 5 factors were related to fruit and leaf traits and the first factor with 22.63% of the variance was related to the berry factor and the second factor with 14.71% of the variance was related to most of the bunch traits.

Cluster Analysis

The cluster analysis was performed based on all measured traits (Table 2) using the Ward method for grouping and comparing 84 grape cultivars and genotypes (Fig. 7). At 5 Euclidean distances the cultivars and genotypes were grouped into four main clusters, which include:

Group 1: This group included 30 cultivars and genotypes out of 84 investigated grapes cultivars and genotypes such as "Lal Khondab", "Lal Enaj", "Siah Enaj", "Asgari Anjudan", "Lorkosh Hazaveh", "Lal Derman", "Sahebi Derman", "Fakhri Anjudan", "Kerak Marzijaran", "Bidaneh Sefid Marzijaran", "Bidaneh Ghermeze Enaj", "Asgari Shahroudi Hazaveh", "Fakhri Aghbolagh", "Fakhri Khondab", "Bidaneh Sefid Enaj", "Sahebi Khondab", "Lal Aghbolagh", "Angore Sefideh Aghbolagh", "Sahebi Anjudan", "Sahebi Enaj", "Bidaneh Ghermeze Derman", "Lal Marzijaran", "Asgari Enaj", "Shirazi Enaj", "Bidaneh Sefid Derman", "Asgari Khondab", "Ghazvini Anjudan", "Yek bazre Marzijaran", "Shirazi Hazaveh" and "Shirazi Derman". These genotypes are characterized by medium budburst time, moderate plant growth vigour and moderate bunch weight. The fruits of this group had mostly low to moderately juicy, and the anthocyanin pigments in their flesh were generally absent. They had thin to medium skin thickness and the color of the berries is mostly yellow-green. The berry shape in this group is usually broad-ovate, and the berries are generally slightly firm to firm with medium to large size. The berry density in the bunch ranges from average to compact and the bunch size was mostly medium to large. Overall, these cultivars showed similarity in most of the measured traits, particularly fruit-related characteristics. The highest amount of brix with 26.96 % in "Bidaneh Ghermeze Derman" cultivar that was included in this group. The cultivars and genotypes of this group were geographically from the same place or close (Fig. 9).

Group 2: This group included 37 cultivars out of 84 investigated cultivars and genotypes, covering; "Kharvand Darman", "Mahdikhani Hazaveh", "Fakhri Darman", "Asgari Darman", "Yaghoti Marzijaran", "Khalili Anjudan", "Yaghoti Anjudan", "Halvaii Anjudan", "Shirazi Khondab", "Shirazi 2 Khondab", "Shirazi Marzijaran", "Bidaneh Sefid Anjudan", "Sahebi Marzijaran", "Kool Bache Anjudan", "Asgari Marzijaran", "Fakhri", "Asgari Enaj", "Siah Khondab", "Yaghoti Khondab", "Siah Marzijaran", "Asgari Gerd Enaj", "Siah Anjudan", "Yaghoti Aghbolagh", "Yaghoti Enaj", "Khalili Enaj", "Moa'melan Darman", "Khalili Marijaran", "Khalili Darman", "Asgari Bi Bazr Anjudan", "Khalilkhani Marzijaran", "Bidaneh Sefid Aghbolagh", "Bidaneh Ghermeze Aghbolagh", "Asgari Aghbolagh", "Khalili Aghbolagh", "Khalili Hazaveh", "Bidaneh Ghermeze Marzijaran", "Koole Aghbolagh" and "Shirazi Aghbolagh". These genotypes have a medium density of trichomes on the main leaf veins. In this group, there are cultivars ranging from very early to late maturity cultivars such as "Khalili Khani", "Yaghoti Marzijaran", "Bidane Sefid Anjudan", "Bidaneh Sefid" and "Bidaneh Ghermez Derman", "Asgari Aghbolagh" and "Asgari Khondab" and "Asgari Derman" have earlier leaves than the rest of the investigated cultivars. Most of the cultivars in this group had seeds and seed separation in this group ranges from difficult to relatively easy. The fruits in this group were usually slightly juicy. The flesh of these fruits usually lacks color and the skin thickness ranges from thin to medium with a seed color predominantly yellow-green. The shape of the fruit in this group was usually oval to round and the texture of the fruits is often slightly firm, with small to medium-sized seeds. The seed density in bunches and the bunch density and size in most cultivars of this group were medium. Most cultivars in this group had complete seeds.

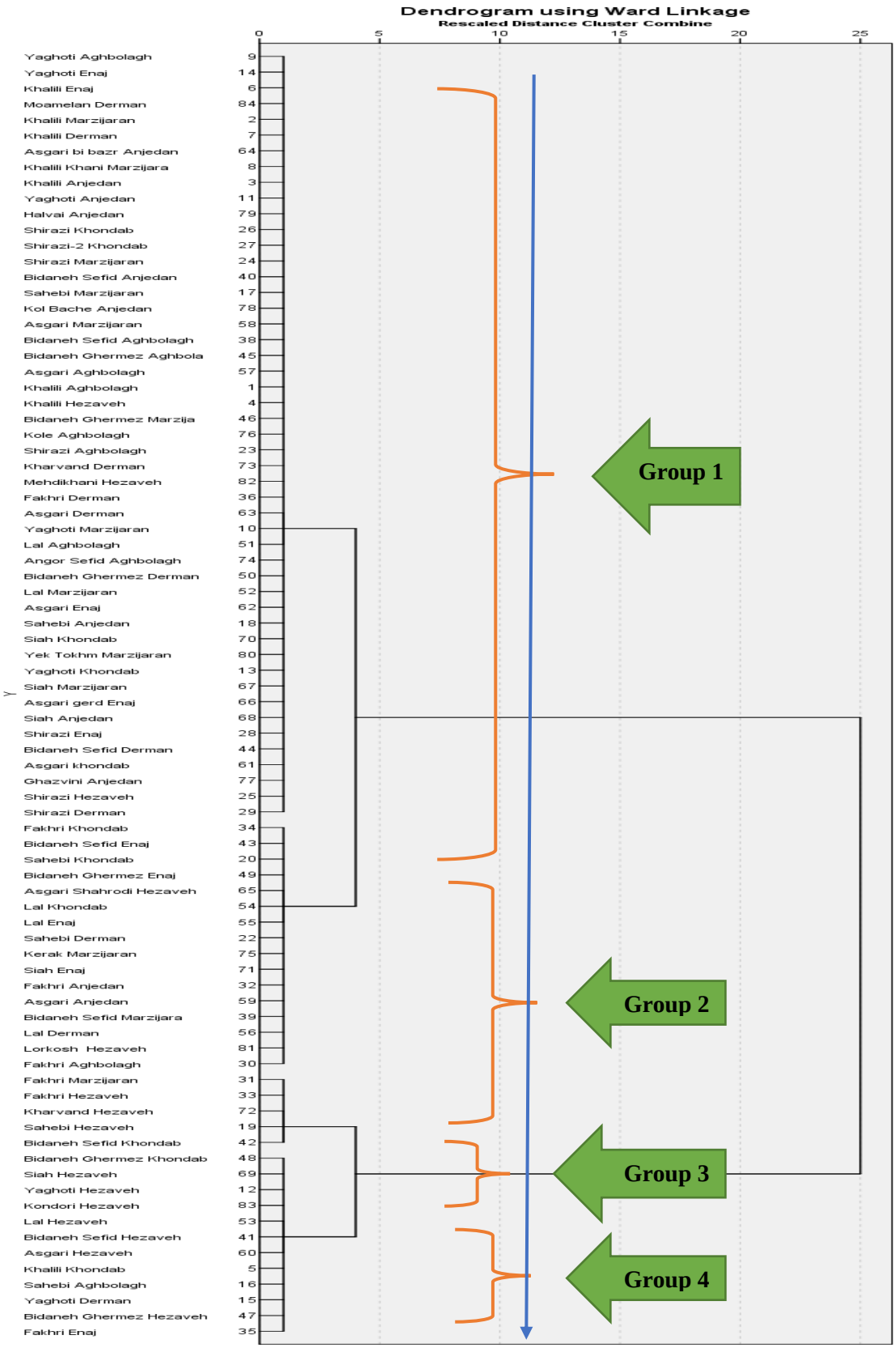

Fig. 7. Dendrogram showing relationship between 84 cultivars and genotypes of grapes, available in the vineyards of Markazi province located in central of Iran, based on studied traits using cluster analysis by Ward method.

Group 3: This group included 5 cultivars and genotypes out of 84 investigated cultivars, such as "Fakhri Marzijaran" and "Fakhri Hazaveh", "Kharvand Hazaveh", "Sahebi Hazaveh" and "Bidaneh Sefid Khondab". The highest bunch weight (1000.71 g) was found in the "Sahabi Hazaveh" cultivar within this group. The "Kharvand Hazaveh" cultivar has a maximum width of the bunch with 126.64 mm that was placed in this group. Also, "Sahebi Hazaveh" has highest average bunch weight with 1000.71 g was included in this group. Among the differences that probably caused the "Bidaneh Sefid" cultivar and "Bidaneh Ghermeze"cultivar of Khondab region to be placed in two separate groups, but one after the other, the difference in Length of teeth, amount of sugar, larger leaf size of the "Bidaneh Sefid" cultivar, amount of anthocyanin in "Bidaneh Ghermeze" cultivar, time the later ripening of "Bidaneh Sefid", slightly firmer berry in "Bidaneh Sefid" cultivar, average flesh anthocyanin "Bidaneh Ghermeze" cultivar and different peduncle separation of these two cultivars were the same. All genotypes in this group had late budburst and flowering times. The berry density in the bunch of these cultivars was compact to very compact and the bunch size was usually large to very large. The cultivars in this group were exhibited vigorous plant growth. The berry size ranges from small to very large and the berry firmness varies from slightly firm to firm. The berry shape in these cultivars ranges from rectangular to oval and broad-oval to round. The color of the berries was mostly yellow-green and the skin of these cultivars was thick.

Group 4: This group included 12 cultivars out of 84 investigated cultivars, such as "Bidaneh Ghermeze Khondab", "Siah Khondab", "Yaghoti Hazaveh", "Lal Hazaveh", "Bidaneh Sefid Hazaveh", "Asgari Hazaveh", "Khalili Khondab", "Sahebi Aqbolagh", "Yaghoti Derman", "Bidaneh Ghermeze Hazaveh" and "Fakhri Enaj". The cultivars "Kondori Hazaveh" with 6.99 g berry weight, "Asgari Hazaveh" with 81.20 bunches per vine, "Fakhri Enaj" with 310.75 mm bunch length were placed in this group (Fig. 9). Most of the cultivars in this group had higher bunch weight and length. They also had a high yield per unit area and larger leaves. Flowering in the cultivars of this group was early to moderate, and the leaf size was usually large. The berries in these cultivars were mostly soft. In this group, most of the cultivars had almost the same internode diameter, number of bunches in the plant was almost high and the length and width of the bunch were almost the same. Most cultivars of this group had seeds, round berry, medium to very large bunch size, medium to very compact bunch density, and slightly juicy berry. One important note that can be seen in these cluster analysis groups was the presence of seedless cultivars in the analysis groups, which was one of the reasons for this division, different recording locations with different altitudes, longitudes and latitudes, environmental effects regions, soil type and genetic potential were high among the cultivars and genotypes studied. The findings regarding the effect on some growth and fruit traits were consistent with the results reported by Zinanlou (1993), Alizadeh (2004), Nejatian (2006), Qobadi et al. (2007), and Rasouli et al. (2015) for cultivars from Kermanshah, West Azerbaijan, Qazvin, Isfahan, and Hamedan provinces in terms of various growth-related characteristics, bunch size and weight, berry density in the bunch, berry color, having seeds or being seedless, ripening time, consumption type and genetic relatedness. However, some cultivars in different geographical and soil conditions showed differences in plant growth vigour and sugar content percentage compared to the results of these researchers. In line with the cluster analysis results of this research, Rasouli et al. (2014) studied the morphological diversity of 32 grapes cultivars and genotypes in Hamedan province and reported the cluster analysis at 5 Euclidean distances has been divided cultivars into 7 groups and some cultivars were different from other cultivars in terms of late flowering, sugar percentage, freshness and shelf life. Also, Rafiei et al. (2015) on seeded and seedless cultivars in some regions of the Markazi central province, they concluded that in these cultivars, the groups were classified

into two main groups, seeded and seedless, at 25 Euclidean distances. They reported, the samples that were placed in the group of quince cultivars had prominent characteristics such as smaller seeds or seed remains. Also, the size of the berry was smaller and the percentage of soluble solids was higher than the in characteristics of “Bidaneh” cultivars. There were 29 samples in this group, which included the same group. The grapes were “Asgari”, “Yaghoti”, “Bidaneh Sefid”, and, “Khalili”. From these researches, it can be concluded that the results of this research are consistent with the researches done on the cultivar of grapes and are in line with the examined cases of this experiment. In a study conducted by Zahedi et al. (2020) on the morphological and pomological characteristics of 55 grape cultivars, significant differences were observed among the studied cultivars for the measured traits. The fruit length ranged from 12.32 to 31.85 millimeters. Additionally, 10 different skin colors were observed, with light green (14 cultivars) and greenish-yellow (15 cultivars) being the predominant colors. Moreover, 20 cultivars initially formed seeds, while seeds were absent in 34 cultivars, and one cultivar had seedless berries. The dendrogram of cluster analysis based on the obtained data revealed three main clusters with several sub-clusters, that their results were somewhat consistent with the cluster analysis results of the present research.

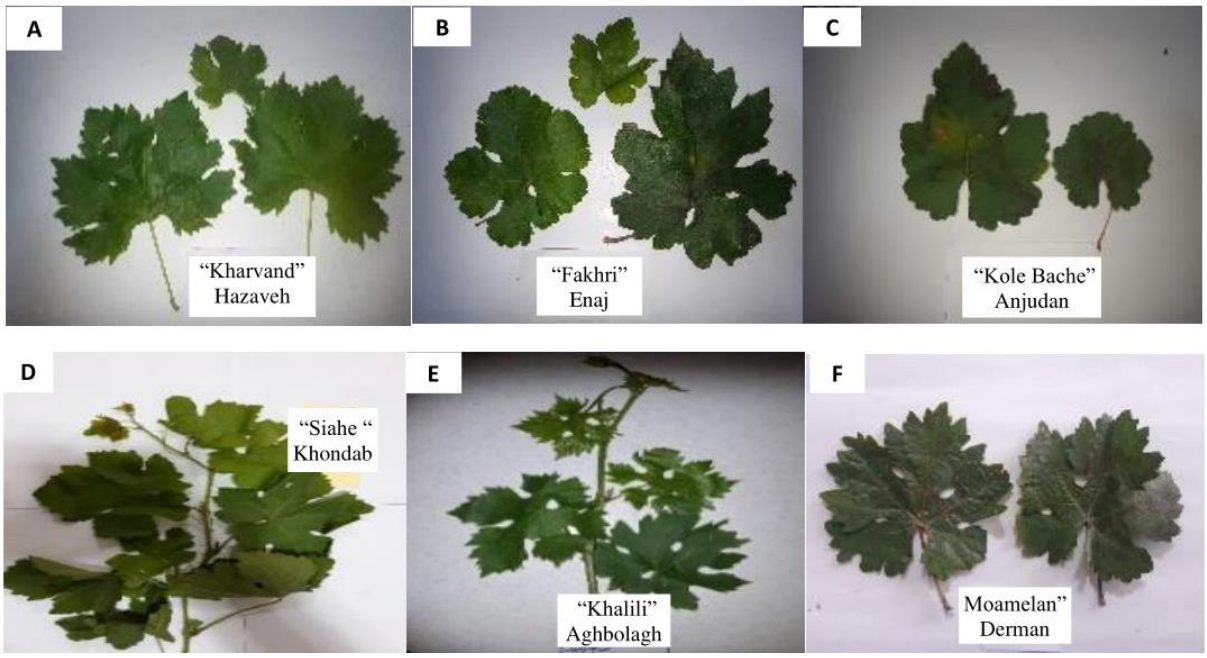

Fig. 8. The leaves of cultivars and genotypes available in vineyards of Markazi province located in central of Iran (A-“Kharvand” Hazaveh, B- “Fakhri”, Enaj, C-“Kole Bache Anjudan”, D-“Siahe Khondab”, E-“Khalili Aghbolagh”, F-“Moamelan Derman”).

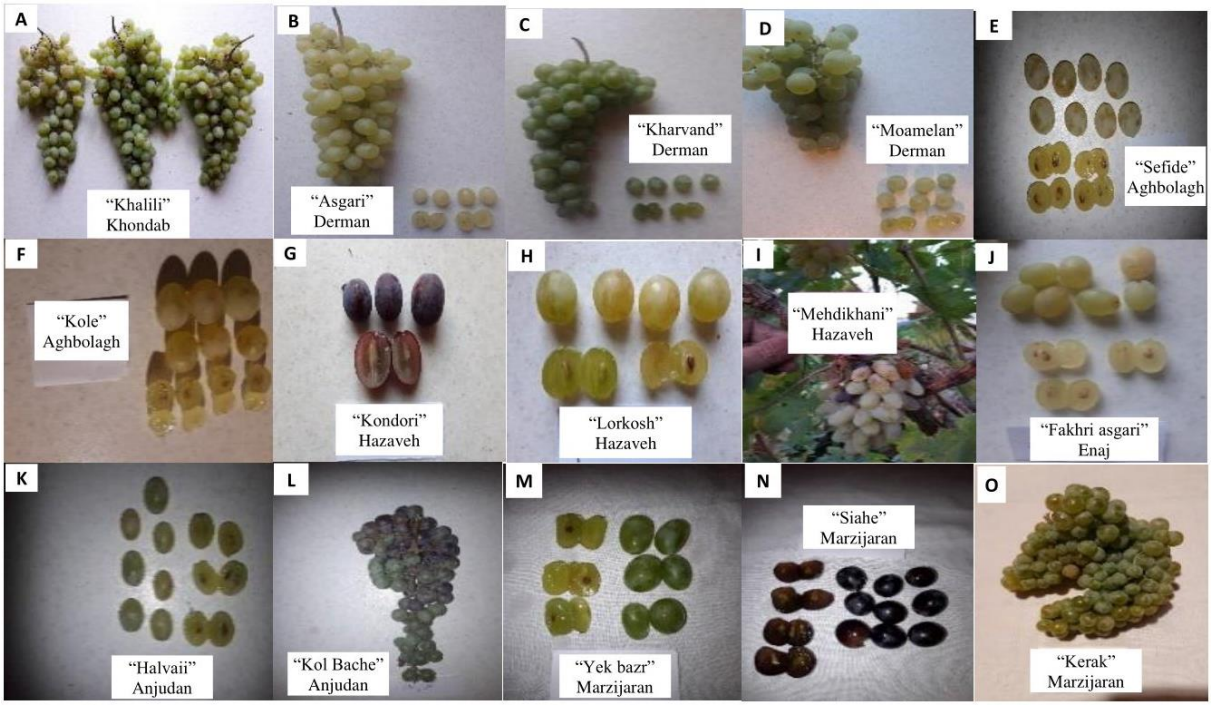

Fig. 9. The fruits of cultivars and genotypes available in vineyards of Markazi province located in central of Iran (A- "Khalili Khondab", B- "Asgari Derman", C- "Kharvand Derman", D- "Moamelan Derman", E- "Sefide Aghbolagh", F- "Kole" Aghbolagh, G- "Kondori" Hazaveh, H- "Lorkosh" Hazaveh, I- "Mehdikhani" Hazaveh, J- "Fakhri asgari" Enaj, K- "Halvaii" Anjudan L- "Kol Bache" Anjudan. M- "Yek bazr" Marzijaran, N- "Siahe" Marzijaran, O- "Kerak" Marzijaran.

CONCLUSION

The main objective of measuring these traits was to assess diversity and identify superior cultivars and genotypes for use in grape breeding programs. Based on the results, cultivars such as ("Yaghoti," Aghbolagh), ("Khalili," Hazaveh, Derman and Khondab), ("Khalilikhani" Marzijaran), ("Mehdikhani" Hazaveh) and ("Kharvand" Darman) exhibited lower density of stomata in the field (25 and 40), while they had higher density of trichome between the main veins and on the main veins. Most cultivars in the Hazaveh and Khondab regions had higher yield, bunch and bunch shoulder weight compared to other regions. The third and fourth groups, including "Khalili Khondab" and "Yaghoti," "Sahebi", "Fakhri", "Kharvand", "Kondori Hazaveh", and "Sahebi" Aghbolagh, were superior to other cultivars and genotypes in terms of yield, bunch length, bunch weight, late budbreak, late flowering, high sugar content, and fruit characteristics. Cultivars such as "Khalili," "Khalilikhani," "Yaghoti" Marzijaran, "Bidaneh Sefid Anjudan", "Bidaneh Sefid" and "Bidaneh Ghermeze" Darman, "Asgari Aghbolagh", "Asgari Khondab" and "Asgari Derman" had earlier budbreak compared to other cultivars and were susceptible to frost damage. The highest sugar content was found in the "Bidaneh Ghermeze" Darman cultivar, which could be attributed to cool night temperatures during the late ripening period in the Derman region. The highest bunch number was observed in the "Asgari Hazaveh" cultivar, which is extensively used for grape syrup production, properly pruned, and well-nourished, resulting in a high number of bunches. The highest bunch weight was found in the "Sahebi Hazaveh" cultivar, as the Hazaveh region followed proper pruning practices, provided timely and appropriate nutrition, and achieved successful stages of development. Most cultivars in this region had very high

bunch weights, with "Asgari" being the dominant cultivar. The remaining cultivars were planted minimally in the surrounding vineyards.

Conflict of interest

The authors (S.M.M. Mirfatah, M. Rasouli, M. Gholami and A. Mirzakhani) declare that they have no competing interests.

Acknowledgments

Authors are thankful Malayer University and Markazi Agricultural and Natural Resources Research and Education Center (MANRREC), Arak. The present study was supported by the Ph.D. thesis entitled "Screening, effect of drought stress, rootstock and scion on some morphological and physiological characteristics (*Vitis vinifera* L.) In climatic conditions of Markazi province", which was funded by the Vice Chancellor for Research, Malayer University.

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JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH
2024, VOL. 7(2), 141-154

Journal of Horticulture and Postharvest Research

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

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Investigating the antifungal effects of essential oils on *Aspergillus* sp. in strawberry (*Fragaria ananassa* Duchesne) fruit

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ARTICLE INFO

Original Article

Article history:
Received 19 December 2023
Revised 5 March 2024
Accepted 16 March 2024

Keywords:
Anthocyanin
Antifungal
Inhibitory
Postharvest

DOI: 10.22077/jhpr.2024.7065.1349
P-ISSN: 2588-4883
E-ISSN: 2588-6169

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ABSTRACT

Purpose: The growing attention and interest in alternatives for chemical preservatives with natural types has led to numerous studies on essential oils and plant extracts. Strawberries, due to their high respiration and metabolic activity, and high water content, are highly sensitive to microbial contamination. **Research Method:** An experiment was conducted to investigate the effect of the essential oils of some medicinal plants on the fungus *Aspergillus* sp. in strawberry fruit in *in vivo* and *in vitro* conditions as a factorial form in a completely randomized design with three replications. The first factor included the type of essential oil: frankincense, ginger, cinnamon, and tarragon essential oils, and the second factor included the concentration of essential oil at five levels (0, 200, 400, 600, and 800 µL.L^{-1}). **Findings:** *In vitro* results showed that with the increase in the concentration of plant essential oils, their antifungal activity increases. As a result, the lowest fungus colony diameter was obtained from the concentration of 800 µL.L^{-1} of essential oil. A comparison of the average type of essential oil showed that cinnamon essential oil had more antifungal activity than other essential oils used, so that at any level (200 to 800 µL.L^{-1}), it caused a 100% inhibition of *Aspergillus* sp. growth. *In vivo,* results showed that the best appearance of the fruit and the highest soluble solids were recorded from the concentration of 800 µL.L^{-1} of essential oil. Cinnamon essential oil treatment resulted in the best fruit appearance, the highest soluble solids, and the highest levels of antioxidants, anthocyanin, and sugar compared to frankincense essential oil. **Research limitations:** There were no limitations. **Originality/Value:** Among the essential oils, cinnamon essential oil showed better antifungal activity against *Aspergillus* sp., which causes strawberry fruit spoilage. Therefore, it can be used as a substitute for chemical fungicides, although other essential oils may also be effective.

INTRODUCTION

Today, the demand for organic fruits is increasing, both domestically and from importing countries. These fruits must not have been exposed to any poisons or chemicals during any stage of their production. After that, some countries that import agricultural products allowed the products to enter their country by of applying non-chemical treatments and ensuring the absence of remaining poisons (Behdad et al., 2013). Today, the global desire to find alternative methods to control post-harvest waste, by prioritizing healthy methods and preventing the adverse effects and side effects of toxins on human health, as well as the existence of resistance to fungicides, reduces the possibility of using chemicals (Hashemi et al., 2008; Kahawattage et al., 2023).

The perennial herbaceous strawberry with the scientific name *Fragaria ananassa* Duchesne belongs to the Rosaceae family (Dris et al., 2003). The strawberry is one of the favorite fruits of consumers in many regions of the world (Fan et al., 2021), and a rich source of antioxidants (Amiri et al., 2022). Due to its respiration, high metabolic activity, and significant water content, strawberry is one of the fruits that are highly susceptible to microbial contamination, against mechanical damage and physiological changes during growth and development, and at postharvest stages during transportation and even in distribution and sales centers; it is consistently exposed to contamination by all kinds of fungi. *Rhizopus*, *Aspergillus*, *Botrytis* and *Penicillium* fungi are the most critical microbial factors that limit the lifespan of strawberries after harvesting (Behnamian & Messiah, 2002). Different postharvest treatments positively affected quality and storage life of various fruits mainly by controlling pre and postharvest pathogens (Dorostkar et al., 2022; Moradinezhad & Ranjbar, 2023). One of the ways to maintain the quality of fruits and vegetables and control decay is using antimicrobial and natural compounds. Due to the increasing concerns about endangering the health of consumers, due to the residual chemical toxins on horticultural products and the increasing resistance of fungi to these toxins, scientists are considering using plant essential oils to control postharvest diseases. This has become a new method and alternative to chemical poisons (Ranjbar et al., 2008).

Plant essential oils include a wide range of secondary metabolites, which in most cases have antimicrobial, allelopathic, and antioxidant properties. Essential oils are chemically complex compounds that contain various types of chemicals, including phenols, alcohols, ketones, aldehydes, esters, ethers, terpenes, and terpenoids (Anthony et al., 2003). Terpenes and terpenoids are the most important compounds that cause the antimicrobial properties of essential oils; there is probably a synergy among the different compounds of essential oils, enhancing the antimicrobial properties of each of the compounds (Lazar et al., 2010). The complexity of the compounds in essential oils has a positive effect on their use in managing plant diseases because the possibility of resistance in the pathogen to such complex compounds will be low. Due to their hydrophobic nature, essential oils can destroy the cell membrane in microorganisms, thereby disrupting the electron transfer chain and the functioning of enzymes, leading to a significant reduction in microbial cell activity (Lanciotti et al., 2004).

According to recent studies, some essential oils have fungicidal properties against certain important plant pathogenic fungi. The researchers reported the minimum inhibition rate of frankincense essential oil on *A. flavus* fungus as 1.75 $\mu L.L^{-1}$ indicating its higher antifungal efficacy compared to other essential oils like *Zanthoxylum alatum*, *Ocimum gratissimum*, and *Piper betle* (Prakash et al., 2014). The antifungal activity of cinnamon plant essential oil against *A. flavus*, *Rhizopus nigricans*, and *Penicillium expansum* fungi in the culture medium was investigated, and it was found that this essential oil has a high antifungal effect against

these plant diseases after harvest (Zhang et al., 2016). Using cinnamon essential oil on strawberry fruit to increase shelf life (Mohammadi et al., 2014), and Shirazi thyme essential oil in controlling fungal diseases at postharvest stage on strawberries (Behdad et al., 2010) are known to be effective. Additionally, using marjoram essential oil on grapes can prevent the growth of *Aspergillus rhizopus* mycelium in storage (de Sousa et al., 2013). Clove essential oil also inhibited the growth of *A. niger* for ten days (Jahani et al., 2020). Considering the promising effect of plant essential oils in controlling fungal diseases of fruits in the post-harvest stage, the sensitivity of strawberries to pathogenic agents, especially *Aspergillus* fungus, and the reduction of desire to use chemicals, the present research aimed to investigate the effect of frankincense, ginger, cinnamon, and tarragon essential oils on *Aspergillus* sp. fungus in strawberry fruit was investigated.

MATERIALS AND METHODS

In this research, the effect of four plant essential oils, including frankincense, ginger, cinnamon, and tarragon essential oils in different concentrations were investigated using a factorial form in a completely randomized design. The study was conducted in two laboratory conditions: the first on the PDA culture medium, and the second on strawberries against *Aspergillus sp.*

Isolation and purification of fungi

Initially, to isolate fungal, infected strawberry fruits that had symptoms, they were collected and transported to the laboratory. Micro-samples were prepared from the infected parts of the fruit, and after disinfection with 1% sodium hypochlorite for 2-3 minutes and three subsequent washes with sterile distilled water, these samples were cultured inside the trays containing potato-dextrose agar culture medium. Subsequently, they were transferred to an incubator with a temperature of 25-28°C. After examining the samples and observing their growth, they were identified based on the fungus's morphology, utilizing microscopic slides with available keys. Water-agar culture medium was employed for purification, using the single sporulation method.

Essential oils used in the experiment

Frankincense, ginger, cinnamon, and tarragon medicinal plant essential oils were obtained from Teb Daru Company, Iran.

Investigation of antifungal activity in laboratory conditions

The antifungal effects of the mentioned essential oils on *Aspergillus* fungus were investigated by mixing essential oils at concentrations of 200, 400, 600, and 800 $\mu L.L^{-1}$ with PDA culture medium. In this method, the PDA culture medium was prepared in one-liter Erlenmeyer flasks and autoclaved. After cooling the environment to 42 to 45°C, essential oils were added at different concentrations to the environment and mixed until a completely uniform emulsion was formed. In order to enhance the solubility of essential oils in a culture medium, Tween 80 was used. The resulting mediums were immediately divided into Petri dishes with a diameter of nine centimeters and allowed to solidify. Also, in all treatments, three Petri dishes of culture medium without added essential oil (zero concentration of essential oil) were considered as the control medium. After freezing the medium, discs with a diameter of five millimeters were removed from the edge of the seven-day-old fungus mycelium with a cork borer and placed upside down on the culture medium (in the center of the Petri dishes containing the culture medium). The fungus was incubated in an incubator at a temperature of

25°C. This experiment was conducted with three repelications for each treatment and control. The vegetative growth rate of the halo of fungi was measured every three days until the surface of the control Petri culture medium was completely occupied by the fungus.

The data related to the average growth were analyzed through the analysis of variance (ANOVA) table, and the test results were investigated and analyzed statistically using SAS 9.4 software in a factorial and completely random design format.

Contamination of fruits with fungus suspension

Initially, the strawberries sourced from a commercial greenhouse in Mashhad were rinsed with sterile distilled water, followed by placement on sterile filter paper for drying. Subsequent experimental procedures were carried out within sterile conditions within a culture room and under a hood. The fruits were subsequently immersed in a suspension of fungal spores (1×10^6 spores per milliliter of sterile distilled water) for three to five minutes, as illustrated in Figure 1A.

To generate the fungal spore suspension at the specified concentration of 10^6 spores per milliliter, following the method outlined by Asgari Marjanlu et al. (2009), initially, 10 milliliters of sterilized distilled water was gently applied onto the surface of 7-day-old fungus trays. Subsequently, a Pasteur pipette, whose tip had been bent over a flame to form a paddle, was employed to wet the surface of the medium. This action facilitated the scraping of the medium's surface to liberate and collect the spores. A suspension solution of 10^6 spores per milliliter was prepared using a Schomar cell slide and a 200 mL beaker was prepared to dip strawberry fruits and contaminate them. Following the removal of the fruits from the fungal suspension, they were transferred onto filter paper and subsequently placed under a hood for two hours to facilitate the fixation of fungal inoculation. This experimental protocol entailed the utilization of three replicates for each treatment, with each replicate consisting of five experimental units (fruits).

The fruits were then submerged in a beaker containing various concentrations of essential oil. The essential oil solution was formulated by blending essential oil with acetone and tween 80 (at a concentration of 0.05%) to enhance solubility and absorption by the fruit. The immersion duration was three minutes, after which the fruits were allowed to dry on sterile filter paper. Subsequently, they were transferred into disposable containers and stored in a refrigerator at a temperature of 4°C for 12 days. At the end of storage period, various characteristics of the fruits were assessed, as outlined in Figure 1B.

Fig. 1. Immersion of strawberry fruits in the beaker containing fungal spores (A) and immersion of fruits infected with fungus in the beaker containing essential oil solution (B).

The appearance characteristics of the fruit were investigated after 12 days of inoculation of the fungus to the fruits and immersion in the essential oils. The percentage of fruit decay was observed and graded on a scale of five categories: 5= fruits with excellent appearance and had preserved their color and condition. 4= Fruits have a tiny percentage of spoilage below 10%. 3= Fruits were rotting was observed, and about 20-25% were crushed and turned black. 2= Fruits with up to 50% damage in appearance and decay. 1= Fruits with more than 50% crushed and black (Shiri et al., 2013).

Soluble solids content (SSC) were measured using a handheld refractometer (RF10, 0-32° Brix, Extech Co., USA), and fruit acidity was calculated using a digital pH meter (Mettler Toledo, Switzerland). Titratable acidity (TA) was measured by titration with 0.1 Normal Sodium hydroxide until reaching pH 8.2 (AOAC, 1980). Total anthocyanin content was determined using the pH change method (Swain, 1965). The antioxidant capacity of fruit juice was determined by the DPPH (2,2-diphenyl 1-picrylhydrazyl) neutralization method (Turkmen et al., 2005). The Antron method used to measure total sugar (McCready et al., 1950).

RESULTS AND DISCUSION

Colony diameter of fungus *Aspergillus* sp.

The test outcomes revealed a significant impact of the singular effect of essential oil concentration on the mean colony diameter of Aspergillus sp (Table 1). Subsequent comparison analysis indicated that the average concentration of 800 µL.L-1 of essential oil exhibited the most minor fungus colony diameter (33 mm). In contrast, the largest fungus colony diameter (59.50 mm) was observed in the control treatment (zero concentration) (Table 2). Furthermore, it was observed that the singular effect of the type of essential oil on the mean diameter of the fungus colony was statistically significant. Specifically, the most minor fungus colony diameter was associated with the cinnamon essence treatment (12 mm), while the largest diameter was recorded in the ginger treatment (52.80 mm) (Table 3).

The interaction effect of the treatments also significantly influenced the average growth of the fungus colony diameter (Table 1), so that the consumption of cinnamon treatment at any level (200 to 800 microliters per liter) caused a 100% inhibition of the fungus growth (Table 4).

In similar studies, cinnamon essential oil demonstrated vigorous, antifungal activity on *Aspergillus flavus*, *Aspergillus niger*, and *Aspergillus fumigatus* (Manso et al., 2013). The effectiveness of cinnamon essential oil in preventing the rotting of tomato, orange, and strawberry fruits infected with *B. cinerea*, *P. digitatum*, and *A. niger* fungi showed that strawberry fruit in the vicinity of this essential oil at concentrations of 200, 400, and 600 $\mu L.L^{-1}$ exhibited contamination rates of 45%, 30%, and 11.53%, respectively. For the tomato at the same concentrations, contamination rates were 50.09%, 25.15%, and 7.30%, and for orange fruit, contamination rates were 45%, 20.30%, and 10.10%, respectively (Mousavian et al., 2018). The positive effect of cinnamon essential oil in controlling four fungi, namely *A.niger*, *Penicillium notatum*, *Mucor hiemalis*, and *Fusarium oxysporum* has also been reported (Mousavian et al., 2018). In the bark of the cinnamon plant, there are compounds such as cinnamaldehyde, eugenol, coumarin, benzaldehyde, benzoic acid, cinnamyl acetate, mannitol, linalool, and thymol. By producing benzoic acid, benzaldehyde, and cinnamaldehyde, this plant exerts fungicidal properties on mentioned fungus; therefore, the cinnamon plant possesses numerous fungicidal properties and the compounds in this plant can be used to control plant diseases. Research has shown that phenols show the most antifungal activity compared to alcohols, aldehydes, and others, and it has been determined that the

inhibitory properties of the essential oils of cloves, thyme, and cinnamon are related to phenolic compounds (Plaza et al., 2004). Also, Vazifedoost et al., (2022) reported the coating of strawberry samples with *Salvia chorassanica* essential oil nanoemulsion at a concentration of 12.5 µL/mL was able to delay the growth of *R. stolonifera* and *B. cinere* mold spores on the surface of strawberries for up to 9 days. In addition, no mold growth was observed on the surface of the strawberry samples until the end of the 12th day in the samples coated with *Salvia chorassanica* essential oil nanoemulsion with a concentration of 25 µL/mL.

Table 1. Analysis of variance of the concentration factor, essential oil and the interaction effect of concentration and essential oil on the colony diameter of *Aspergillus* sp. fungus.

Sources of variation	df	Radial growth of fungus
Repetition	2	29.26^{ns}
Concentration	4	1439.77^{**}
Essential oil	3	$5515/09^{**}$
Concentration× Essential oil	12	446.92^{**}
Error	38	21.75
C.V.	-	11.49

ns: non-significant, ** significant at P ≤0.01 probability level, df: degree of freedom.

Table 2. Means comparison of the effect of concentration of essential oils on radial growth for *Aspergillus* sp. fungi treatments in *in vitro* conditions.

Concentration (µL.L^{-1})	0	200	400	600	800
Radial growth of fungus (mm)	59.50^{a}	40.00^{b}	36.83^{bc}	33.50^{c}	33.00^{c}

Similar letter indicates non-significant difference between treatments at 5% levels.

Table 3. Means comparison of the effect of type of essential oils on radial growth for *Aspergillus* sp. fungi treatments in *in vitro* conditions.

Essential oil	Ginger	Cinnamon	Tarragon	Frankincense
Radial growth of fungus (mm)	52.80^{a}	12.00^{c}	47.33^{b}	50.13^{ab}

Similar letter indicates non-significant difference between treatments at 5% levels.

Table 4. Means comparison of the effect of type and concentration of essential oils on radial growth for *Aspergillus* sp. fungi.

Essential oil	Concentration (µL.L^{-1})	Radial growth of fungus (mm)
Ginger	0	60^{a}
	200	57.33^{ab}
	400	53.33^{abcd}
	600	45.33^{ef}
	800	48^{cdef}
Cinnamon	0	60^{a}
	200	0^{h}
	400	0^{h}
	600	0^{h}
	800	0^{h}
Tarragon	0	60^{a}
	200	47.33^{def}
	400	44^{ef}
	600	48^{cdef}
	800	53.33^{abcd}
Frankincense	0	58^{a}
	200	55.33^{abc}
	400	50^{bcde}
	600	40.66^{f}
	800	30.66^{g}

Similar letter indicates non-significant difference between treatments at 5% levels.

Fruit appearance

The simple effect of concentration and type of essential oil as well as the interaction effect of treatments had a significant effect on the appearance of strawberry fruits (Table 5). It was observed that with 800 µL.L^{-1}, the best appearance of the fruit was obtained and the lowest index was obtained in the control treatment (Table 6). Regarding the essential oil type factor, the best fruit appearance was obtained in strawberries impregnated with cinnamon essential oil, and the least favorable appearance was observed in ginger essential oil (Table 6). The results of the interaction of the treatments showed that the consumption of all concentrations of cinnamon essential oil resulted in the best appearance of the fruit (Table 7).

Consistent with our findings, previous studies have also documented the efficacy of cinnamon essential oil in preserving and enhancing the visual quality of fruits, as well as inhibiting fungal growth on strawberries throughout the storage duration (Mohammadi et al., 2014; Tzortzakis, 2007). Xing et al. (2010) also stated that cinnamon essential oil reduced the activity of *Penicillium expansum*, *Aspergillus flavus*, and *Rhizopus nigricans* on jujube and orange fruits. The decrease in the decay rate corresponds to the antibacterial and antifungal properties of the essential oil. The antimicrobial activity of essential oils can be related to an aromatic nucleus and OH group, which can affect the hydrogen bonds of enzymes in microorganisms (Sharma & Tripathi, 2008). The antifungal activity of cinnamon is due to the presence of cinnamaldehyde which is an aromatic aldehyde that prevents the activity of amino acid decarboxylase and is highly electronegative. Such compounds interfere with biological processes associated with electron transfer and react with nitrogen-containing compounds such as proteins and nucleic acids, which prevent the growth of microorganisms (Gupta et al., 2008).

Total soluble solids

The outcomes derived from the analysis of variance revealed a notable impact of the singular effects of both essential oil concentration and type of essential oil on total soluble solids. However, the interaction effect among the treatments was found to be statistically non-significant (Table 5). The concentration effect on the amount of total soluble solids showed that the highest amount of this trait (4.61) was obtained at concentrations of 600 and 800 µL.L^{-1} (Table 6). Among the essential oils used in the experiment, cinnamon essential oil showed the highest amount of total soluble solids (Table 6).

In this regard, the researchers announced in their experiments on bananas, papayas, and strawberries that the fruits treated with the tested essential oils during the storage period lost the amount of soluble solids less than the control. Our results are consistent with increasing the concentration of essential oil, a smaller decrease was observed compared to the control (Maqbool et al., 2011). In similar findings, Tzortzakis (2007) reported an increase in soluble solids in strawberry fruit during treatment with cinnamon essential oil. Also, the results of Mohammadi and Aminifard (2011) also showed that the fruits treated with cinnamon essence had a higher percentage of soluble solids than the control fruits, which is consistent with the results of the present study. Aminifard and Bayat (2018) also reported the highest amount of soluble solids in orange fruit from a concentration of 800 µL.L^{-1} of anise and black cumin essential oil. The amount of soluble solids is one of the critical indicators that has a direct relationship with the edible quality of the fruit at the time of ripening, and consumers have an excellent desire for ripe fruits with a high TSS level. Soluble solids increase at the beginning of storage due to biochemical changes and then decrease drastically due to fruit tissue respiration or fungal contamination and fruit decay. Essential oils play a role in reducing fungal contamination and fruit rot, preventing excessive respiration, and consequently, reducing soluble solids (Sharafi et al., 2011). Abbasi et al. (2021) also showed that the lowest

amount of soluble solids in lemon fruit with 5.58% Brix was obtained in the treatment of 0.2% garden thyme essential oil.

pH of fruit juice

The simple effect of concentration, type of essential oil, and their mutual effect on the pH of fruit juice infected with fungus was significant (Table 5). According to the results of the comparison of the average of mutual effects, it was observed that the infected fruits treated with tarragon essential oil at a concentration of 400 µL.L^{-1} with a pH of 3.74 had the highest pH (Table 7). Sharafi et al. (2011) stated that the use of plant essential oils had a significant effect on the pH of apple fruit. The pH value reflects the acidity level of the fruit extract; the higher the amount of organic acids in the fruit, the lower the pH will be, so the decrease in pH due to essential oil treatments compared to the control indicates the preservation of organic acids in the fruit during the storage period (Alikhani et al., 2009). Essential oils, similar to edible coatings, establish a semi-permeable barrier around the fruit, thereby diminishing the ingress and egress of gases and consequently retarding the respiration process. This delay in respiration curtails the ripening process of the fruit and the metabolism of organic acids, thus contributing to the maintenance of the fruit's pH level. Moreover, the presence of phenols in essential oils plays a pivotal role in pH maintenance by attenuating ethylene production and moderating the pace of metabolic processes (Nasrullah Zade Asl, 2013). Mohammadi and Aminifard (2013) in different results reported the highest pH value in the control treatment and the lowest pH value of tomato juice with a concentration of 800 µL.L^{-1} of essential oil.

Table 5. Analysis of variance of the investigated qualitative traits of strawberry fruits infected with *Aspergillus* sp. fungus.

Sources of variation	df	Appearance of the fruit	TSS	pH	Titratable acidity	Antioxidant capacity	Total anthocyanin content	Total sugar
Repetition	2	0.35^{ns}	0.018^{ns}	0.009^{ns}	0.004^{ns}	136.2^{ns}	3409^{ns}	8.11^{ns}
Concentration	4	9.80**	0.99**	0.033**	0.108**	205.02^{ns}	20980**	342.3**
Essential oil	3	14.19**	0.95*	0.146**	0.473**	667.1**	42.70^{ns}	705.1**
Concentration× Essential oil	12	0.87**	0.19^{ns}	0.016**	0.051**	372.6**	3914*	121.8**
Error	38	0.17	0.22	0.004	0.005	117.4	1685	24.99
C.V.	-	11.44	10.77	1.88	8.44	28.62	29.39	9.91

ns: non-significant, * significant at P ≤0.05 and ** significant at P ≤0.01 probability level, df: degree of freedom.

Table 6. Means comparison of the effect of concentration and essential oil on the investigated qualitative traits of strawberry fruits infected with *Aspergillus* sp. fungus.

Treatments	Appearance of the fruit	TSS (%)	pH	Titratable acidity (%)	Antioxidant capacity (%)	Total anthocyanin content (mg.g^{-1})	Total sugar (mg.gfw^{-1})
Concentration (µL.L^{-1})							
0	2.16^{d}	4.12^{bc}	3.45^{b}	1.02^{a}	32.33^{a}	206.1^{a}	55.86^{a}
200	3.66^{c}	4.00^{c}	3.55^{a}	0.98^{a}	34.89^{a}	112.7^{c}	53.87^{ab}
400	3.75^{bc}	4.50^{ab}	3.58^{a}	0.91^{b}	39.66^{a}	153.0^{b}	51.10^{bc}
600	4.08^{b}	4.61^{a}	3.55^{a}	0.84^{c}	39.89^{a}	124.7^{bc}	49.17^{c}
800	4.58^{a}	4.61^{a}	3.56^{a}	0.79 ^{c}	42.50^{a}	101.5^{c}	42.02^{d}
Essential oil							
Ginger	2.26^{c}	4.34^{b}	3.39^{c}	1.13^{a}	30.69^{c}	138.4^{a}	45.98^{c}
Cinnamon	4.53^{a}	4.74^{a}	3.56^{b}	0.77^{c}	33.98^{bc}	138.0^{a}	43.40^{c}
Frankincense	4.00^{b}	4.18^{b}	3.58^{ab}	0.77^{c}	45.13^{a}	141.5^{a}	57.98^{a}
Tarragon	3.80^{b}	4.22^{b}	3.62^{a}	0.97^{b}	41.62^{ab}	140.6^{a}	54.26^{b}

The same letter indicates no significant difference between treatments at 5% levels.

Titratable acidity (TA)

According to the results of this experiment, it was evident that both the individual effects of concentration and type of essential oil, as well as their combined effect, exerted a significant influence on TA. (Table 5). Fruits infected with *Aspergillus* fungus not treated with essential oil (control) had the highest amount of TA (1.02 %), which had no statistically significant difference with a concentration of 200 µL.L^{-1}, and the lowest amount. This index (0.79 %) was obtained from a concentration of 800 µL.L^{-1} (Table 6). The fruits treated with ginger essential oil had the highest TA (Table 6). Additionally, mutual effects indicated that ginger essential oil at a concentration of 400 µL.L^{-1} had the highest total acidity (Table 7). Organic acids play a crucial energy storage source in fruit tissue. During the ripening process, a considerable portion of these organic acids undergo decomposition due to respiration. (Wills et al., 1998). Organic acids possess a higher oxygen-to-carbon ratio compared to carbohydrates or fatty acids. This characteristic renders them more readily utilized as a source of energy during respiration. Along with the results of this experiment, the results of Ghafouri (2013) on pomegranate fruit showed that the lowest amount of total acidity in cinnamon essence is 500 mg.L^{-1} (0.9), and the highest amount of total acidity was observed in the control treatment (1.2). Vesal Talab and Gholami (2012) also reported that clove essential oil gradually reduced the amount of total acidity in treated grapes during the post-harvest period. Similar findings have also been documented by other researchers in studies conducted on grapes and strawberries (de Sousa et al., 2013; Hernández-Muñoz et al., 2006).

Antioxidant capacity

The results indicate that both the effect of essential oil and the interaction between concentration and essential oil had a significant impact on antioxidant percentage. However, the effect of concentration alone did not exhibit a significant influence on this index (Table 5). The results showed that the infected fruits treated with tarragon essential oil with a concentration of 800 µL.L^{-1} had the highest antioxidant (52.18%) (Table 7). Antioxidants are crucial in mitigating physiological damage and enhancing tissue resistance against stress and microbial contamination by neutralizing free radicals and diminishing oxidative stress (Wang & Lin, 2000).

The results of the current investigation reported by Sazvar et al. (2022) stated that the effect of concentration on the antioxidant percentage of barberry fruits infected with *Alternaria* fungus was not significant, however, the interaction of concentration and essential oil confirmed that the highest antioxidant percentage was obtained from the concentration of 200 µL.L^{-1} of chamomile essential oil. Raspberries treated with plant essential oils had higher levels of phenolics, anthocyanin content, and stronger antioxidant activity than untreated raspberries (Jin et al., 2012), aligning with the findings of this research.

Total anthocyanin content

The concentration and the interaction effect of concentration and essential oil on the amount of anthocyanin in strawberries infected with fungus were significant (Table 5). The highest amount of anthocyanin was observed in the control treatment (zero), while the concentration of 800 µL.L^{-1} had the lowest amount (Table 6). The results of the mutual effects of the treatments showed that the fruits infected with the fungus without the addition of essential oil had the highest amount of anthocyanin. In contrast, the fruits infected with fungus treated with ginger essential oil with a concentration of 800 µL.L^{-1} had the lowest amount of this index (Table 7).

Table 7. Means comparison of the interaction effect of concentration and essential oil on the investigated qualitative traits of strawberry fruits infected with *Aspergillus* sp.

Essential oil	Concentration ($\mu L.L^{-1}$)	Appearance of the fruit	pH	Titratable acidity (%)	Antioxidant capacity (%)	Total anthocyanin content ($mg.g^{-1}$)	Total sugar ($mg.g^{-1}$)
Ginger	0	2.00^{ef}	3.47^{cde}	0.96^{ef}	30.97^{f}	236^{a}	54.97^{abc}
	200	1.66^{f}	3.41^{ef}	1.25^{ab}	42.06^{abcd}	143^{cde}	52.26^{abcd}
	400	2.00^{ef}	3.35^{f}	1.27^{a}	19.00^{fg}	155^{bcde}	48.41^{cde}
	600	2.33^{ef}	3.36^{ef}	1.16^{abc}	33.87^{bcdef}	103^{defg}	40.95^{efg}
	800	3.33^{cd}	3.38^{ef}	1.05^{cde}	27.57^{defg}	53.8g	33.29^{gh}
Cinnamon	0	2.66^{de}	3.44^{ef}	1.05^{cde}	33.69^{bcdef}	203^{abc}	60.22^{a}
	200	5.00^{a}	3.57^{bc}	0.71^{ijk}	12.33^{g}	97.33^{efg}	47.23^{cde}
	400	5.00^{a}	3.63^{b}	0.75^{hij}	41.87^{abcd}	128^{def}	42.43^{ef}
	600	5.00^{a}	3.60^{b}	0.64^{jk}	37.97^{abcde}	142^{cde}	36.03^{gh}
	800	5.00^{a}	3.57^{bcd}	0.69^{jk}	44.03^{abcd}	118^{defg}	31.08^{h}
Frankincense	0	2.33^{ef}	3.46^{def}	1.02^{de}	42.30^{abcd}	214^{ab}	56.68^{ab}
	200	4.00^{bc}	3.61^{b}	0.84^{fgh}	50.57^{ab}	142^{cde}	57.51^{ab}
	400	4.00^{bc}	3.60^{b}	0.67^{jk}	50.87^{ab}	162^{bcde}	59.17^{ab}
	600	4.66^{ab}	3.60^{b}	0.69^{jk}	35.66^{abcdf}	118^{defg}	59.42^{ab}
	800	5.00^{a}	3.63^{ab}	0.62^{k}	46.24^{abc}	69.55^{fg}	57.13^{ab}
Tarragon	0	1.66^{f}	3.41^{ef}	1.07^{cde}	22.36^{efg}	169^{abcd}	51.58^{cd}
	200	4.00^{bc}	3.62^{b}	1.13^{bcd}	36.60^{abcdef}	68.25^{fg}	58.47^{ab}
	400	4.00^{bc}	3.74^{a}	0.96^{ef}	46.90^{abc}	166^{bcd}	54.39^{abcd}
	600	4.33^{ab}	3.66^{ab}	0.89^{fg}	52.06^{a}	135^{def}	60.30^{a}
	800	5.00^{a}	3.66^{ab}	0.82^{ghi}	52.18^{a}	163^{bcde}	46.58^{de}

Similar letter indicates non-significant difference between treatments at 5% levels.

In line with this experiment, the results of Amiri et al. (2019) research showed that control strawberry fruits inoculated with fungus showed the highest amount of anthocyanin and the same treatment also demonstrated the highest reduction in fruit weight. Additionally, Jahani et al. (2020) also showed that grape fruits infected with *Penicillium* fungus untreated with essential oil had the highest amount of anthocyanin. The increase in the amount of anthocyanin during the harvest period is related to the weight loss and moisture loss of the fruit, and as a result, the concentration of anthocyanin content (Meighani et al., 2018). Anthocyanins, a class of phenolic compounds, contribute to the red-blue hues observed in various vegetables and fruits, and are known for their significant impact on human health. The findings imply that the synthesis of these compounds continues even after harvest (Wang & Gao, 2013).

Total sugar

According to the results of this experiment, the simple effect of the concentration, the type of essential oil, and the interaction effect of the treatments on the sugar content of the treated strawberries were significant (Table 5). By increasing the concentration of essential oils, the amount of sugar in the treated fruits decreased. The control treatment exhibited the highest amount of 55.86 $mg.g^{-1}$ fresh weights, while the fruits treated with a concentration of 800 $\mu L.L^{-1}$ of essential oil had the lowest amount of sugar with 42.02 $mg.g^{-1}$ fresh weight (Table 6). Statistical comparison of the averages revealed that the frankincense essential oil treatment exhibited the highest sugar content, whereas the cinnamon essential oil treatment displayed the lowest sugar content (Table 6). The results of the interaction of the treatments showed that the fruits treated with tarragon essential oil with a concentration of 600 $\mu L.L^{-1}$ had the highest amount of sugar at the end of the experiment (Table 7). Hatfi et al. (2013) reported that the highest amount of sugar was observed in the coated samples compared to the control. By reducing the amount of respiration, the coating delays the consumption of sugar in the

respiratory enzymatic reactions. Soluble sugars act as a defense mechanism, limiting the penetration of pollutants into the fruit tissue. Additionally, higher sugar levels in the fruit tissue correlate with reduced water content, resulting in less water loss due to evaporation and transpiration.This, in turn, contributes to the increased shelf life of strawberry fruit (Amal et al., 2010).

CONCLUSION

In general, this research demonstrated that essential oils have antifungal properties in controlling postharvest fungal diseases. *In-vitro* tests showed that ginger essential oil had a shallow fungicidal effect, while cinnamon essential oil had the most fungicidal effect in controlling *Aspergillus* sp. Antifungal properties increased with increasing the concentration of essential oil, but the antifungal property of cinnamon essential oil was 100% in all concentrations used. Under natural conditions, the use of essential oils had a significant effect on maintaining the appearance characteristics and preserving soluble solids during the storage period of strawberry fruit. Among the essential oils, cinnamon essential oil showed commendable antifungal activity against the fungus *Aspergillus* sp. a common cause of strawberry fruit spoilage. This suggests its potential as an alternative to chemical fungicides, although other essential oils also possess such potential. Considering the biodegradability and low toxicity of plant essential oils, it is recommended to consider using cinnamon essential oil to enhance the shelf life of food products to increase the shelf life of food products.

Conflict of interest

The authors declare that there is no conflict of interest to report.

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JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH
2024, VOL. 7(2), 155-170

Journal of Horticulture and Postharvest Research

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

University of Birjand

Effect of compost tea on the quality promotion of sweet corn (*Zea mays* var. Rugosa) in organic cultivation

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ARTICLE INFO

Original Article

Article history:
Received 29 February 2024
Revised 21 April 2024
Accepted 23 April 2024

Keywords:
Chemical
Physical
Properties
Sensory

DOI: 10.22077/jhpr.2024.7362.1367
P-ISSN: 2588-4883
E-ISSN: 2588-6169

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ABSTRACT

Purpose: A field experiment was designed to determine the effects of compost tea on the quality parameters of sweet corn produced without the use of mineral fertilizers. **Research method:** This research was conducted in the Taraba State University Teaching and Research Farm, Jalingo, Nigeria in 2023. The fertilizer treatments in this study were 500kg ha^{-1} NPK fertilizer (Control), 1 kg compost per 10 L water compost tea, 1 kg compost per 20 L water compost tea and 1 kg compost per 30 L water compost tea, arranged in Randomized Complete Block Design, replicated thrice. **Findings:** The results indicated that the treatments had significant ($P \leq 0.05$) effects on the physical, chemical and sensory characteristics of the sweet corn evaluated. The mineral (NPK) fertilizer treatment gave highest mean total soluble sugar content (33.13 mg g^{-1}), followed by 1 kg compost per 10 L water compost tea (33.10 mg g^{-1}), then 1 kg compost per 20 L water compost tea (31.72 mg g^{-1}) and 1 kg compost per 30 L water compost tea gave the lowest total soluble sugar content (29.88 mg g^{-1}). Yet, the effects of 1 kg compost per 10 L water compost tea treatment and mineral (NPK) fertilizer treatment were the same ($p > 0.05$). **Research limitations:** There were no limitations to the report. **Originality/Value:** This study illustrated the possibility of utilizing 1 kg compost per 10 L water compost tea concentration to produce a good yield and quality of sweet corn without mineral fertilizers.

INTRODUCTION

Sweet corn (*Zea mays* var. Rugosa) is an increasingly popular vegetable for consumption in many countries i.e. Australia, Canada, USA, Europe, Japan and South-East Asia. Sweet corn has high sugar content in the early dough stage, where the sugars accumulate in the kernels two or three times more than the normal maize (Abou-El-Hassan & El-Batran, 2020) and serves as raw material to many manufacturing industries in the production of materials such as corn syrup and starch as well use as biofuel (Emam et al., 2020). Sweet corn is a very profitable crop for farmers because it is harvested after short time as 65-90 days depending on the cultivars and has a high price for local market and exportation. So it must be considered as important crops in Nigerian economy in the future. Economic value of sweet corn might be double when it is organically produced due to increased consumer demand and limited product availability (Fahrurrozi et al., 2016). Generally, vegetables or crops produced using organic fertilizers are more attractive to the consumer than those produced using inorganic fertilizers. This is due to its free of synthetic chemicals that harm the environment and human health (Abou-El-Hassan & El-Batran, 2020).

The current environmental issues are capturing the world's attention focusing on improving the environmental quality through the adoption of techniques and measures that have reduced impacts on the environment (Kim et al., 2015; Waliczek & Wagner, 2023). Organic cultivation techniques for crops production in the field and greenhouses have been developed in alternative production techniques which employ biological or organic compounds for disease and pest control (Kim et al., 2015). Organic compost has already been established as a recommended fertilizer for improving the productivity of several crops due to its high organic matter content (El-Shaieny et al., 2022).

Compost is a humus-like material produced from aerobic microbiological decomposition of organic material derived from plants and/or animal residues by mesophilic and thermophilic microorganism (Somerville et al., 2018; Kranz et al., 2020). The role of organic matter is very important. Its high content in the soil influences physical properties; assures good value of soil cation exchange capacity (CEC); reduces the mobility of nutrients in soil solution, prevents the loss of useful substances by means of the action of enzymes; avoids the pollution of water table; improves soil porosity; helps the chemical stabilization of structure; and reduces the processes of soil erosion and increases the micro-organisms and enzymatic activity (Kim et al., 2015).

Composts teas are oxygenated compost water extracts obtained through a suitable liquid-phase blowing process. Compost tea is one sources of plant nutrition which is prepared by fermenting compost in water for a period of time in order to extract soluble organic matter, beneficial microorganisms and nutrients in the watery solution (Zaccardelli et al., 2018). Study on compost tea technology began in 1980's in USA, but field practices comparing the brewing methods are few. Historically, home-made extract of compost tea called "passive" or non-aerated compost tea (NCT) were prepared by suspending a bag of compost in a container of water for 14 days to extract anaerobic microbes and nutrients which are used and applied to promote plant health and vitality in plants (Kim et al., 2015). Recently, aerated compost tea has been brewed in large-scale mechanized equipment for shorter period of time and often supplemented with oxygen, nutrients, and microbial starter cultures to enhance the biological activity of the compost tea which contains aerobic microbes and nutrients (Sujesh et al., 2017). Compost tea is rich of nutrients, organic compounds and beneficial microbes that positively effect on the plant rhizosphere, besides improves soil physical and chemical properties as well as suppress some plant pathogens. It has beneficial effects on plant growth and considered as a soil amendment (Abou-El-Hassan & El-Batran, 2020).

Direct use of compost can create waste and aesthetic problem in small scale gardening and indoor plants. Therefore, compost tea has beneficial effects on plant growth by providing plant nutrients directly. Compost extract is used as an organic foliar fertilizing material. It is used directly on foliage without using compost to the soil. Compost tea inoculates the leaf surface and soil with beneficial microorganisms and to add soluble nutrients to the foliage or to the soil for organisms and the plants present (Abou-El-Hassan & El-Batran, 2020). Compost teas as an effective source of nutrients but there is little scientific evidence to support or disprove this claim.

Compost tea has been cited as an option for conventional and organic growers thought to enhance crop fertility by introducing microorganisms that might aid in soil nutrient retention and extraction, and by adding soluble nutrients, further adding to their potential value as a part of an integrated crop management plan (Kim et al., 2015; Sujesh et al., 2017; Zaccardelli et al., 2018; Palese et al., 2021; Oyewusi & Osunbitan, 2021). Many studies indicated that application of organic extracts enhanced the growth, yield and quality for many crops such as Jasson (2017) on Hypoxis Hemerocallidea and Siphonochilus Aethiopicus, Giménez, et al. (2020) on baby leaf lettuce, Ros et al. (2020) on baby spinach, Khoerunnisa et al. (2022) on Black Rice (*Oryza sativa* L. indica), Carricondo-Martínez et al. (2022) on tomato and Jasson et al. (2023) on Siphonochilus aethiopicus (Schweinf.) BL Burtt. Therefore, the objective of this study was to determine and evaluate the efficacy dose of application of aerated compost teas prepared from mixtures of three composts on the quality promotion of sweet corn.

MATERIALS AND METHODS

Field experiment on sweet corn was carried out at the Teaching and Research Farm of the Taraba State University, Jalingo, Nigeria in 2023. The study site is geographically coordinated at latitude 8° 53′ and 34.2672″ North and longitude 11° 22′ and 37.74″ East. This experiment was performed to improve the efficiency of compost tea by using mix feed stock for producing sweet corn without mineral fertilizers.

Plant material

Seeds of sweet corn (Meilan F1 Hybrid) were planted in the field on flat beds. The experimental area was prepared into flat beds by plowing, harrowing and leveling; each plot measures 2 m×2m (4 m^2). The seeds were planted in September, 2023 at a distance of 0.3 m×0.3 m. The experiment was designed in complete randomized blocks with three replicates.

Table 1. Physical and chemical properties of the used farmland soil, compost and compost tea.

Parameters	Soil	Compost	Compost Tea
Bulk Density (kg/m^3)	1630.00	243.67	-
pH	6.74	8.15	7.52
Electrical Conductivity (dS/m)	2.98	2.36	2.88
Organic carbon (g/kg)	5.41	152.14	29.68
Organic matter (g/kg)	9.20	271.38	51.35
Total N (g/kg)	1.45	16.47	29.74
Total P (g/kg)	6.33	8.96	10.26
K (g/kg)	0.10	8.14	10.47
Ca (g/kg)	0.51	10.52	12.95
Mg (g/kg)	0.16	1.68	1.88
Na (g/kg)	0.05	0.27	0.43

Experimental treatments

i. 500kg ha^{-1} NPK fertilizer (Control) (Sidiky et al., 2019; Donald et al., 2021).
ii. 1 kg of compost per 10 L of water compost tea.
iii. 1 kg of compost per 20 L of water compost tea.
iv. 1 kg of compost per 30 L of water compost tea.

Composting process

Compost used was produced from cow dung, poultry droppings and vegetable wastes collected from the animal farm of the Taraba State University, Jalingo. The composting pile/above ground heap method (2 × 1.5 × 2 m^3) was prepared on a clean ground surface, located under shade. Cow dung, Poultry manure and vegetable wastes in a ratio 5:5:1 was used as feedstock to produce compost. The materials were arranged by keeping proper thickness and finally the heap was covered with banana leaves. The pile was turned manually and watered after every two weeks, during composting process of three months (Girshe et al., 2018).

Preparation of compost tea

From the mature compost obtained, compost tea was produced as described by Ngakou et al. (2014), based on the ratios of 1/10, 1/20 and 1/30 (kg/L: compost/water). Mixture was homogenized for 10 minutes and then filtered through a 0.001 mm mesh sieve. Filtrate obtained was then being left to ferment for four (4) days in a non-aerated condition. The physical and chemical properties of the used farmland soil, compost and compost tea are listed in Table 1.

Sweet corn harvest and sample preparations

At early dough stage (75 to 85 days from planting) the ears were manually harvested. Four ears from each plot were taken randomly at harvest and were husked to measure ear parameters as weight of ear, length of ear, diameter of ear, number of kernel in row and number of row. Sweet corn was directly husked for physicochemical and sensory evaluation. Samples of ear kernels were randomly bulked from each experimental unit to determine some physical properties of the ear kernels which include; Moisture content, Kernel texture (hardness) and Kernel colour. Samples of ear kernels were also randomly bulked from each experimental unit to determine kernel chemical compositions of Total soluble solids, Total soluble sugar, Carotenoids, pH value, Total titratable acidity and Mineral (Iron, Potassium, Calcium, Phosphorus and Zinc) content. Sweet corn for sensory evaluation were also husked and steamed for 30 minutes.

Determination physical properties of sweet corn ear and kernels

Ear mass and dimensional properties

The sweat corn ear mass was determined using a digital precision balance with accuracy of 0.001 g. The dimensional parameters, i.e. the length and diameter of corn ears, were determined using a ruler gauge and a slide calliper. The corn ear diameter was measured in the middle of the corn ear length. Number of kernel in row and number of row per ear were determining by manual counting.

Moisture content

The percentage retention of moisture was analyzed by the process of oven drying method. As much as two grams of corn kernels were weighed and then dried for three hours in the oven at

105°C. This drying is carried out until a constant weight is obtained. The difference between initial and final was considered as moisture and percentage calculated.

$$\text{Moisture content (\%)} = \frac{\text{Initial weight} - \text{Final weight after drying}}{\text{Initial weight}} \times 100 \quad (1)$$

Texture (hardness)

Hardness was measured by a texture analyzer (TA. XT Plus, Stable Micro Systems, UK). The experiment was conducted using the P6 probe of the texture analyzer with pre-test speed set to 1 mm/s, test speed set to 2 mm s^{-1}, strain set to 40%, and time set to 5 s (Saengrayap et al., 2015).

Color characteristics

The color attributes L* (darkness/lightness), a* (redness/blueness), and b* (yellowness/greenness) of the sweet corn were valuated using a colorimeter (CR-5, Konica Minolta, Osaka, Japan). The sample was equilibrated to room temperature. The colorimeter was calibrated before measurement and the color was measured from three different random positions for each measurement (Kumar et al., 2020). Yellowness Index (YI), Chroma (C*) and Hue (H*) were expressed as shown in Equations (2), (3) and (4).

$$YI = \frac{142.86 \times b^*}{L^*} \quad (2)$$

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (3)$$

$$H^* = \tan^{-1}\left(\frac{b^*}{a^*}\right) \quad (4)$$

Determination chemical properties of sweet corn kernels

Total Soluble Solids

Sweet corn kernels were homogenized and centrifuged at 3,000 rpm at 4°C for 20 minutes. The supernatants were collected and tested using a digital display refractometer (WYT-32, Quanzhou optical, Quanzhou, China). For each experiment, five sweet corns for each sample were used to determine the total soluble solids (TSS) content (Liu et al., 2021).

Total Soluble Sugar

Experiments were performed according to the method of Calvo-Brenes and O'Hare (2020). Five grams of the ground sample was placed into a 50 mL tube and extracted twice with 25 mL of a mixture of ethanol and water (50: 50, v/v) and centrifuged at 3,000 rpm at 4°C for 20 minutes. The supernatants were combined, and 2 mL of the supernatant was filtered through a 0.2 µm water syringe filter to measure the sugar content.

Carotenoids

Carotenoids were determined according to the method of Deng et al. (2022). The samples were well ground in hexane: acetone (1:1, v/v) solution using a mortar and pestle and extracted on a shaker at 270 rpm for 1 h at room temperature. The supernatant was measured at 450 nm using a spectrophotometer.

pH and titratable acidity

The pH of sweet corn kernel was determined on a filtrate of a 5 g ground sample (80 mesh size) in 20 ml distilled water using a glass electrode digital pH meter (PYE Umicam, England). The meter was switched on and allowed to equilibrate for about 15 minutes and calibrated with pH 4.0 and pH 7.0 buffer solutions before the measurement. The electrode of the pH meter was rinsed with distilled water. The electrode and temperature probe was dipped into each sample and was allowed to display. The displayed pH value for each sample was recorded. The titratable acidity was expressed as sodium hydroxide required neutralizing the acids in a 100 g sample using phenolphthalein as an indicator. Titratable acidity (TA) of reconstituted sample was estimated by diluting the aliquot of the sample with water to a fixed volume and then titrated with 0.1N NaOH using phenolphthalein as an indicator. Percentage acidity was calculated as the percentage of anhydrous citric acid using following formula (Kadam et al., 2010).

$$\text{TA} = \frac{\text{Titre} \times \text{normality of alkali} \times \text{volume made up} \times \text{equivalent weight of acid} \times 100}{\text{Volume of sample taken for estimation} \times \text{weight or volume of sample taken} \times 1000} \quad (5)$$

Maturation index

Maturation index was obtained by dividing total soluble solids by total titratable acidity.

Mineral content

The mineral content (iron, potassium, calcium, phosphorus and zinc) were determined by Atomic Absorption spectrophotometer according to the method of AOAC (2019). Samples (1.0 g) were digested with 6 mL of HNO_3 (65 %), 2 mL of H_2O_2 (30 %) in microwave digestion system for 31 min and diluted to 10 mL with deionized water. A blank digest was carried out in the same way. Due to higher accuracy with respect to time and recovery values, this procedure preferred. The recovery values were nearly quantitative (> 95%) for above mentioned digestion method. An atomic absorption spectrometer with deuterium background corrector was used for elemental analysis. All measurements were carried out in an air/acetylene flame.

Determination sensory properties of sweet corn kernels

A comprehensive survey of the sensory characteristics was conducted based on nine-point category scale where higher score indicates better quality (1 = none; 9 = extreme). Sensory attributes such as sweetness, tenderness, juiciness and general acceptability were evaluated. Random samples of different treatments were presented in three-digit coded plates and were separately evaluated by 12 untrained consumer panelists. The mean scores for each attribute were finally summed to give an overall sensory score known as the Quality Index (QI).

Statistical analysis

The average and standard deviation of each physical, chemical and sensory parameters result were subjected to a mono-factorial variance analysis (ANOVA), and the significance of differences ($P \leq 0.05$) among means was determined with Fisher's Least Significant Difference (FLSD) test. Statistical analysis was performed using an SPSS version 19.0 for Windows (SPSS, Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

The effects of compost tea application on the physical, chemical and sensory characteristics of freshly harvested sweet corn variety were determined to show sweet corn as a good source of nutrients and antioxidants. Results showed that the fertilizer treatments significantly affected the various physical, chemical and sensory characteristics of freshly harvested sweet corn variety determined at $P \leq 0.05$.

Effects of compost tea on physical properties of sweet corn cobs and kernels

The effects of compost tea on physical properties of sweet corn cobs and kernels are illustrated in Table 2. Results showed that the different fertilizer treatments affected the various physical characteristics of sweet corn cobs and kernels evaluated significantly ($P \leq 0.05$). The treatments of mineral (NPK) fertilizer and 1 kg of compost per 10 L of water compost tea gave the highest values in most of the physical characteristics of sweet corn cobs and kernels compared to other treatments.

Ear characteristics

Effects of different treatments on ear characteristics of sweet corn showed that all the fertilizer treatments produced sweet corn ear with significant ($P \leq 0.05$) differences between them. The 1 kg of compost per 10 L of water compost tea and mineral (NPK) fertilizer treatments produced the highest ear mass, ear length, ear diameter, number of kernel per row and number of row per ear, which gave the best ear characteristics compared to other treatments. The 1 kg of compost per 20 L of water compost tea treatment came in second order, whereas the 1 kg of compost per 30 L of water compost tea treatment produced the lowest values of ear characteristics. The results mentioned that treatment of 1 kg of compost per 10 L of water compost tea produced sweet corn ear diameter similar to the ear diameter produced by mineral (NPK) fertilizers without significant differences between them. The superior treatments of 1 kg of compost per 10 L of water compost tea over 1 kg of compost per 20 L of water compost tea and 1 kg of compost per 30 L of water compost tea treatments can be attributed to its superiority in stimulating vegetable growth of plants, resulting in an increase in photosynthesis and better carbohydrate construction, thus improved yield and ear characteristics of sweet corn. These results are consistent with those obtained by Kim et al. (2015) who investigated the effect of aerated compost tea on the growth promotion of lettuce, soybean, and sweet corn in organic cultivation and reported that application of aerated compost tea from organic compost based using MOVR (the mixture of rice straw compost, vermicompost, and Hinoki cypress bark compost) to the root zone increased the plant shoot and root growths and yield of the red leaf lettuce, sweet corn, and soybean. They mentioned that application of compost teas leads to the production of plants vigor growth, higher nutrient uptake, more tolerant of stress conditions, better in the productivity and yield quality. Thus, compost tea could be used as an agent for promoting plant growth and yield in organic cultivation of crops.

Moisture content

The results of the effects of different fertilizer treatments on mean moisture content of sweet corn kernels showed that all the fertilizer treatments produced sweet corn kernels with significantly ($P \leq 0.05$) different moisture content. The results indicated that the percentages of mean corn kernel moisture content significantly ($P \leq 0.05$) increased with increasing concentration of compost tea. The 1 kg of compost per 10 L of water compost tea treatment

gave the highest mean kernel moisture content of 76.77%, followed by the mineral (NPK) fertilizer treatment with mean moisture content of 76.46%, then the 1 kg of compost per 20 L of water compost tea treatment with mean moisture content of 74.80% and lastly the 1 kg of compost per 30 L of water compost tea treatment with mean moisture content of 72.43%. Differences could be from variations in conditions of cultivation. This result is in line with the works of Ndiaye et al. (2017) who carried out the physical and biochemical characterization of sweet corn ears of four varieties grown in Senegal and reported moisture content range of 75.03 ± 1.04% to 80.70 ± 1.2%. Water content in a food is related to the stability index of the food, especially related to food storage. If the water content is higher, the quality and storability of food will be lower (Sinay & Harijati, 2021). This means that the corn cultivar evaluated cannot be stored based on its moisture content.

Kernel hardness

Hardness can reflect the maturity and aging degree of corn during storage and it is likewise a major classification criterion for sweet corn (Zhang et al., 2023). The results of the effects of different fertilizer treatments on sweet corn kernel hardness show that all the four treatment levels of fertilizer concentration were significantly ($P \leq 0.05$) different in their effects on the kernel harness. With regard to sweet corn kernel hardness as affected by different sources of nutrient (organic or inorganic), it was noticed that, kernel hardness increased with the concentration of nutrient. Where, mineral (NPK) fertilizer treatment gave the highest mean value of kernel harness (1874.14 g), followed by 1 kg of compost per 10 L of water compost tea treatment (1872.86 g), then 1 kg of compost per 20 L of water compost tea treatment (1857.63 g) and 1 kg of compost per 30 L of water compost tea treatment gave the lowest mean value of kernel harness (1841.27 g). It remarked that, the increase of hardness of seeds which increase the storage period after harvesting was associated with their high contents of calcium which translocate from leaves to seeds during the period of seeds development. For this reason, average hardness has similar trends as their contents from calcium. Where, increase of calcium concentration causes a strong membranes structure. Since a high proportion of calcium in plant tissue is located in the middle lamella, which gives the strength to the cell walls.

Table 2. Effects of compost tea on physical properties of sweet corn cobs and kernels.

Physical properties	Treatment				P-Value	F-LSD $_{0.05}$
	500 kg ha^{-1} NPK Fertilizer	1kg 10L^{-1} Compost tea	1kg 20L^{-1} Compost tea	1kg 30L^{-1} Compost tea		
Ear Mass (g)	362.63^{a}	360.45^{b}	331.33^{c}	303.58^{d}	0.000	0.0000
Ear Length (cm)	21.50^{a}	21.11^{b}	19.80^{c}	18.56^{d}	0.000	0.1258
Ear Diameter (cm)	4.90^{a}	4.88^{a}	4.19^{b}	3.63^{c}	0.000	0.0316
Number of Kernel in Row	38.52^{b}	39.47^{a}	32.40^{c}	32.34^{d}	0.000	0.0154
Number of Row	16.36^{a}	15.80^{b}	15.24^{c}	14.58^{d}	0.000	0.0377
Moisture Content (%)	76.46^{b}	76.77^{a}	74.80^{c}	72.43^{d}	0.000	0.0154
Kernel Hardness (g)	1874.14^{a}	1872.86^{b}	1857.63^{c}	1841.27^{d}	0.000	0.0844
Kernel Colour						
Lightness (L*)	62.86^{d}	69.64^{a}	66.89^{b}	65.38^{c}	0.000	0.0377
Greenness (a*)	-2.27^{a}	-5.38^{d}	-4.14^{c}	-2.95^{b}	0.000	0.0129
Yellowness (b*)	30.73^{d}	35.80^{a}	33.44^{b}	32.52^{c}	0.000	0.0218
Yellowness Index (YI)	69.84^{d}	73.44^{a}	71.42^{b}	71.06^{c}	0.000	0.0253
Chroma (C*)	30.81^{d}	36.20^{a}	33.70^{b}	32.65^{c}	0.000	0.0000
Hue Angle (°)	85.78^{a}	81.45^{d}	82.94^{c}	84.82^{b}	0.000	0.0000

Row means with the same letters are not significantly different at 5% level.

Kernel colour

Statistical analysis carried out on colour parameters of sweet corn samples cultivated using different type of fertilizer treatment, highlighted that all the samples showed different values with significance differences ($P \leq 0.05$) among them. The L* and b* are used to reflect the freshness of sweet corn (Calvo-Brenes & O'Hare, 2020). The greater the positive L* and b* value, the more the colour tends to be brighter and yellow.

The results from this study showed that 1 kg of compost per 10 L of water compost tea treatment had the best colour, with significant differences compared to the other treatment groups. The L* and b* values of 1 kg of compost per 10 L of water compost tea treatment samples were greater than those of other samples at harvest, which indicated that 1 kg of compost per 10 L of water compost tea treatment could better maintain the brightness of sweet corn. The sweet corn color is mainly due to the presence of a huge number of secondary metabolites, such as phenolic acids, carotenoids, and flavonoids. The different expression of these pigments imparts to sweet corn tissues different colors, from yellow-orange to dark purple-blue, as well as ivory, and cream colors (Zhang et al., 2021).

Effects of compost tea on chemical properties of sweet corn kernels

The effects of compost tea on chemical properties of sweet corn kernels are shown in Table 3. Results showed that the different fertilizer treatments affected the various chemical characteristics of sweet corn kernels evaluated significantly ($P \leq 0.05$). The treatments of mineral (NPK) fertilizer and 1 kg of compost per 10 L of water compost tea gave the highest values in all the chemical characteristics of sweet corn kernels evaluated compared to other treatments except for the pH values.

Total soluble solids

The results in reveal that the mineral and organic fertilizers treatments had a significant ($P \leq 0.05$) effect on total soluble solids percentage. This study confirms that total soluble solids levels of sweet corn kernels were affected by the different fertilizers concentration. For the total soluble solids trait, data clearly show that treatments of mineral (NPK) fertilizer gave the highest total soluble solids of 17.68%, followed by treatment of 1 kg of compost per 10 L of water compost tea with total soluble solids of 17.63%, treatment of 1 kg of compost per 20 L of water compost tea with total soluble solids of 17.32% and lastly, the treatment of 1 kg of compost per 30 L of water compost tea producing the lowest value of total soluble solids of 17.12%. Yet, the effects of 1 kg of compost per 10 L of water compost tea treatment and mineral (NPK) fertilizer treatment were the same ($p > 0.05$) while that of 1 kg of compost per 20 L of water compost tea treatment and 1 kg of compost per 30 L of water compost tea treatment were significantly ($p \leq 0.05$) different. Observed TSS was in close agreement with the value obtained for sweet corn kernels by Evangelista et al. (2017), and Abou-El-Hassan and El-Batran (2020). These results might be due to the role of mineral fertilizers and organic manures in increasing soil porosity, aeration, water holding capacity and cation exchange capacity (CEC), which encourage the biological activities of soil microorganisms and led to break down of organic matter releasing N, P and K and other nutrients to the soil solution. As these nutrients are available in the soil solution, absorption would be higher and nutrients uptake might be stimulated. These results agreed with those reported by Sarhan et al. (2011), Adebayo et al. (2014), and Shafeek et al. (2015) who reported that the highest TSS, total sugars, protein, vitamin C and moisture contents in cucurbits fruits were obtained by increasing the levels of compost used.

Total soluble sugar

The amount of the total soluble sugar detected in sweet corn kernels cultivated by different treatments, was reported in Table 3. Control samples (NPK fertilizer) exhibited higher total soluble sugar content than those treated by different concentration of compost teas. The concentration of the total soluble sugar changed in function of the treatment employed during the cultivation of the plant. In fact, the sweet corn samples treated by mineral (NPK) fertilizer treatment gave the highest mean value of total soluble sugar content (33.13 mg g^{-1}), followed by 1 kg of compost per 10 L of water compost tea treatment (33.10 mg g^{-1}), then 1 kg of compost per 20 L of water compost tea treatment (31.72 mg g^{-1}) and 1 kg of compost per 30 L of water compost tea treatment gave the lowest value of total soluble sugar content (29.88 mg g^{-1}). Yet, the effects of 1 kg of compost per 10 L of water compost tea treatment and mineral (NPK) fertilizer treatment were the same ($p > 0.05$) while that of 1 kg of compost per 20 L of water compost tea treatment and 1 kg of compost per 30 L of water compost tea treatment were significantly ($p \leq 0.05$) different. The results indicated that application of mineral fertilizers as well as 1 kg of compost per 10 L of water compost tea treatment increased kernel compositions of total soluble sugars compared to other treatments. This result may be attributed to the positive role of mineral fertilizers and enriched compost tea in improving vegetable growth and nutritive status of sweet corn plants, which led to increase photosynthesis products that translocation to corn kernels. These results are in harmony with those reveled by Abou-El-Hassan and El-Batran (2020).

Total carotenoids

Statistical analysis carried out on total carotenoids of sweet corn samples cultivated using different type of fertilizer treatment, highlighted that all the samples showed different values with significance differences ($P \leq 0.05$) among them. With regard to total carotenoids of sweet corn sample as affected by different sources of nutrient (organic or inorganic), it noticed that, total carotenoids content of sweet corn increased significantly ($P \leq 0.05$) with the concentration of nutrient. Where, mineral (NPK) fertilizer treatment gave the highest mean value of total carotenoids (449.82 µg g^{-1}) followed by 1 kg of compost per 10 L of water compost tea treatment (447.58 µg g^{-1}), then 1 kg of compost per 20 L of water compost tea treatment (360.97 µg g^{-1}) and 1 kg of compost per 30 L of water compost tea treatment gave the lowest mean value (330.18 µg g^{-1}). These results are in line with those reported by More et al. (2018). Carotenoids concentration is influenced by various factors like species/variety, stage of maturity, part of the plant consumed, cultivar, cooking preparation, time of harvesting (Zhang et al., 2023).

pH value

Concerning the effect of organic and inorganic sources of nutrients on the pH value of sweet corn as were illustrated in Table 3, the pH values generally increased with decrease in nutrients concentration. The Analysis of Variance (ANOVA) showed that fertilizer treatments had significant effects ($p \leq 0.05$) on the pH values of sweet corn kernels produced. The Fisher's Least Significant Difference (FLSD) test indicates that 1 kg of compost per 30 L of water compost tea treatment had the highest mean pH value (6.61) followed by 1 kg of compost per 20 L of water compost tea treatment (6.60), 1 kg of compost per 10 L of water compost tea treatment (6.55) and mineral (NPK) fertilizer treatment (6.54). Yet, the effects of 1 kg of compost per 10 L of water compost tea treatment and mineral (NPK) fertilizer treatment were the same ($p > 0.05$) and that of 1 kg of compost per 20 L of water compost tea treatment and 1 kg of compost per 30 L of water compost tea treatment were also the same ($p > 0.05$). These results are in agreements with the results documented by More et al. (2018).

Titratable acidity

With regard to titratable acidity of sweet corn sample as affected by different sources of nutrient (organic or inorganic), it noticed that the titratable acidity generally decreased with decrease in nutrients concentration. The Analysis of Variance (ANOVA) showed that fertilizer treatments had significant effects ($p \leq 0.05$) on the total titratable acidity of sweet corn kernels produced. The Fisher's Least Significant Difference (FLSD) test indicates that mineral (NPK) fertilizer treatment had the highest mean value of titratable acidity of 0.53%, followed by 1 kg of compost per 10 L of water compost tea treatment with titratable acidity of 0.51%, then 1 kg of compost per 20 L of water compost tea treatment with titratable acidity of 0.47% and 1 kg of compost per 30 L of water compost tea treatment with the lowest mean value of titratable acidity of 0.46%. Yet, the effects of mineral (NPK) fertilizer treatment and 1 kg of compost per 10 L of water compost tea treatment were significantly ($p \leq 0.05$) different while that of 1 kg of compost per 20 L of water compost tea treatment and 1 kg of compost per 30 L of water compost tea treatment were not significantly ($p > 0.05$) different.

Maturation index

Data shown in (Table 3) indicated that the maturation index of sweet corn kernels increased with decreasing concentration of the fertilizer treatments. Statistical analysis carried out on maturation index of sweet corn samples cultivated using different type of fertilizer treatment, highlighted that all the samples showed different values with significance differences ($P \leq 0.05$) among them. The Fisher's Least Significant Difference (FLSD) test indicates that 1 kg of compost per 30 L of water compost tea treatment had the highest mean maturation index (37.23) followed by 1 kg of compost per 20 L of water compost tea treatment (36.85), 1 kg of compost per 10 L of water compost tea treatment (34.57) and mineral (NPK) fertilizer treatment (33.36) and their effects were found to be significantly ($p \leq 0.05$) different.

Mineral composition

In our study (Table 3), the mineral composition of sweet corn showed higher concentrations of phosphorus and potassium; and low concentrations of calcium, zinc and iron. The low concentration of calcium, zinc and iron recorded in this study tallies with the findings of Prasanthi et al. (2017), who found that cereal are poor in these minerals. However, the observed differences in mineral composition in these products may be due to genetic factor and environmental factors like irrigation frequency, soil composition and fertilizer used (Ikram et al., 2010). In our study among the fertilizer treatments, it was found that the mineral contents of the sweet corn variety increased with the concentration of nutrient. The concentration of all the mineral contents evaluated changed in function of the treatment employed during the cultivation of the plant. The Analysis of Variance (ANOVA) showed that fertilizer treatments had significant effects ($p \leq 0.05$) on the mineral composition of sweet corn kernels produced. The Fisher's Least Significant Difference (FLSD) test indicates that the sweet corn samples treated by mineral (NPK) fertilizer treatment gave the highest mean values of phosphorus, potassium, calcium, zinc and iron, followed by 1 kg of compost per 10 L of water compost tea treatment, then 1 kg of compost per 20 L of water compost tea treatment and 1 kg of compost per 30 L of water compost tea treatment gave the lowest mean values of phosphorus, potassium, calcium, zinc and iron. However, the effects of all the fertilizer treatments on the phosphorus, potassium, calcium, zinc and iron contents of sweet corn kernels were found to be significantly ($p \leq 0.05$) different, except the iron contents of mineral (NPK) fertilizer treatment and 1 kg of compost per 10 L of water compost tea treatment which were found to be the same ($p > 0.05$). This finding showed that sweet corn is a good source of these essential minerals, particularly for the iron and zinc which are of

public health significance and this further enhanced the nutritional values of the sweet corn hence, its utilization in the production of various corn products or any other cereal-based meal products would not have any detrimental effects on the consumers.

Effects of compost tea on sensory (organoleptic) properties of sweet corn kernels

The organoleptic characteristic is of great importance from the point of food material acceptability by the consumer. The sensory characteristics together with the assessors' ratings are shown in Table 4. The Analysis of Variance (ANOVA) showed that fertilizer treatments had significant effects ($p \leq 0.05$) on the sensory characteristics of sweet corn kernels produced. The Fisher's Least Significant Difference (FLSD) test indicates that the means of scores for all quality attributes, overall acceptability and quality index of all samples were significantly different ($p \leq 0.05$). The degree of Sweetness, Tenderness, Juiciness, overall acceptability and quality index were generally found to be significantly higher in the treatment of 1 kg of compost per 10 L of water compost tea followed by treatment of 1 kg of compost per 20 L of water compost tea, then treatment of 1 kg of compost per 30 L of water compost tea treatment and lastly, treatment of mineral (NPK) fertilizer.

Table 3. Effects of compost tea on chemical properties of sweet corn kernels.

Chemical properties	Treatment				P-Value	F-LSD $_{0.05}$
	500 kg ha^{-1} NPK Fertilizer	1kg 10L^{-1} Compost tea	1kg 20L^{-1} Compost tea	1kg 30L^{-1} Compost tea		
Total Soluble Solids (%)	17.68^{a}	17.63^{a}	17.32^{b}	17.12^{c}	0.000	0.0503
Total Soluble Sugar (mg g^{-1})	33.13^{a}	33.10^{a}	31.72^{b}	29.88^{c}	0.000	0.0818
Total Carotenoids (µg g^{-1})	449.82^{a}	447.58^{b}	360.97^{c}	330.18^{d}	0.000	0.0000
pH Value	6.54^{b}	6.55^{b}	6.60^{a}	6.61^{a}	0.000	0.0252
Titratable Acidity (%)	0.53^{a}	0.51^{b}	0.47^{c}	0.46^{c}	0.000	0.0189
Maturation Index	33.36^{d}	34.57^{c}	36.85^{b}	37.23^{a}	0.000	0.0377
Iron (mg 100g^{-1})	0.62^{a}	0.61^{a}	0.55^{b}	0.52^{c}	0.000	0.0126
Potassium (mg 100g^{-1})	298.53^{a}	296.87^{b}	281.55^{c}	278.02^{d}	0.000	0.0000
Calcium (mg 100g^{-1})	3.87^{a}	3.80^{b}	2.41^{c}	2.01^{d}	0.000	0.0129
Phosphorus (mg 100g^{-1})	121.71^{a}	119.49^{b}	97.93^{c}	89.76^{d}	0.000	0.0000
Zinc (mg 100g^{-1})	0.80^{a}	0.79^{b}	0.60^{c}	0.45^{d}	0.000	0.0064

Row means with the same letters are not significantly different at 5% level.

Table 4. Effects of compost tea on sensory (organoleptic) properties of sweet corn kernels.

Sensory (Organoleptic) properties	Treatment				P-Value	F-LSD $_{0.05}$
	500 kg ha^{-1} NPK Fertilizer	1kg 10L^{-1} Compost tea	1kg 20L^{-1} Compost tea	1kg 30L^{-1} Compost tea		
Perceived Sweetness Scores	7.17^{d}	9.00^{a}	8.02^{b}	7.48^{c}	0.000	0.0129
Perceived Tenderness Scores	7.38^{c}	8.46^{a}	7.60^{b}	7.34^{d}	0.000	0.0069
Perceived Juiciness Scores	8.15^{d}	8.53^{a}	8.40^{b}	8.22^{c}	0.000	0.0126
Overall Acceptability Scores	7.50^{c}	9.00^{a}	7.59^{b}	7.33^{d}	0.000	0.0630
Quality Index (QI)	30.20^{d}	34.99^{a}	31.61^{b}	30.37^{c}	0.000	0.1261

Row means with the same letters are not significantly different at 5% level.

CONCLUSION

The number of kernels per ear is one of the most important physical parameters for producing sweet corn suitable for canning. The 1 kg of compost per 10 L of water compost tea and mineral (NPK) fertilizer treatments gave the best ear characteristics compared to other treatments. Water and sugar contents, reported as the main attributes of the sweet corn quality for processing, also represent important parameters to be considered in selecting variety to avoid Mallard reaction and loss in kernel tenderness. The 1 kg of compost per 10 L of water compost tea and mineral (NPK) fertilizer treatments exhibited the higher content of total soluble sugars compared to other treatments. With regards to analysis of the sensory characteristics of sweet corn, the 1 kg of compost per 10 L of water compost tea treatment specifically, provided improvement for Quality Index which represented the overall sensory score of the sweet corn and thus higher preferences by the consumers. The data obtained in this study allowed presuming that the 1 kg of compost per 10 L of water compost tea should be used as a valid and promising alternative to the use of chemical stimulants in sweet corn cropping systems. It could be concluded possibility utilizing 1 kg of compost per 10 L of water compost tea to produce a good yield and quality of sweet corn without mineral fertilizers. Nevertheless, other studies need to be carried out over several years in order to assess the behavior of studied cultivars in longer terms for canning processing. It would be relevant to consider the edaphic characteristics, in particular by advanced physical and chemical analyses of the soil, for a better adaptation to amounts of fertilization. Further tests need to be conducted in order to collect data on post-harvest storage and suitability for sweet corn production as well as canning processing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgments

This study was carried out with the support of the Tertiary Education Trust Fund (TETFund), Institutional Based Research (IBR) Project Grant, 2023.

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JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH
2024, VOL. 7(2), 171-182

Journal of Horticulture and Postharvest Research

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

University of Birjand

Effect of biofertilizer inoculation on the growth and physiological traits of Red Angel and Wonderful pomegranate plantlets under salinity stress

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ARTICLE INFO

Original Article

Article history:
Received 13 January 2024
Revised 17 February 2024
Accepted 6 April 2024

Keywords:
Cell membrane injury
Glomus mosseae
Pseudomonas fluorescens
Relative water content
Sodium chloride

DOI: 10.22077/jhpr.2024.7168.1356
P-ISSN: 2588-4883
E-ISSN: 2588-6169

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ABSTRACT

Purpose: The study aimed to explore the effects of biofertilizer inoculation on the growth and morphophysiological traits of Red Angel and Wonderful pomegranate cultivars under salinity stress. **Research method**: The experiment utilized a factorial design based on a completely randomized design with four replications to assess the effects of salinity stress at three levels (control, 4, and 8 dS/m) and biofertilizer at four levels (control, *Pseudomonas fluorescens*, *Glomus mosseae*, and *P. fluorescens* + *G. mosseae*) on pomegranate plantlets. **Findings**: The results showed that the highest percentage of symbiosis was observed in *P. fluorescens* + *G. mosseae*, with 89.16% and 90.55% in the Red Angel and Wonderful cultivars, respectively. Salinity did not have any influence on the percentage of symbiosis in both cultivars. Furthermore, the application of biofertilizers increased the stem diameter, number of lateral branches, total number of leaves, leaf fresh and dry weight, root diameter, number of lateral roots, and relative water content of leaves in both cultivars. Additionally, all biofertilizers reduced cell membrane injury at all salinity levels by approximately 40%. Salinity decreased the leaf fresh and dry weight, root fresh and dry weight, and number of lateral roots, while increasing cell membrane injury in both cultivars. **Research limitations**: No limitations were identified. **Originality/Value**: The results highlight the potential of biofertilizers in mitigating the adverse effects of salinity stress on pomegranates, particularly when *P. fluorescens* and *G. mosseae* are combined.

INTRODUCTION

Pomegranate (*Punica granatum* L.), a member of the Lythraceae family and native to Iran and North Africa, thrives in dry, semi-tropical, and Mediterranean climates (Feyzi et al., 2018; Sarkhosh et al., 2020). The global cultivation of pomegranate spans an area of over 500 thousand hectares, yielding more than six and a half million tons of various cultivars annually (FAO, 2021).

Salinity affects more than 80% of the world's land, with saline areas on the rise due to improper irrigation water management, climate change, reduced annual rainfall, increased evaporation, and the use of inappropriate irrigation systems (Zheng et al., 2009). This poses a significant challenge in agriculture, impacting plant growth and development. Salinity stress causes structural changes in plant organs, reduces chlorophyll and photosynthesis, and diminishes plant growth and efficiency (Keshavarzi et al., 2022; Poury et al., 2023). It also imposes nutritional restrictions, reduces water absorption, and disrupts ion balance, leading to decreased plant production (Anahita et al., 2015).

In pomegranate trees, salinity can result in reduced fruit formation and growth, an increase in incomplete flowers, reduced branch growth, and fruit drop (Fattahi et al., 2021). High salinity can reduce pomegranate fruit size, weight, and quality, leading to issues like cracking, browning, and sunburn (Tavousi et al., 2016; Momenpour et al., 2022). Soori et al. (2019) studied the effect of different sodium chloride concentrations in irrigation water on the growth indices and physiological characteristics of selected pomegranate cultivars. The research revealed that as salinity increased, plant height, leaf size, fresh and dry leaf weight, and roots decreased, while the percentage of leaf necrosis increased. The findings indicated that the studied cultivars exhibited acceptable tolerance to salinity up to an electrical conductivity of 6.21 dS/m. Ibrahim (2016) evaluated and compared the salinity resistance of two pomegranate cultivars, Wonderful and Manfalouty, under hydroponic cultivation conditions. The research demonstrated that under salt stress conditions, the Wonderful cultivar displayed higher chlorophyll content, branch length, and growth ratio compared to the Manfalouty cultivar.

The growth-promoting rhizosphere bacteria, including *Azetobacter*, *Azospirillum*, and *Pseudomonas*, are vital soil microorganisms that stimulate plant growth through direct and indirect mechanisms (Azarmi-Atajan & Sayyari-Zohan, 2022). *P. fluorescens* bacteria, in particular, optimize cell processes, produce a wide range of plant growth regulators, organic acids, and exopolysaccharides, and facilitate the absorption of essential nutrients, demonstrating adaptation and resistance for survival and growth in saline conditions (Saleem et al., 2012).

Symbiotic fungi offer an effective method for enhancing resistance to adverse environmental conditions, particularly salt stress, by increasing seedling survival, growth, and resistance to abiotic stresses such as salinity (Seifi et al., 2014). Arbuscular mycorrhizal (AM) fungi play a significant role in improving plant growth under saline conditions by reducing the adverse effects of salinity stress on plants through various physical, nutritional, physiological, and cellular effects. Pomegranate has been shown to be a suitable host for mycorrhizal fungi, with their use resulting in increased plant establishment, improved shoot and root growth, enhanced chlorophyll production in pomegranate tissue culture, and increased resistance to adverse moisture conditions and osmotic stress (Bompadre et al., 2015). Inoculation with AM fungi has been found to enhance the growth of pomegranate trees and improve their vegetative and nutritional characteristics in saline conditions (Parvin et al., 2017).

The use of biological stimulants is an effective strategy for mitigating the adverse effects of salinity stress on plants. AM fungi improving the nutritional, physiological, and morphological conditions of the host plant and enhance the plant's resistance to salt stress through symbiosis and various other mechanisms (Evelin et al., 2019). Similarly, growth-stimulating bacteria in symbiosis with the root environment can enhance the physical conditions of the rhizosphere, modify the selectivity of sodium and potassium, produce 1-aminocyclopropane-1-carboxylate (ACC) deaminase, and employ specialized mechanisms to directly or indirectly improve plant growth and development (Dominguez-Nunez et al., 2013). However, there is a lack of comprehensive research on the effect of the symbiotic relationship of AM fungi and plant growth-promoting bacteria (PGPB) on pomegranate cultivars under salinity stress conditions. Therefore, the aim of this experiment was to assess the influence of a PGPB (*Pseudomonas fluorescens*) and an AM fungi (*Glomus mosseae*), as well as their combination, on some morphological and physicochemical traits in pomegranate cultivars Red Angel and Wonderful under salinity conditions. The Red Angel is a popular cultivar known for its distinctive flavor and beautiful appearance. The Wonderful cultivar is popular for its large, sweet, and tangy arils, as well as its high antioxidant content. Both cultivars are adaptable to various growing conditions (Hooks et al., 2021; Yan-hui et al., 2022).

MATERIALS AND METHODS

Plant materials and treatments

The research was conducted in 2021 at the Department of Horticulture Science, Gorgan University of Agricultural Sciences and Natural Resources in Gorgan, Iran. The experiment utilized a factorial design based on a completely randomized design with four replications and two pots for each replication (each pot had one rooted cutting). The first factor comprised three levels of irrigation water salinity: non-saline control, 2.5, and 6.4 g/l of NaCl, corresponding to EC values of 1.4, 4, and 8 dS/m, respectively. The second factor consisted of four levels of biofertilizer: non-inoculated control, *P. fluorescens*, *G. mosseae*, and *P. fluorescens* + *G. mosseae*. In January, one-year-old hardwood cuttings from Red Angel and Wonderful cultivars were prepared and rooted in 7 L pots filled with a 1:1 mixture of agricultural soil and sand. The potting mixture had the following composition: N (0.11 mg/kg), K (625 mg/kg), P (98 mg/kg), pH of 7.1, and EC of 1.1 dS/m. To ensure sterility, the prepared potting mixture was autoclaved at 121°C and 1.5 bar for one hour. The plantlets were cultivated in a controlled greenhouse environment with a relative humidity of 80 ± 5% and a daytime temperature of 28 ± 5°C.

The bacterium *P. fluorescens* was obtained from the Department of Soil Biology at the Soil and Water Research Institute in Tehran, Iran. A liquid inoculation suspension (75 ml) containing the bacteria were added to each pot (Aalipour et al., 2018). The *G. mosseae* AM fungi were sourced from Toran Biotechnology Company in Shahroud, Iran. The inoculum included rhizosphere soil, AM fungi spores (approximately 50-60 spores per gram of dry clay soil), as well as hyphae, arbuscules, and root segments from the host plant *Trifolium repens* L. Each pot was inoculated with 100 g of fungi powder mixed with 6 kg of the potting mixture.

Once the plantlets had established and achieved sufficient growth, salinity stress was gradually introduced over a 10-week period. To prevent sudden shock to the plantlets, the salt concentration in the irrigation water was gradually increased until it reached the final desired concentration (Hariadi et al., 2011). The pots were irrigated once a week, and the amount of water applied was adjusted based on changes in pot weight. Regular monitoring of substrate EC was conducted every week following irrigation to ensure effective management of soil

salinity, preventing excessive salt accumulation in the soil over time. At the end of the experiment, the EC of the potting mixture was measured.

Morphophysiological assessments

The quantification of symbiosis percentage involved staining fresh root segments three months after inoculation and examining them under a microscope to identify and quantify the presence of symbiotic structures, following the procedures outlined by Phillips and Hayman (1970). Stem and root diameters were measured in millimeters using a digital caliper (El-Khawaga & Yossef, 2013). The number of leaves, lateral branches, and lateral roots were recorded at the end of the experiment. After determining the fresh weight of leaves and roots, the leaf dry weight and roots were obtained by following the method of Ganjeali and Kafi (2007) after placing the plant samples at 70°C for 48 hours. The relative water content (RWC) of leaves was determined using the method described by Dhanda and Sethil (1998). Cell membrane injury was assessed based on the method outlined by Sairam et al. (1997). In this method, the conductivity of leaf samples was measured before and after autoclaving to evaluate cellular injury using the related equation.

Data analysis

The data was statistically analyzed using SAS (version 9.3), and mean comparisons were conducted using the Duncan's multiple range test. Prior to the analysis, the data underwent suitable transformations to meet the assumptions of normality and homogeneity of variance.

RESULTS AND DISCUSSION

The results of the study showed that the interaction effect of biofertilizers (*P. fluorescens*, *G. mosseae*, and *P. fluorescens* + *G. mosseae*) and salinity treatments on the percentage of symbiosis and the morphological traits, including stem diameter, the number of lateral branches, total number of leaves, fresh and leaf dry weight, root diameter, number of lateral roots, fresh and dry weight of roots in both the Red Angel and Wonderful cultivars were statistically non-significant. Consequently, the main effects of the biofertilizers and salinity treatments were assessed. However, the interaction effect of biofertilizer and salinity on RWC of leaves and cell membrane injury was found to be significant in both cultivars, and therefore, the interaction effects of the treatments were compared.

Table 1. The effect of biofertilizer and salinity on symbiosis, stem diameter and number of lateral branches in pomegranate plantlets of Red Angel and Wonderful cultivars.

Treatments	Symbiosis (%)		Stem diameter (mm)		Lateral branches (n)	
	Red Angel	Wonderful	Red Angel	Wonderful	Red Angel	Wonderful
Biofertilizer	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
Control	29.66±0.89^{d}	30.11±1.28^{d}	3.05±0.15^{c}	3.83±0.21^{d}	0.91±0.08^{d}	1.41±0.15^{d}
P. fluorescens	87.41±0.51^{b}	88.25±0.73^{b}	5.33±0.23^{b}	5.81±0.23^{b}	2.83±0.17^{b}	3.08±0.15^{b}
G. mosseae	80.00±0.98^{c}	80.35±1.20^{c}	5.33±0.19^{b}	5.66±0.22^{c}	2.25±0.13^{c}	2.83±0.21^{c}
P. fluorescens + *G. mosseae*	89.16±0.78^{a}	90.55±1.11^{a}	6.33±0.14^{a}	6.25±0.13^{a}	3.16±0.21^{a}	3.58±0.19^{a}
Salinity (dS/m)	P=0.156	P=0.431	P=0.144	P=0.594	P=0.131	P=0.371
Control	70.81±6.64	71.03±6.66	4.56±0.30	5.36±0.32	2.13±0.30	2.81±0.27
4	71.56±6.23	71.38±6.41	4.33±0.29	5.37±0.27	2.13±0.26	2.78±0.25
8	72.31±6.11	73.50±5.98	4.33±0.29	5.31±0.30	2.05±0.18	2.63±0.24

Different letters in each column represent significant differences at P=0.01. Mean values ± standard error.

Table 2. The effect of biofertilizer and salinity on number of leaves, leaf fresh weight, and leaf dry weight in pomegranate plantlets of Red Angel and Wonderful cultivars.

Treatments	Leaves (n)		Leaf fresh weight (g)		Leaf dry weight (g)	
	Red Angel	Wonderful	Red Angel	Wonderful	Red Angel	Wonderful
Biofertilizer	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
Control	110.41±1.31^{d}	115.91±2.01^{d}	0.48±0.02^{d}	0.59±0.02^{d}	0.23±0.01^{c}	0.27±0.01^{d}
P. fluorescens	177.58±1.73^{a}	183.98±2.17^{a}	0.58±0.02^{c}	0.68±0.02^{c}	0.33±0.01^{b}	0.38±0.01^{b}
G. mosseae	162.75±2.24^{c}	170.75±2.15^{c}	0.62±0.01^{b}	0.70±0.01^{b}	0.34±0.01^{a}	0.37±0.01^{c}
P. fluorescens + *G. mosseae*	173.66±1.55^{b}	180.50±2.31^{b}	0.67±0.01^{a}	0.77±0.01^{a}	0.34±0.01^{a}	0.39±0.01^{a}
Salinity (dS/m)	P<0.001	P=0.069	P=0.008	P=0.019	P<0.001	P<0.001
Control	159.56±6.92^{a}	162.75±6.92	0.63±0.02^{a}	0.65±0.02^{a}	0.32±0.01^{a}	0.36±0.01^{a}
4	157.00±7.42^{b}	160.06±7.53	0.56±0.02^{b}	0.64±0.02^{b}	0.31±0.01^{b}	0.34±0.01^{b}
8	151.75±6.84^{c}	159.25±7.39	0.55±0.02^{c}	0.62±0.02^{c}	0.29±0.01^{c}	0.34±0.01^{b}

Different letters in each column represent significant differences at P=0.01. Mean values ± standard error.

Table 3. The effect of biofertilizer and salinity on root diameter and number of lateral roots in pomegranate plantlets of Red Angel and Wonderful cultivars.

Treatments	Root diameter (mm)		Lateral roots (n)	
	Red Angel	Wonderful	Red Angel	Wonderful
Biofertilizer	P<0.001	P<0.001	P<0.001	P<0.001
Control	1.66±0.14^{c}	2.66±0.14^{d}	22.25±0.59^{d}	24.33±0.62^{d}
P. fluorescens	2.33±0.14^{b}	3.16±0.17^{c}	32.08±0.63^{b}	34.16±0.64^{b}
G. mosseae	2.33±0.28^{b}	3.33±0.28^{b}	28.50±0.76^{c}	33.66±0.81^{c}
P. fluorescens + *G. mosseae*	2.83±0.21^{a}	3.66±0.23^{a}	42.91±0.75^{a}	44.91±0.87^{a}
Salinity (dS/m)	P<0.001	P<0.001	P<0.001	P=0.003
Control	2.87±0.18^{a}	3.16±0.19^{a}	33.12±2.13^{a}	35.22±2.20^{a}
4	1.93±0.14^{c}	3.00±0.14^{b}	30.62±1.91^{b}	32.75±1.90^{b}
8	2.06±0.19^{b}	2.81±0.18^{b}	30.51±1.97^{b}	32.56±1.90^{b}

Different letters in each column represent significant differences at P=0.01. Mean values ± standard error.

The study revealed that the highest percentage of symbiosis was observed in *P. fluorescens* + *G. mosseae*, with 89.16% and 90.55% in the Red Angel and Wonderful cultivars, respectively (Table 1). These findings are consistent with previous research by Al-Khaliel (2010), who reported symbiosis with *Glomus* spp. fungi in most plants under various environmental stress conditions. Esna-Ashari and Bahrami (2018) also noted that the combination of fungi and bacteria led to increased nodulation and nutrient absorption under osmotic and salinity stress conditions. In both cultivars, salinity did not have any influence on the percentage of symbiosis. Notably, even in the absence of biofertilizer treatments, non-inoculated plants exhibited a certain degree of symbiosis (Table 1). This could be attributed to potential contamination from indigenous bacteria and AM species present in the irrigation water and greenhouse environment, a finding consistent with previous research (Aseri et al., 2008; Eftekhari et al., 2012). These findings underscore the adaptability of pomegranate cultivars to form symbiotic relationships with beneficial microorganisms, even under salinity stress. The consistent symbiosis percentages across salinity treatments highlight the complex interactions between pomegranate plants and microbial communities

The study's results also indicated an increase in stem diameter with the use of biofertilizer (Table 1). The highest stem diameter of the Red Angel and Wonderful cultivars was observed in *P. fluorescens* + *G. mosseae* (6.33 and 6.25 mm, respectively). These findings align with the research of Williams et al. (2010), who reported that inoculation with AM fungi under increased levels of salinity stress led to an increase in stem diameter due to enhanced production of hormones, cell division and elongation, water and nutrient absorption, cell

development, and photosynthesis. Additionally, the independent effect of salinity on stem diameter was statistically non-significant in both cultivars. This could be attributed to the specific tolerance of these cultivars to salinity stress. Pomegranate plants may have developed mechanisms to cope with moderate salinity levels without significant changes in stem diameter.

According to the research findings, the independent effect of biofertilizer on the number of lateral branches in both cultivars was found to be significant (Table 1). The application of biofertilizers led to an increase in the number of lateral branches in both cultivar (Table 3). The highest number of lateral branches in the Red Angel and Wonderful cultivars was observed in *P. fluorescens* + *G. mosseae* (3.16 and 3.58, respectively). These results are in line with the findings of Tadayon and Maafpourian (2018), who reported that biofertilizer and mycorrhiza inoculation under salinity stress conditions resulted in a 20.8% increase in the number of lateral branches of the studied genotypes. They also demonstrated that inoculation with *P. fluorescens* and *Azospirillum* spp increased the number of lateral branches of studied genotypes through nitrogen fixation and the production of growth regulators such as auxin. In contrast, the independent effect of salinity on the number of lateral branches of both cultivars was not found to be significant (Table 3). The lack of significant effect of salinity on the number of lateral branches in pomegranate cultivars could be attributed to the genetic resilience of these cultivars to salinity stress. Additionally, the experimental conditions, such as the duration of exposure to salinity or the specific growth stage of the plants during the study, could have influenced the results.

The effect of salinity on the total number of leaves was significant only in the Red Angel cultivar, with the lowest number of total leaves observed at 8 dS/m salinity (151.75) (Table 2). Salinity mainly influences the number of leaves through stimulating leaf abscission. Elevated salinity levels commonly lead to water stress in plants, prompting leaf abscission. Nevertheless, biofertilizers can improve the plant's ability to withstand stress and manage salinity-induced challenges. In contrast, the application of biofertilizers led to an increase in the total number of leaves in the Red Angel and Wonderful cultivars, with the highest total number of leaves observed in *P. fluorescens* (177.58 and 183.98, respectively). These results are consistent with the findings of Fattahi and Mohammadkhani (2019), who reported that the use of biofertilizer is an effective strategy in reducing salinity losses, and with the treatment of *G. mosseae* and *G. versiform*, the total number of leaves in citrus seedlings increased. Additionally, the results of this research align with the findings of Dominguez-Nunez et al. (2013), who reported that the combined use of *P. fluorescens*, *G. mosseae*, and *G. intraradices* led to an increase in the number of leaves in silver cedar genotypes through increased absorption of water and nutrients, synthesis of auxin, gibberellic acid, and cytokinin, and production of antioxidant enzymes.

With the application of biofertilizer, an increase in leaf fresh and dry weight was observed (Table 2), with the highest values in *P. fluorescens* + *G. mosseae*. Conversely, salinity led to a decrease in both leaf fresh and dry weight, with the lowest values observed at 8 dS/m salinity. In a study by Bahrani et al. (2020), it was noted that at the highest concentration of salinity, the leaf fresh weight decreased by 32% in grape studied genotypes due to a decrease in cell water content. Additionally, Wen-Bo et al. (2008) reported that inoculation with *G. mosseae* resulted in an increase in the leaf fresh and dry weight of iris due to enhanced absorption of water and nutrients by the roots and an increase in the root surface area with the hyphae of the fungi under salt stress conditions. The results are also consistent with the findings of Naseri et al. (2012), who demonstrated that the combination of *P. putida* bacteria and *Funneliformis mosseae* fungi increased the leaf fresh and dry weight of *Trigonella foenum-graceum* medicinal plant by 52% compared to the control treatment under osmotic stress conditions.

Furthermore, Mardhiah et al. (2016) reported that salinity led to a significant decrease in photosynthesis, biomass, and shoot growth, resulting in a decrease in leaf dry weight and shoots.

The results also revealed that the lowest root diameter in the Red Angel and Wonderful cultivars was observed at 8 dS/m salinity (2.06 and 2.81 mm, respectively) (Table 3). With the application of biofertilizer, the root diameter of both cultivars increased, with the largest root diameter observed in *P. fluorescens* + *G. mosseae* (2.83 and 3.66 mm, respectively). These findings align with the results of Khosrovjerdi et al. (2013), who reported that application of *Sinorhizobium meliloti* × *Azotobacter chrococcum* × *Azospirillum lipoferum* × *P. fluorescens* and the AM fungi of *G. mosseae* and *F. mosseae*, individually and in a binary combination, led to an increase in the root diameter of chickpeas under salinity condition. The synergistic relationship between AM fungi and bacteria stimulates the growth of nitrogen fixers and the production of plant hormones such as auxin, cytokinin, and gibberellin, resulting in an increase in root diameter and the number of lateral roots.

The increase in salinity resulted in a decrease in the number of lateral roots (Table 3). The lowest number of lateral roots in the Red Angel and Wonderful cultivars was observed at 8 dS/m salinity (30.51 and 32.56, respectively). However, the application of biofertilizer increased the number of lateral roots, with the highest numbers observed in *P. fluorescens* + *G. mosseae* (42.91 and 44.91, respectively). These findings are consistent with the results of Naseri et al. (2012), who demonstrated that under osmotic stress conditions, treatment with *F. mosseae* increased biomass and the number of lateral roots by enhancing the absorption of nutrients from the soil solution. They also concluded that the number of lateral roots is highly dependent on the growth environment, as salt stress reduces the water content of cells and makes their elongation difficult. Furthermore, they reported that the combined treatment of *F. mosseae* and *P. putida* increased root length and the number of lateral roots through the expansion of the root system and soil exploration by external hyphae in the hairy roots and the root surface. Lateral roots play a crucial role in water and nutrient uptake, anchoring plants in the soil, and providing stability to the plant. Boosting root diameter and lateral roots with biofertilizers can enhance nutrient absorption, water uptake, and plant resilience. This leads to higher yields, better stress tolerance, and improved overall plant performance, benefiting agricultural sustainability.

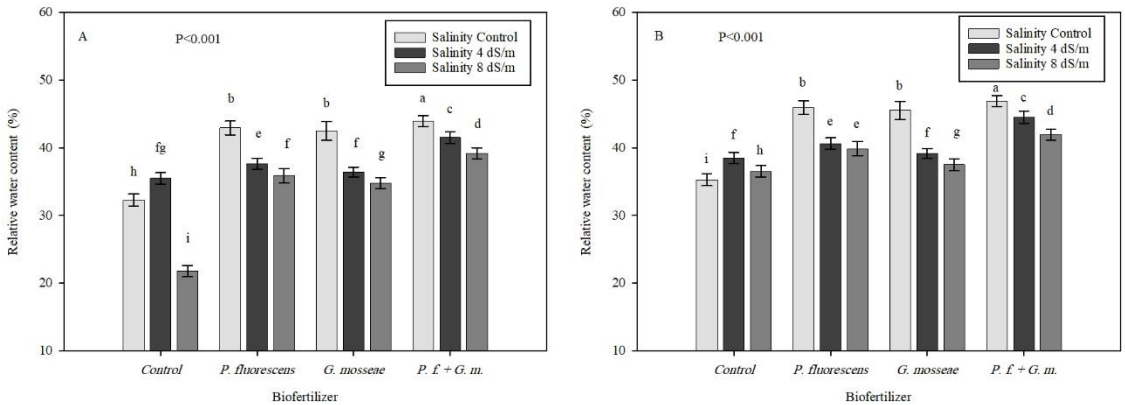

Fig. 1. The interaction effect of biofertilizer and salinity on relative water content of leaves in pomegranate plantlets of Red Angel (A) and Wonderful (B) cultivars. Different letters represent significant differences at P=0.01. Error bars indicate ± standard error.

Table 4. The effect of biofertilizer and salinity on root fresh and dry weight in pomegranate plantlets of Red Angel and Wonderful cultivars.

Treatments	Root fresh weight (g)		Root dry weight (g)	
	Red Angel	Wonderful	Red Angel	Wonderful
Biofertilizer	P<0.001	P<0.001	P<0.001	P<0.001
Control	39.20±0.90^{d}	41.20±0.90^{d}	18.61±0.61^{c}	19.27±0.64^{d}
P. fluorescens	44.33±0.63^{c}	46.24±0.67^{c}	23.55±0.42^{b}	24.33±0.43^{c}
G. mosseae	45.30±0.63^{b}	47.30±0.63^{b}	23.49±0.51^{b}	25.83±0.51^{a}
P. fluorescens + *G. mosseae*	46.03±0.50^{a}	48.03±0.50^{a}	24.89±0.60^{a}	25.41±0.64^{b}
Salinity (dS/m)	P<0.001	P<0.001	P<0.001	P<0.001
Control	45.59±0.54^{a}	47.59±0.54^{a}	24.26±0.62^{a}	24.86±0.64^{a}
4	43.71±0.98^{b}	45.71±0.98^{b}	22.26±0.73^{b}	23.30±0.73^{b}
8	41.84±0.85^{c}	43.78±0.86^{c}	21.01±0.68^{c}	21.70±0.72^{c}

Different letters in each column represent significant differences at P=0.01. Mean values ± standard error.

The increase in salinity resulted in a significant decrease in the root fresh and dry weight in both cultivars (Table 4), with the lowest weights observed at 8 dS/m salinity. However, with the application of biofertilizer, particularly *P. fluorescens* + *G. mosseae*, the root fresh and dry weight of both cultivars increased. These findings are consistent with the results of Rydlová et al. (2016), who demonstrated that inoculation with AM fungi increased root fresh and dry weight under salinity conditions. Additionally, Soori et al. (2019) reported a 57% decrease in the fresh root weight of the Shahwar pomegranate cultivar due to the decrease in cellular water content and root expansion area with an increase in salinity. The results are also in line with the findings of Cruz and Husain (2008), who concluded that AM fungi increased the root fresh and dry weight of many plants due to the effect of mycorrhiza on the absorption of nutrients such as nitrogen, phosphorus, and potassium. Furthermore, Esna-Ashari and Bahrami (2018) reported that inoculation with AM fungi, including *G. hoi*, *G. intraradices*, and *G. mosseae*, increased the root fresh and dry weight of *Poncirus trifoliata* seedlings under salinity conditions. Bahrani et al. (2020) also demonstrated a decrease in the root dry weight of Chafteh grape cultivar with an increase in osmotic stress caused by salinity.

The application of biofertilizers increased the RWC of leaves at all salinity levels (Fig. 1). The highest RWC of leaves in the Red Angel and Wonderful cultivars was observed in *P. fluorescens* + *G. mosseae* at the non-saline control (43.9% and 46.9%, respectively). However, the lowest RWC in the Red Angel cultivars was observed at 8 dS/m salinity and non-inoculated control (21.79%), while in the Wonderful cultivar, the lowest RWC of leaves was observed at zero salinity and non-inoculated control (35.29%). Increasing the RWC through biofertilizer application is practically significant as it indicates improved water status in plants. This enhancement in water retention can contribute to enhanced salinity tolerance by helping plants maintain proper hydration levels even under saline conditions. These results are consistent with the findings of Kumar et al. (2015), who demonstrated a 26% increase in the RWC in pomegranate genotypes under salinity conditions with treatment using AM fungi. Similarly, Ghasemi and Zahedi (2018) showed an increase in the RWC in all sorghum genotypes under salinity stress as a result of inoculation with mycorrhiza. However, they noted that mycorrhiza did not have a significant effect on the RWC in some genotypes under non-saline conditions. Additionally, Alipour et al. (2019) reported an increase in the RWC in *Cupressus arizonica* as a result of the combination of *P. fluorescens*, *G. mosseae*, and *G. intraradices*, attributed to the increase in root length and changes in root morphology for water search. The observed increase in RWC suggests improved water uptake and retention, which may contribute to enhanced salinity tolerance and overall plant resilience.

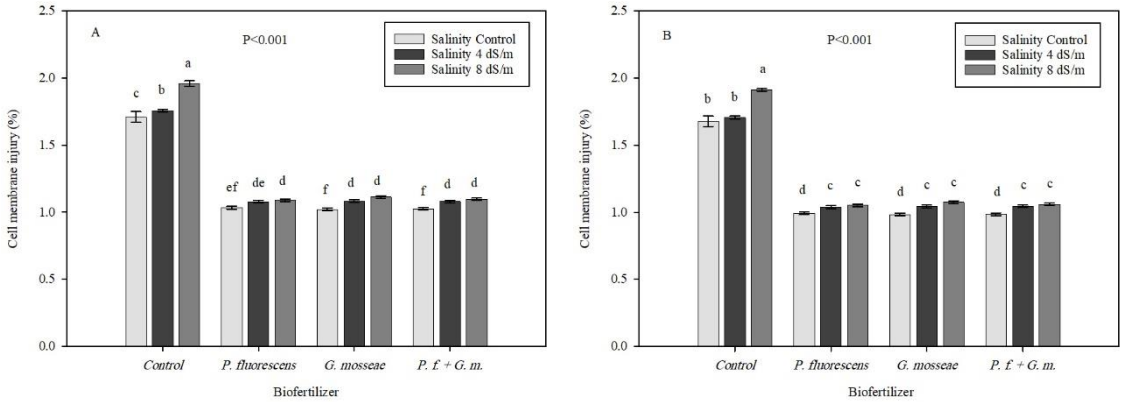

Fig. 2. The interaction effect of biofertilizer and salinity on cell membrane injury in pomegranate plantlets of Red Angel (A) and Wonderful (B) cultivars. Different letters represent significant differences at P=0.01. Error bars indicate ± standard error.

At all salinity levels, the application of biofertilizer resulted in a decrease in cell membrane injury (Fig. 2). The highest injury in the Red Angel and Wonderful cultivars was observed in the non-inoculated control at 8 dS/m salinity (1.96% and 1.91%, respectively), followed by the non-inoculated control at 4 dS/m salinity. These results are consistent with the findings of Ziaei et al. (2020), who reported that the use of AM fungi increased the membrane stability index and decreased cell membrane injury by 16% in all studied iris genotypes under salt stress. Additionally, the results align with the findings of Shirinzadeh et al. (2021), who reported that inoculation with *F. mosseae*, *F. caledonius*, *Rhizophagus intraradices*, and *R. iranicus* prevented the production of free radicals and cell membrane peroxidation, thereby preserving cell membrane proteins and enzymes and decreasing cell membrane injury in pear genotypes under salinity conditions. These results emphasize the function of biofertilizers in maintaining membrane integrity during periods of stress. Biofertilizers can enhance the plant's stress tolerance by promoting the synthesis of protective compounds such as antioxidants and osmoprotectants. These compounds help to stabilize cell membranes and protect them from damage caused by salinity stress.

CONCLUSION

The study revealed that the symbiosis percentage was highest in the Red Angel and Wonderful cultivars when using *P. fluorescens* + *G. mosseae* biofertilizer, and salinity did not affect this percentage in either cultivar. All studied biofertilizers contributed to increased stem and root diameter, number of lateral branches, roots, and leaves, as well as leaf and root fresh and dry weight, and relative water content in both cultivars. Additionally, biofertilizers reduced cell membrane injury at all salinity levels. These findings highlight the complex interplay between biofertilizer application and pomegranate physiological responses under salinity stress. Overall, the research results demonstrated that the applied biofertilizers, particularly *P. fluorescens* + *G. mosseae*, had positive effects in mitigating the harmful influences of salinity in both cultivars. Conducting a comparative analysis of different biofertilizers and their effects on pomegranate tree growth and development under varying abiotic stresses would provide valuable insights and contribute to the existing body of knowledge.

Conflict of interest

The authors declare that they have no conflict of interest to report.

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JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH
2024, VOL. 7(2), 183-196

Journal of Horticulture and Postharvest Research

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

University of Birjand

Influence of hydrogel composite applications and irrigation intervals on the yield and fruit quality of Valencia orange (*Citrus sinensis* L.) trees under semi-arid conditions

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ARTICLE INFO

Original Article

Article history:
Received 29 January 2024
Revised 7 April 2024
Accepted 8 April 2024

Keywords:
Citrus trees
Fruit characteristics
Polymer
Productivity
Tree canopy

DOI: 10.22077/jhpr.2024.7222.1362
P-ISSN: 2588-4883
E-ISSN: 2588-6169

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ABSTRACT

Purpose: Under fluctuations in climatic conditions, sustaining production with excellent fruit quality is the main objective of citrus producers in the arid regions. This experiment was conducted on twelve-year-old Valencia orange trees (*Citrus sinensis* L) budded on Volkamer lemon rootstock (*Citrus volkameriana*), cultivated in sandy soil in the Eastern Desert of Egypt. **Research method:** This work studies the influence of hydrogel and irrigation intervals on the growth, yield, and fruit quality of Valencia orange trees. The experiment consists of four levels of hydrogel (0, 750, 1000, and 1250 g/tree) with three irrigation intervals (daily, day-by-day, and every 2 days) during three seasons (2020-2022). **Findings:** All applications affected tree canopy volume, shoot length, leaf number, yield (kg/tree), and the physical and chemical fruit characteristics. While, the application of 1000 g/tree hydrogel and every two-day irrigation interval produced the highest values when compared to other treatments during the experimental seasons. With respect to yield and fruit characteristics, treatment of 1250 g/tree hydrogel with irrigation day-by-day resulted in the highest tree yield (113.58 kg/tree) and total yield (18.74 tons/feddan) and improved various physical and chemical fruit characteristics. **Research limitations:** There was no limitation. **Originality/Value:** Hydrogel applications mitigated the impact of prolonging irrigation intervals on the vegetative growth, productivity, and fruit quality of the Valencia orange trees compared to untreated trees.

INTRODUCTION

Rising temperatures and water shortages are the main abiotic threats facing the agricultural sector in arid and semi-arid regions. Under these conditions, there is a greater need to reserve water consumption in the agricultural sector and increase water use efficiency for the future expansion of agriculture in the water-scarce arid region (Karandish et al., 2015). There is more interest in improving practice management that reserves water, i.e., adapting irrigation intervals, using advanced irrigation methods, and using hydrogel substances to increase soil water retention, which could save huge amounts of water and sustain agricultural production, particularly in fruit orchards (Rabbani & Kazemi, 2022).

Egypt, which is located in an arid region with limited water resources, is experiencing water scarcity conditions along with rising temperatures, which increase evapotranspiration in sandy soil. This crisis is considered one of the determining factors for the agricultural sector, especially fruit cultivation in desert areas (Gado & El-Agha, 2021).

Under such conditions, proper practice management, such as using polymeric substances and controlling irrigation quantity, must be used to reduce water loss in sandy soils in order to sustain citrus production and increase the productivity of the available water (Malik et al., 2022; Abd El-Aziz et al., 2020).

Deficit irrigation is a strategy that is more efficient in reducing water consumption than the water requirements of the crop without harming plant productivity, depending on the amount of water reduction, variety, and growth stage (Solanki et al., 2021; Abdelraouf et al., 2020). Using different irrigation intervals is considered a modified technique of deficit irrigation that aims to save water without having a harmful effect on tree growth and productivity (Galindo et al., 2018).

Citrus is an evergreen tree growing in a warm climate that requires a continuous water supply throughout the year. Under slight water stress, trees undergo physiological responses to adapt to water shortages and complete the growth season satisfactorily (Consoli et al., 2017). Therefore, in arid and semi-arid regions, the supply of adequate irrigation water is a determined factor for the economic production of citrus under these conditions (Abou Ali et al., 2023).

In citrus orchards, water shortages diminish vegetative growth, reduce yield, and produce poor fruit quality, causing significant economic losses. Sustaining citriculture with superb fruit quality is the main target of citrus growers worldwide, particularly in arid conditions such as the Mediterranean climate, which suffers from rainfall reduction and increases evapotranspiration.

Citrus is considered the most important fruit in Egypt and is regarded as a key pillar of the agricultural economy. Given the cultivated area, which reaches 519,788 Feddan, its annual productivity (about 4.7 million tons) represents 36.2% of total fruit production and its commercial value in both domestic and international markets, whereas citrus fruits occupy the first position in fruit export with a total of approximately 1,8 million tons in 2022. Moreover, orange exports accounted for about 25.07% of the total citrus production. Valencia orange occupies the second position as the most cultivated citrus variety in Egypt, with a fruitful area reaching 125,152 Feddan and producing 1,34 million tons (Annual Reports, 2022).

In arid and semi-arid areas, using hydrogel substances as soil applications improves soil-holding capacity and reserves water for a longer time, which could increase water intervals and enhance water productivity by reducing runoff (Alshallash et al., 2022). Furthermore, hydrogel retains nutrients and reduces leaching with drainage water, consequently enhancing the growth and productivity of fruit crops in sandy soil (Pattanaaik et al., 2015). Therefore, using hopeful practices such as control of irrigation intervals and hydrogel ingredients to

maintain citrus productivity is necessary to sustain citriculture in such regions (AbdEl-Aziz et al., 2020).

This investigation was conducted during the 2020-2022 seasons to study the effect of combinations of irrigation intervals and rates of hydrogel composite on vegetative growth, productivity, and fruit quality of Valencia orange trees grown under arid region conditions.

MATERIALS AND METHODS

In Egyptian conditions with limited water resources, managing irrigation intervals could be a modified strategy to control water productivity without significant crop reduction.

This research was conducted in a private orchard located at Wadi-Almollak region of Ismailia Governorate, Egypt to assess the impact of two irrigation intervals and three rates of hydrogel composite on the growth and productivity of Valencia orange trees (*Citrus sinensis* L.) budded on Volkamer lemon (*Citrus volkameriana*) rootstock. Trees were planted at 5 × 5 m apart, for 165 trees/Feddan. The study was conducted for three consecutive years (2020, 2021, and 2022). Soil analysis, according to (Wild et al., 1979), was carried out at the department of soil sciences, faculty of Agriculture, Al-Azhar University (Table 1).

The quantity of hydrogel composite for each replicate was mixed with (1 kg) of fine sand and added under irrigation lines of 30 cm depth during mid-January each season. Trees were watered by a drip irrigation system with two adjustable emitters/trees (8 litter.h^{-1}) through two irrigation lines. Other agricultural practices were according to the recommendations of the Egyptian Ministry of Agriculture and Land Reclamation. This field experiment aimed to evaluate the impact of hydrogel polymers with irrigation intervals on the growth and productivity of Valencia orange trees under arid regional conditions.

Twenty-one trees were selected and grouped into seven treatments; each treatment was represented by three replicates (tree/each).

Table 1. Chemical and mechanical analysis of the experimental soil.

Chemical analysis

Date	pH	EC	Soluble cations (meq / L)				Soluble anions (meq / L)			Macro elements (ppm)			Microelements (ppm)			
			Ca^{+2}	Mg^{+2}	Na^{+1}	k^{+1}	Cl^{-1}	HCO_3^-	SO_4^{-2}	N	P	K	Fe	Mn	Zn	Cu
January 2020	7.90	1.93	3.8	1.8	1.45	0.27	6.3	1.6	3.97	980	0.66	200.5	3.6	2.7	7.8	22.74
October 2022	7.98	1.88	3.65	1.9	1.24	0.29	0.7	2.44	4.44	1204	50.77	7927	7.2	3.2	8.0	24.3

Mechanical analysis

Date	Rough sand (%)	Fine sand (%)	Silt (%)	Clay (%)	Soil density
January 2020	33.5	36.5	18.4	11.6	1.53
October 2022	33.8	36.8	17.3	12.1	1.55

Treatments

T1: Control (Irrigation daily without hydrogel composite).
T2: Irrigation day-by-day (I1) + hydrogel composite (750 g /tree) (HC1).
T3: Irrigation every 2 days (I2) + hydrogel composite (750 g /tree) (HC1).
T4: Irrigation day-by-day (I1) + hydrogel composite (1000g/tree) (HC2).
T5: Irrigation every 2 days (I2) + hydrogel composite (1000 g /tree) (HC2).
T6: Irrigation day-by-day (I1) + hydrogel composite (1250 g /tree) (HC3).
T7: Irrigation every 2 days (I2) + hydrogel composite (1250 g /tree) (HC3).

The response of trees to the effects of the soil application of hydrogel and irrigation intervals was studied by comparing changes in growth, yield, and chemical agents. The following parameters were determined.

Vegetative growth parameters

Tree canopy volume (m^3) was calculated according to Zekri (2000) by the formula (1):

$$TV = 0.5236 \times HD^2 \quad (1)$$

Where H = tree height and D = tree diameter.

Shoot length (cm), **e**ach season, in the spring, four main branches similar in length and diameter were chosen, one in each direction of each replicate was labeled for measuring shoot length from the 1st of March to the 1st of November during each season. At the end of spring cycle of each season, leaves number/ shoot was counted, then, ten leaves were calculated from tagged shoots to determine leaf area (cm^2) by using the equation of Chou (1966) (2)

$$\text{Leaf space} = 2/3 \text{ (length} \times \text{width)} \quad (2)$$

Four branches (one-year-old) similar in growth were chosen, one branch in each original direction, and twelve shoots per main branch were tagged at the balloon stage of the flower each seasons. At blooming, all opened flowers/shoot were counted. At the end of fruit set, the number of fruitlets was recorded, and the fruit set percentage was calculated according to the following equation (3):

$$\text{Fruit set (\%)} = \text{Total fruit number/ Total flowers number} \times 100 \quad (3)$$

Nutritional status

Ten leaves were taken from non-fruiting shoots on the outer canopy, washed with distilled water, dried at 70°C, and then digested according to (Wolf, 1982) to determine leaf mineral content.

Total nitrogen was determined by the semi-micro Kjeldahl methods (Bremner & Mulvaney, 1983). Phosphorus % was estimated colorimetrically by the method of (King, 1951). Potassium % was determined by the flame-photometer according to Jackson method (1969).

Yield parameters

Harvesting was achieved in mid-February every season, and yield was recorded as total fruit weight ($Kg.tree^{-1}$), average fruit weight (g), fruit number per tree, and yield efficiency ($Kg.m^3$) were calculated.

Fruit yield increment or reduction percentage was compared with the control was calculated according to equation of (Hifny et al., 2017) (4):

$$\text{Fruit yield increment or reduction (\%)} = \frac{\text{Fruit yield (kg)/treatment- Fruit yield (kg) / control} \times 100}{\text{Fruit yield (kg) / control}} \quad (4)$$

Physical and biochemical fruit characteristics

Samples of ten fruits were picked at harvesting time from the outer canopy of each replicate and used to determine both physical and chemical fruit characters that include fruit weight (g), fruit volume (ml), peel thickness (mm), and flesh firmness (lb.inch^2). Furthermore, total soluble solids (TSS) was determined using a hand-held refractometer (Dorostkar et al., 2020). Titratable acidity percentage in fruit juice was determined as grams of citric acid per 100 ml of juice by titration against (0.1 N) NaOH in presence of phenolphthalein as an indicator, and Vitamin C (as mg/ 100 g pulp) was determined according to (AOAC, 1995), then TSS/acid ratio was calculated.

Soil analysis

Soil samples were collected from the experiment site in January 2020, before the start of the experiment, and at the end of the third season (October 2022), to determine the physical and chemical properties of the used soil, which are shown in Tables 1 and 2 according to Sparks et al. (2020).

Statistical analysis

A completely randomized block design was conducted on mature Valencia orange trees (14 years old). Twenty-one trees were organized into seven treatments with three replicates for each to investigate the aforementioned variables. All data obtained during experiment seasons were subjected to analysis of variance (ANOVA) according to (Ott & Longnecker, 2015), and significant differences among means were determined by L.S.D. at the level of 5% probability according to (Snedecor & Cochran, 1980).

RESULTS AND DISCUSSION

According to observational data on Valencia orange growth characteristics, treatments had a significant impact on growth parameters, including canopy volume, shoot length, and leaf area. Data obtained in Table 2, shows that trees exposed to T4 had the largest tree canopy volume (19.72, 23.55, & 29.48 m^3) throughout the trial seasons, followed by T7 (16.61 m^3) in the first season and T3 (22.87 & 27.50 m^3) during the second and third seasons, and control treatment showed the least significant values (14.68, 20.33, & 24.80 m^3). On the other hand, over the experiment's three years, the effectiveness of different therapies varied. While the control treatment had the lowest significant values during the experimental seasons, there was a fluctuation in the effect of other treatments during the three years of the experiment.

Regarding shoot length, T6 recorded the longest shoots in every growth cycle compared to control throughout the experiment (Fig. 1).

Data in Table 2 showed that, across seasons, the highest leaf area (42.13, 59.37, & 60.81 cm^2) and number of leaves per shoot values (13.30, 14.27, & 12.53) were recorded from trees subjected to T4, respectively. While T5 recorded the lowest values of both parameters whereas leaf area recorded (36.27, 38.36, & 39.78 cm^2) and number of leaves per shoot was (9.00, 9.67, & 10.00) in all seasons.

The outcome data showed that polymer applications improved growth parameters despite long irrigation intervals by enhancing nutrients and water use. Our findings are in agreement with Alshallash et al. (2022) on Mango trees, AbdEl-Aziz et al. (2020) on 'Murcott' mandarin trees, Abobatta and Khalifa (2019), and Zoghdan and Abo El-Enien (2019) on Navel orange, who reported that the application of hydrogel composite improves the vegetative growth parameters. Furthermore, Solanki et al. (2021) claimed that the scheduled irrigation water

requirement of acid lime with polymer applications enhances canopy volume and increases vegetative growth.

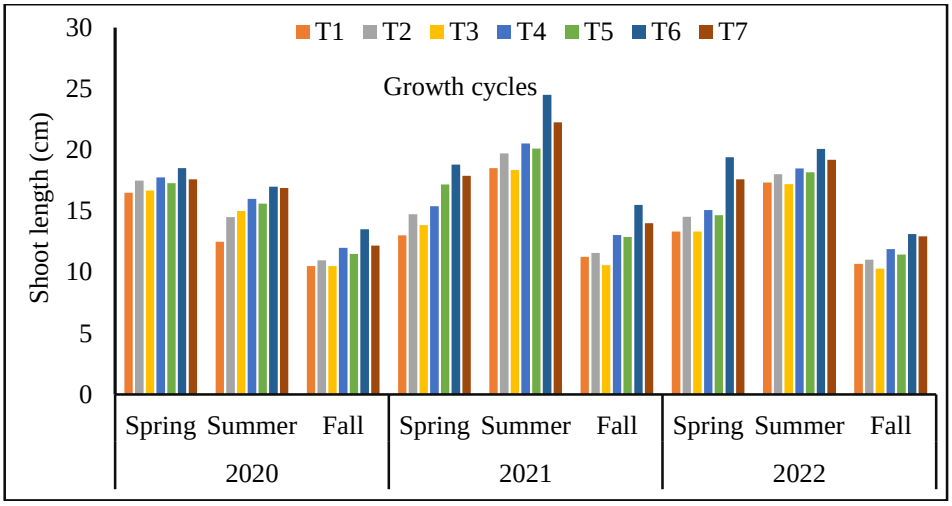

Fig. 1. Effect of various treatments on shoot growth of Valencia orange trees.
*T1 (Control), T2 (I1+HC1), T3 (I2 +HC1), T4 (I1 + HC2), T5 (I2 + HC2), T6 (I1 + HC3), T7 (I2 +HC3).

Table 2. Effect of various treatments on vegetative growth parameters and leaf mineral contents of Valencia orange trees.

Treatment									
Characters	Season	T1	T2	T3	T4	T5	T6	T7	LSD
Tree canopy	2020	14.68 b	14.94 b	15.61 a	19.72 a	14.75 b	15.40 b	16.61 b	2.06
	2021	20.33 d	20.80 cd	22.87 ab	23.55 a	20.92 cd	21.02 cd	21.95 bc	1.22
	2022	24.80 d	26.00 bcd	27.50 b	29.48 a	25.22 cd	26.58 bc	27.12 b	1.55
No. of leaves.shoot^{-1}	2020	10.57 b	101.10 bc	12.63 a	13.30 a	9.00 c	10.71 b	10.90 b	1.54
	2021	10.50 b	10.72 b	13.80 a	14.27 a	9.67 b	11.23 b	11.50 b	2.26
	2022	10.75 bc	10.13 c	10.73 bc	12.53 a	10.00 c	10.96 bc	12.00 ab	1.40
Leaf area (cm^2)	2020	36.36 b	38.94 ab	40.68 ab	42.23 a	36.27 b	39.45 ab	39.72 ab	5.67
	2021	41.25 c	44.94 bc	49.79 b	59.37 a	38.36 c	44.50 bc	45.73 bc	7.38
	2022	45.85 cd	46.27 cd	56.47 ab	60. a	39.78 d	49.27 bc	51.19 bc	7.54
N%	2020	2.12 ab	2.06 b	2.17 ab	2.22 a	2.13 ab	2.14 ab	2.18 ab	014
	2021	2.10 ab	2.08 b	2.14 ab	2.26 a	2.16 ab	2.17 ab	2.13 ab	0.18
	2022	2.13 b	2.11 b	2.15 b	2.29 a	2.14 b	2.18 ab	2.20 ab	0.12
P%	2020	0.15	0.14	0.14	0.13	0.13	0.13	0.12	N. S.
	2021	0.16	0.14	0.14	0.12	0.14	0.13	0.13	N. S
	2022	0.15 a	0.15 a	0.14 bc	0.12 d	0.14 bc	0.13 cd	0.12 d	0.013
K%	2020	2.00 ab	2.19 a	2.01 ab	2.03 ab	1.95 b	1.97 b	1.96 b	0.21
	2021	1.96 b	2.06 ab	2.08 ab	2.11 a	2.00 ab	1.99 ab	2.01 ab	0.13
	2022	1.99 c	2.09 ab	2.10 a	2.15 a	2.02 bc	2.01 c	2.03 bc	0.07

*T1 (Control), T2 (I1+HC1), T3 (I2 +HC1), T4 (I1 + HC2), T5 (I2 + HC2), T6 (I1 + HC3), T7 (I2 +HC3).

The effect of different treatments on tree growth was monitored by estimating mineral elements in the leaves. A large variation in available nutrients in leaves was noticed through the investigation, viz., N% (2.22 to 2.06; 2.26 to 2.08; & 2.29 to 2.11 %), K% (2.19 to 1.95; 2.11 to 1.96; & 2.15 to 1.99 %), while in P% the variation was slight (0.15 to 0.12; 0.16 to 0.12; & 0.15 to 0.12%). These variations were statistically highly significant when compared with the responses of different treatments, except for P content in the first season.

Data in Table 2 showed that the lowest leaf N content was observed in the tree that was subjected to T2 (2.06, 2.08, & 2.11 %). Untreated trees recorded the highest leaf P content (0.15, 0.16, & 0.15 %). On contrary, T4 recorded the lowest values (0.12 & 0.12%) in the second and third seasons. also; T7 recorded the lowest P content in the first and third seasons (0.12 & 0.12%). Control treatment recorded the lowest K values (1.96 & 1.99%) in the second and third seasons, respectively. Furthermore, T4 recorded the highest concentrations of N (2.22, 2.26, & 2.29%) during the experiment and maximum values of K (2.11 & 2.15%) in the second and third seasons compared to the rest of the treatments.

The stimulating effect of the polymeric substances on the leaf mineral content may be due to improved plant nutrition status through increased availability of water and nutrients in the rhizosphere for a longer period, consequently enhancing the supply of nutrients and improving the nutritional status of the trees under arid conditions (Patra et al., 2022). Furthermore, outcome data from this work were consistent with the findings of (Abobatta & Khalifa, 2019), who claimed that hydrogel treatment improved the chemical composition of navel orange leaves. Furthermore Shirgure et al. (2014) on Nagpur mandarin claimed that treatment irrigation schedules recorded the highest leaf content of macronutrients (N, P, & K). Considering the impact of hydrogel and irrigation interval treatments on flowering and fruit set, it is quite evident that flowering and fruit set parameters responded positively to both investigated factor treatments.

The results obtained in Figure 2A showed that all treatments affected the number of opened flowers per shoot. T6 recorded the highest values (89.81, 91.47, & 115.17), followed by T7 (81.74, 85.07, & 110.98), the lowest values were recorded from trees subjected to T2 (78.68, 81.19, 84.60) throughout the experiment.

According to Figure 2B, data clearly showed that all treatments affected the fruit set ratio positively and had the same trend, whereas trees grown under T6 had the highest fruit set ratio (19.73, 23.39, & 20.98 %), followed by those under T7 (18.00, 22.09, & 19.23 %). The differences between both treatments and the other treatment were statistically significant. While T2 recorded the lowest values (13.45, 14.06, & 14.05 %), there were fluctuating responses in other treatments during the investigation.

Yield of trees treated with hydrogel at the most moderate intervals was much higher than that of other treatments due to the increased availability of water and nutrients for a longer time during various phenological stages. Consequently, stimulating fruit retention and increasing fruit weight in the trees that received polymers led to an increase in tree yield, consequently likely leading to a reduction in the effects of extending irrigation intervals. Our results concur with those of (Shirgure et al., 2014) who reported that irrigation schedules had a substantial impact on the yield and fruit quality parameters of Nagpur mandarin trees. The results in hand are in agreement with Solanki et al. (2021) on acid lime, Abd El-Aziz et al. (2020) on Murcott mandarin, and Zoghdan and Abo El-Enien (2019) on navel orange.

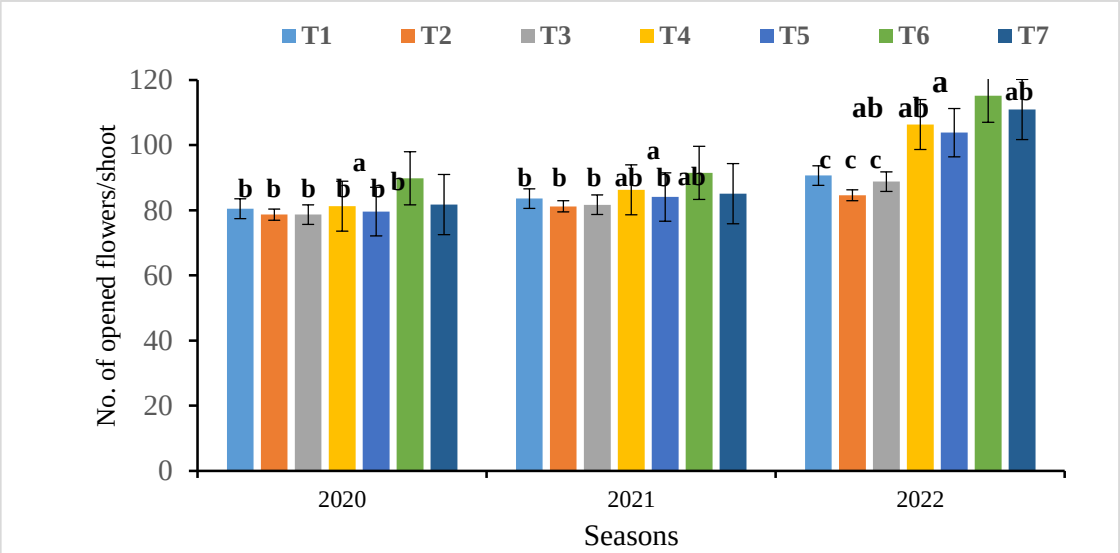

Fig. 2A: Flower number per shoot

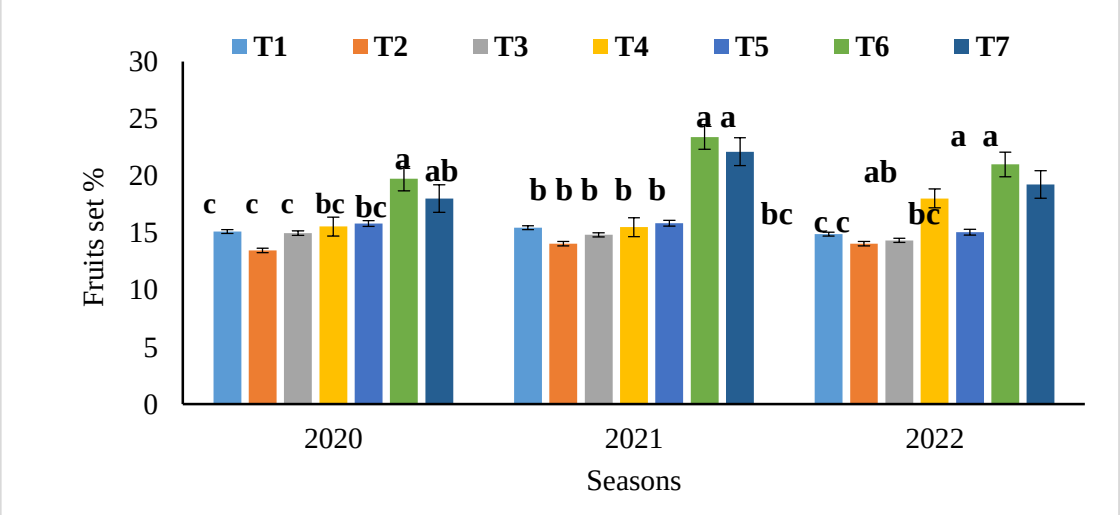

Fig. 2B: Fruit set %

Fig. 2. Effect of various treatments on flowering and fruit set of Valencia orange trees.
*T1 (Control), T2 (I1+HC1), T3 (I2 +HC1), T4 (I1 + HC2), T5 (I2 + HC2), T6 (I1 + HC3), T7 (I2 +HC3).

Regarding yield efficiency, Figure 3 showed that T6 recorded the highest value (7.39, 5.69, & 5.04 kg/m^3), while T3 recorded the lowest value (4.78 kg/m^3) in the first season and control treatment recorded the lowest value (4.38 & 3.97 kg/m^3) in the second and third seasons.

Concerning fruit quality, Data in Table 4 illustrate a significant relationship that was identified in the number of fruits per plant, fruit weight, TSS, juice %, TSS/acidity ratio, and vitamin C during experiment. Data in hand showed a positive effect of various treatments on fruit weight, whereas the average fruit weight was 201.29 to 253.79 g across the seasons, heaviest fruits were produced from trees subjected to T5 (237.31 g), followed by T4 (227.77 g) in the first season. Trees subjected to T6 produced the heaviest fruit (214.02 & 253.79 g) compared to the rest of the treatments in the second and third seasons, followed by T4 (220.45 & 253.79 g), while the minimum fruit weight was recorded from untreated trees (209.91 & 201.29 g) in the first and third seasons and from T2 (203.26 g) in the second one. The results illustrate how different soil conditioner and irrigation interval treatments affect fruit quality

parameters, which may be due to increasing soil moisture content in the rhizosphere of trees subjected to T6, which increases the availability of nutrients and reduces the effect of long irrigation intervals.

Table 3. Effect of various treatments on yield and yield parameters of Valencia orange trees.

Treatment		T1	T2	T3	T4	T5	T6	T7	LSD
Characters	Season								
Tree yield ($Kg.tree^{-1}$)	2020	73.13 d	76.80 d	74.68 d	102.00 bc	94.40 c	113.58 a	108.73 ab	8.69
	2021	89.08 e	99.50 d	102.42 d	112.25 b	107.67 c	119.58 a	111.08 bc	3.47
	2022	100.00 e	114.17 cd	112.42 d	124.00 b	118.73 c	133.75 a	125.83 b	4.70
Total Yield ($ton.fed^{-1}$)	2020	12.07 d	12.67 d	12.32 d	16.83 bc	15.58 c	18.74 a	17.94 ab	1.43
	2021	14.70 e	16.42 d	16.90 d	18.52 b	17.77 c	19.73 a	18.33 bc	0.57
	2022	16.50 e	18.84 cd	18.55 d	20.46 b	19.59 c	22.07 a	20.76 b	0.78
Fruit yield increasing %	2020	0.00 d	5.02 d	3.08 d	40.81 bc	30.34 c	56.67 a	50.02 ab	11.49
	2021	0.00 e	11.47 d	15.02 cd	26.04 b	20.92 bc	34.32 a	24.78 b	6.90
	2022	0.00 d	14.30 c	12.45 c	24.09 b	18.84 bc	33.80 a	25.91 ab	8.32
Yield Efficiency	2020	4.99 c	5.15 c	4.78 c	5.23 c	6.42 b	7.39 a	6.56 b	0.73
	2021	4.38 d	4.78 c	4.49 d	4.77 c	5.15 b	5.69 a	5.06 b	0.25
	2022	3.97 e	4.39 cd	4.09 de	4.21 de	4.79 ab	5.04 a	4.65 bc	0.32

*T1 (Control), T2 (I1+HC1), T3 (I2 +HC1), T4 (I1 + HC2), T5 (I2 + HC2), T6 (I1 + HC3), T7 (I2 +HC3).

Table 4. Effect of various treatments fruit quality parameters of Valencia orange.

Treatment		T1	T2	T3	T4	T5	T6	T7	LSD
Fruit Parameters	Season								
Fruit weight (g)	2020	209.91 c	222.33 bc	224.00 abc	227.77 ab	237.31 a	220.31 bc	223.72 abc	14.39
	2021	212.97 b	203.26 c	203.90 c	220.45 a	208.94 bc	214.02 ab	207.36 bc	7.24
	2022	201.29 c	223.51 b	223.30 b	219.64 b	214.57 b	253.79 a	223.78 b	11.53
Fruit volume (ml)	2020	224.00 c	238.00 b	236.50 b	247.00 ab	257.70 a	238.80 b	238.30 b	10.76
	2021	227.20 bc	214.67 d	219.00 cd	240.60 a	218.00 cd	230.00 ab	218.27 cd	10.62
	2022	214.22 d	225.60 c	238.07 a	234.17 ab	232.83 abc	227.07 bc	237.67 a	8.29
Fruit firmness ($lb/inch^2$)	2020	8.53	8.91	8.56	8.86	8.75	8.57	8.42	N. S.
	2021	8.93	9.03	8.75	8.65	8.73	8.60	8.54	N. S.
	2022	8.73 ab	9.01 a	8.49 bc	8.64 abc	8.53 bc	8.47 bc	8.32 c	0.40
Juice volume (ml)	2020	42.57 c	44.23 bc	41.50 c	48.33 b	45.93 bc	54.77 a	48.70 b	4.90
	2021	49.37 bc	48.27 cd	45.80 d	52.27 ab	48.53 cd	54.53 a	52.77 ab	4.42
	2022	45.53 e	49.50 d	59.87 b	53.50 c	52.37 cd	65.17 a	59.50 b	3.80
TSS%	2020	11.43 ab	11.53 ab	11.67 a	11.37 ab	11.63 a	11.20 b	11.50 ab	0.350
	2021	9.95 e	11.11 c	10.81 d	11.58 a	11.24 b	11.60 a	10.90 d	0.127
	2022	11.44 b	11.41 b	11.49 b	11.55 ab	11.93 a	11.69 ab	11.72 ab	0.285
Total Acid %	2020	1.13 ab	1.01 cd	1.16 a	0.94 d	1.04 bcd	1.04 bcd	1.10 abc	0.117
	2021	1.16 a	1.05 bcd	1.08 b	1.01 d	1.06 bc	1.02 cd	1.07 b	0.040
	2022	1.15 a	1.09 bc	1.12 ab	1.04 d	1.08 c	1.03 d	1.09 bc	0.039
TSS/Acid ratio	2020	10.08 e	11.40 b	10.03 e	12.38 a	11.18 b	10.80 c	10.43 d	0.327
	2021	8.56 d	10.61 c	10.01 c	11.43 a	10.63 b	11.37 a	10.16 c	0.392
	2022	9.95 f	10.47 d	10.26 e	11.11 b	11.05 b	11.35 a	10.75 c	0.062
VC	2020	42.40 abc	43.60 a	42.43 abc	43.20 ab	42.83 abc	41.83 bc	41.43 c	1.67
	2021	42.07 ab	43.50 a	41.67 ab	40.25 b	41.50 ab	42.50 ab	40.25 b	2.46
	2022	42.43 abc	43.77 a	42.90 ab	41.97 bc	42.57 abc	42.63 abc	41.17 c	1.51

*T1 (Control), T2 (I1+HC1), T3 (I2 +HC1), T4 (I1 + HC2), T5 (I2 + HC2), T6 (I1 + HC3), T7 (I2 +HC3).

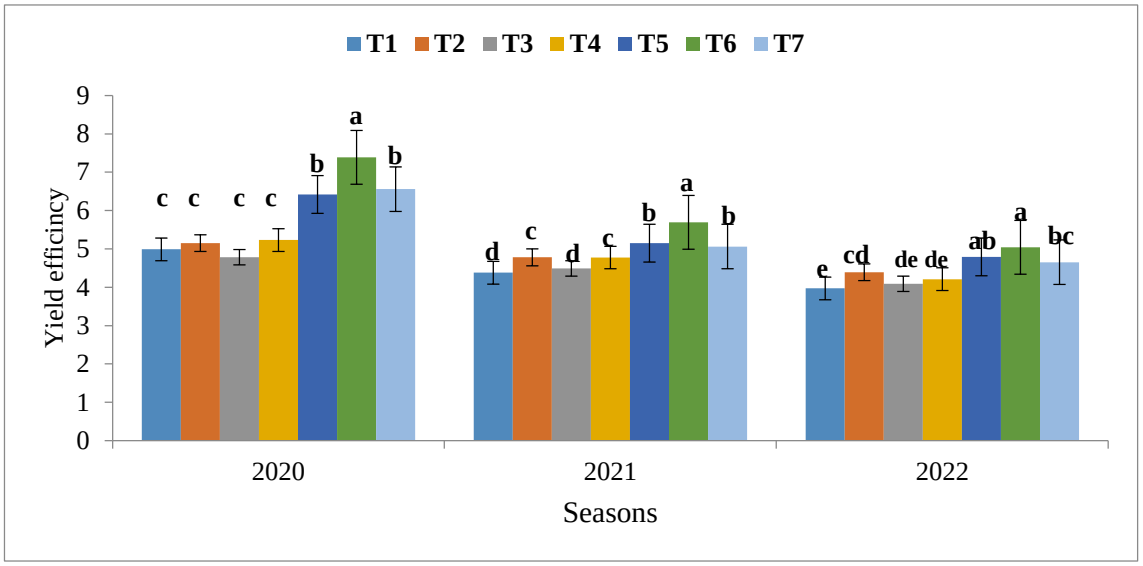

Fig. 3. Effect of various treatments on yield efficiency of Valencia orange trees.
*T1 (Control), T2 (I1+HC1), T3 (I2 +HC1), T4 (I1 + HC2), T5 (I2 + HC2), T6 (I1 + HC3), T7 (I2 +HC3).

Outcome data from Table 4 revealed that various treatments affected fruit volume. T5 had the biggest fruits (257.70 ml), followed by T4 (247.00 ml) in the first season, and T6 recorded the biggest fruits (240.60 & 237.07 ml) the second and third seasons. However, the control treatment produced the smallest fruits (224.00 & 214.22 ml) during the first and third seasons, while T2 recorded the lowest value (214.67 ml) in the second season.

Furthermore, the results obtained throughout the experiment showed that the differences between all coefficients of fruit firmness were non-statistical in 2020 and 2021, while they were statistically significant in 2022. Whereas, T2 recorded the highest significant values (9.01 lb/inch^2) compared to the rest of the treatments, and T7 recorded the lowest values (8.32 lb/inch^2), which may be due to higher soil moisture content throughout the investigation.

Data from Table 4 showed that there was a gradual increase in juice volume associated with the increased doses of the polymer during the experiment seasons, whereas T6 recorded the highest values (54.77, 54.53, & 65.17 ml) of juice volume. While, T3 recorded the least juice volume (41.50 & 45.80 ml) in the first and second seasons, and control treatment has the lowest value (45.53 ml) in the third season.

Data obtained in Table 4 showed that various treatments have a positive impact on TSS % compared to the control treatment, which had the lowest values (11.43, 9.95, & 11.44 %) during the experiment. Furthermore, the control treatment gave the highest total acidity (1.16 & 1.15 %) in the second and third seasons, while, T3 recorded the highest value (1.16 %) in the first season. In contrary, T4 recorded the lowest values (0.94 & 1.01%) in the first and second seasons, and T6 recorded the minimum acidity value (1.03 %) in the third season.

Regarding TSS/Acidity ratio, the differences between all treatments were statistical in 2020-2022, while the control treatment recorded the lowest significant values (10.08, 8.56, & 9.95 %). The differences in TSS/Acidity ratio may have stemmed from different acidity levels in fruit at harvest, such variation in TSS/Acidity ratio of fruits could be explained by differential available water for trees, particularly during the cell enlargement stage, which raises the acidity ratio in fruit juice from control trees.

The treatments carried out significantly affected fruit quality parameters and had a positive impact on most physical and chemical fruit parameters compared to the control, particularly T6. This could be due to the differential availability of water for trees during the fruit growth stages. Our findings are in the same line as those of Alshallash et al. (2022) on

Mango, Solanki et al. (2021) on acid lime, AbdEl-Aziz et al. (2020) on Murcott mandarin, Abobatta and Kahlifa (2019) and Zoghdan and Abo El-Enien (2019) on navel orange, Consoli et al. (2017) on orange and Shirgure et al. (2014) on acid lime.

Economic study

Data in Table 5 shows outstanding yield figures i.e. yield reaching 18.74, 19.73, and 22.07 tons of fruits in the recommended treatment, while it was 12.07, 14.70, and 16.50 tons in the control trees during the experimental seasons. Therefore, applying the suggested treatment (T6) in one feddan, the total expenses amounted to 16,160, 18,825, and 22,650 Egyptian pounds, while the total expenses for the control treatment amounted to 15,500, 18,00, and 22,000 Egyptian pounds.

Thus, total income per feddan with the recommended treatment reached 65,590, 78,920, and 88,280, while the untreated treatment reached 42,245, 58,800, and 66,000 L.E. during the investigation, respectively.

The expected net profit for the recommended treatment when applied in one feddan containing 165 Valencia orange trees reached 49,430, 60,095, and 64,630 L.E., while it reached 26.745, 40,800, and 44,000 L.E. in the control treatment during the experimental seasons, respectively.

Table 5. Economic study of productivity, total cost, and net profit per feddan.

Seasons	Control			Recommended treatment		
	2020	2021	2022	2020	2021	2022
Yield (ton)	12.07	14.70	16.50	18.74	19.73	22.07
Total cost (L.E.)	15,500	18,000	22,000	16,160	18,825	23,650
Total income	42,245	58,800	66,000	65,590	78,920	88,280
Net profit	26,745	40,800	44,000	49,430	60,095	64,630

CONCLUSION

Due to water shortage crises, citrus growers need to adapt new irrigation strategies to sustain citrus production in Egypt. The implementation of day-by-day irrigation with 1000 g of hydrogel composite/tree would be recommended to sustain Valencia orange production and save water. The results of this work could be recommended for citrus plantations in arid areas. Furthermore, vegetative growth parameters (tree canopy, shoot length, leaf area, and leaf number) demonstrate the unique role that polymer treatments play in regulating water and nutrient absorption. Finally, adopted irrigation schedules accompanied by soil application of hydrogel composite produced a clear differentiation within the other treatments.

Treatment of 1000 g/tree hydrogel composite and every two-days irrigation interval achieved the best results when compared to other treatments during the experiment. While application of 1250 g/tree hydrogel with irrigation day-by-day produced the highest tree yield (113.58 kg/tree) and total yield (18.74 tons/feddan) and improved various physical and chemical fruit characteristics, it could be a promising strategy for managing orange orchards in areas that are suffering from water shortages and are similar to the experiment area. More work is required to explain the different effects of hydrogel composite on plant physiology and soil characteristics.

Conflict of interest

The authors declare that there is no conflict of interest.

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JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH
2024, VOL. 7(2), 197-212

Journal of Horticulture and Postharvest Research

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

University of Birjand

Optimizing callus induction and analyzing *in vitro* phytochemicals in San Pedro cactus (*Echinopsis pachanoi*)

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ARTICLE INFO

Original Article

Article history:
Received 22 January 2024
Revised 6 April 2024
Accepted 8 April 2024

Keywords:
Explants
Growth regulators
Tissue culture

DOI: 10.22077/jhpr.2024.7199.1359
P-ISSN: 2588-4883
E-ISSN: 2588-6169

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ABSTRACT

Purpose: The objective of the present study was to examine the impact of explant type and varying concentrations of 2,4-Dichlorophenoxyacetic acid and 6-Benzyladenine growth regulators on the San Pedro cactus callus morphological and biochemical characteristics. **Research method:** Four types of explants were used *i.e.* explants with areola, without areola, with truncated areola, and with central tissue. Additionally, five combinations of BA and 2,4-D, were tested (0 mg/L BA + 2 mg/L 2,4-D, 2 mg/L BA + 2 mg/L 2,4-D, 3 mg/L BA + 3 mg/L 2,4-D, 4 mg/L BA + 4 mg/L 2,4-D, 0 mg/L BA + 0 mg/L 2,4-D). **Findings:** The results indicated that callus formation induced in all treatments 6 days after inoculation. There were significant differences in growth parameters, including fresh weight, volume, moisture, tissue firmness, total phenols, total flavonoids and antioxidant activity of the callus ($P < 0.01$) and dry weight of callus ($P < 0.05$). Explants holding a segment of central tissue, yielded the least favorable results in most of experimental treatments, and the application of 2,4-D in the absence of BA had an inhibitory and toxic effect on the San Pedro cactus explants. **Research limitations:** No limitations were found. **Originality/Value:** Specifically, use of 2 mg/L BA + 2 mg/L 2,4-D and explants with areola resulted in callus with higher fresh weight, volume and total flavonoids, as well as good tissue integrity and firmness. The reported results are a valuable resource for future research related to cell tissue culture and the elicitation of secondary metabolites in *Echinopsis* spp.

INTRODUCTION

The San Pedro cactus, with the scientific name *Echinopsis pachanoi* is a native plant found in the southwest of America, Mexico, and Indonesia. It has been historically used for its hallucinogenic properties. Additionally, it is recognized for its diverse biological activities, including antimicrobial effects, analgesic and psychoactive properties. The cactus also contains specific biochemical compounds such as the peptide Ep-AMP1, mescaline, bridgexigenin A, B, and C, and pachanols A, B, and C (Agte et al., 1995; Kinoshita et al., 1995).

In recent years, the production of plant secondary metabolites has garnered significant attention as a potential source of effective pharmaceutical compounds (Dias et al., 2016; Hussain et al., 2012; Wang et al., 2017). The utilization of *in vitro* culture methods for extracting pharmaceutical compounds offers numerous benefits, including overcoming the limitations imposed by seasonal and geographical changes, environmental factors, and the potential for optimal and rapid production, as well as the establishment of a continuous production system in terms of quantity and quality (Rameshi, 2015). Therefore, enhancing our understanding of the growth behavior of undifferentiated cells is crucial for determining optimal subculture stages and harvesting periods for maximum biomass or metabolite accumulation (Cabanas-García et al., 2021). The literature contains limited scientific reports on the growth behavior of *in vitro* cultures of cactus species, with most focusing on *Nopalea cochenillifera* (Adki et al., 2012), cell suspension cultures of *Opuntia ficus-indica* (Llamoca-Zárate et al., 1999), *Mammillaria candida*, and *Turbinicarpus laui* (Reyes-Martínez et al., 2019). *E. pachanoi*, a medicinal cactus containing specific biochemical compounds, necessitates the study of tissue culture and callus production.

Research has shown that equal or nearly equal amounts of auxin and cytokinin can lead to favorable results in callus production. For instance, in a study on *E. chamaecereus*, treatment with 2.97 mg/L of both growth regulators BAP and NAA resulted in the highest amount of callus production. Additionally, callus formation was observed to occur more on cut surfaces (Téllez-Román et al., 2020). In another study by Angulo-Bejarano and Paredes-López (2011), it was found that among 20 combined treatments of 2,4-D and BA, the best callus induction occurred with the combination of 2.26 µM of 2,4-D and 2.21 µM of BA. Similarly, experiments on six cactus pear genotypes showed no difference among treatments in terms of the day of callus induction (Mengesha et al., 2016).

Furthermore, studies on *O.streptacantha*, *O. megacantha*, and *O. ficus-indica* revealed that the best callus production was achieved with 3 mg/L 2,4-D and 0.5 mg/L BA (Robles-Martinez et al., 2016). Notably, experiments on *Cereus peruvianus*, *Echinocactus mihanovichi*, *E. chamaecereus*, and *Aylostera heliosa* demonstrated the impact of growth regulators on callus formation (Karimi et al., 2010; Vidican et al., 2009). Additionally, hormone treatments involving 2,4-D and BAP were successfully used to induce callus in cacti such as *Notocactus magnificus* and *Curifanta macromeris* (Medeiros et al., 2006). Given the importance of the San Pedro cactus in traditional and modern medicine, the present study aimed to optimize callus induction using different explant types and plant growth regulators. In addition, according to our literature review, no study has earlier been conducted on different explant types for callus induction in this cactus species.

MATERIALS AND METHODS

The present study was conducted as factorial experiment based on completely randomized design with four replicates. The *in vitro* studies were undertaken at the Tissue Culture Laboratory, Kesht Sanat Veshtakesht Co., situated in the Science and Technology Park of Semnan University, during the period of 2020-2021. Biochemical parameters were measured at the Horticultural Science Laboratory, Gorgan University of Agriculture and Natural Resources. The first factor was the explant type, including those containing areola (A), without areola (WA), with a cut areola (CA), and those consisting of central tissue (T). The second factor involved a combination of different concentrations of BA and 2,4-D, as detailed in Table 1.

Two-year-old San Pedro cactus plants, which have approximately 20 cm length and 5 cm diameter, were procured from the Cactus House, Semnan, Iran. To ensure uniformity in size and age, the apical part of the plants was removed. Such a treatment also can eliminate apical dominance and promote rapid meristem growth. The cactus stems were then carefully disinfected to maintain the sterility of the explants. After rinsing the stems for 30 minutes to remove any dirt or debris, the samples were treated with a 2.5% active chlorine sodium hypochlorite solution for 5 minutes to eliminate microorganisms. Subsequently, the explants were sterilized with a 70% ethanol solution for 1 minute and rinsed three times with sterile distilled water for 3, 5, and again 3 minutes, respectively.

The cactus stem was cut into 1 cm^2 explants, and three of them were placed in a jar containing Murashige and Skoog (MS) culture medium, supplemented with different hormonal concentrations, agar (8 g/l), and sucrose (30 g/l), which was then autoclaved at 121°C for 20 minutes under 1 atmosphere Pressure. Subsequently, the samples were transferred to a growth chamber with a photoperiod of 16/8 hours, and a temperature of 25±1°C. The explants growth development was monitored daily, and the explants were sub-cultured every four weeks on the same medium. After 60 days, the calli obtained from the different hormonal treatments were evaluated based on the days of callus formation, callus color, tissue firmness, callus fresh and dry weights, callus moisture percentage, callus volume, total phenols and flavonoid content, and antioxidant activity.

Measurement of total phenols

The total phenolic compounds were quantified using the Folin-Ciocalteu colorimetric method. Briefly, 0.1 g of the fresh callus tissue was ground in 5 ml of 95% ethanol and left in the dark for 24 h. Then, 1 ml of 95% ethanol was added to 1 ml of the supernatant solution, and the solution volume was adjusted to 5 ml with distilled water. Subsequently, 0.5 ml of 10% Folin reagent and 1 ml of 5% sodium carbonate were added. The resulting mixture was kept in the dark for one hour. Finally, the absorbance of each sample at a wavelength of 765 nm was measured using an Analytic Jena model spectrophotometer, and the total phenol content was calculated in mg/g fresh weight. Gallic acid concentrations ranging from 0 to 500 mg/L were used as standards. This extract was utilized to measure the total phenol content using a spectrophotometer in terms of mg/g of fresh weight (Singleton et al., 1999).

Measurement of total flavonoid content

The total flavonoid content was quantified based on the Rutin standard curve. One ml of the methanolic extract was mixed with 250 µl of 10% aluminum chloride solution and 250 µl of one-molar potassium acetate. The absorbance of the samples was measured at a wavelength of 415 nm (Akkol et al., 2008).

Table 1. Description of experimental treatments.

Hormonal treatment (mg/L)	Type of explant			
	A	WA	CA	T
A_1: 0 mg/L BA + 2 mg/L 2,4-D	A_1A	A_1WA	A_1CA	A_1T
A_2: 2 mg/L BA + 2 mg/L 2,4-D	A_2A	A_2WA	A_2CA	A_2T
A_3: 3 mg/L BA + 3 mg/L 2,4-D	A_3A	A_3WA	A_3CA	A_3T
A_4: 4 mg/L BA + 4 mg/L 2,4-D	A_4A	A_4WA	A_4CA	A_4T
A_5: 0 mg/L BA + 0 mg/L 2,4-D	A_5A	A_5WA	A_5CA	A_5T

A : explants containing areola /WA: without areola / CA: cut areola & T :central tissue

Measurement of antioxidant activity through DPPH method

The free radical inhibition was measured using the 2,2-diphenyl-2-picrylhydrazyl (DPPH) method. Initially, one ml of the methanolic extract was mixed with one ml of DPPH at a concentration of 0.1 mM. For the control, one ml of pure methanol was used as a blank. The samples were then kept in the dark for 30 minutes. Subsequently, the absorbance of the samples was measured at a wavelength of 517 nm using a spectrophotometer (Ebrahimzadeh et al., 2011).

Measurement of fresh weight, dry weight and callus moisture percentage

The callus tissue was weighed immediately after taking out of jar. Then a part of the callus was cut and after recording its weight, was transferred to an oven with a temperature of 70°C. It was remained in the oven until the weight of the sample became fixed. The difference between fresh weight and dry weight of callus was used to calculate the moisture percentage of callus (Salemlian, 2017).

Measurement of callus volume

Callus tissues after exiting from jars and weighing, were transferred to a graduated cylinder containing 20 ml of water. By calculating the difference in water volume in the cylinder before and after adding the callus, the volume of the callus was obtained (Mashayekhi & Atashi, 2018).

Data analysis

The experimental data were organized and processed using Excel, and statistical analysis was conducted using SAS software (9.1). The means were compared using Duncan's test at a significance level of $P < 0.05$.

RESULTS AND DISCUSSION

Initially it was found that there was no significant difference in the day of callus formation among the different treatments; all treatments-initiated callus after 6 days of cultivation (Fig. 1). Previous studies by Robles-Martinez et al. (2016) reported that callus formation from embryos culture of *O. streptacantha*, *O. Megacantha*, and *O. ficus-indica* was observed in all media supplemented with 2,4-D but not in media containing only BA. The combination of 3 mg/L 2,4-D and 0.5 mg/L BA produced the best response, with 70% of callus induction in *O. streptacantha* and 100% in *O. megacantha* and *O. ficus-indica* explants at day 15 of culture. Another experiment conducted on six cactus pear genotypes showed that there was no significant difference among genotypes with respect to time taken to callus induction (22-23.3 days after culture) (Mengesha et al., 2016).

Our findings revealed that the callus induction in San Pedro cactus is a rapid occurrence, while the maximum biomass accumulation is slow, as observed at 8 weeks after culture in the

current experiment. This behavior mirrors that of callus cultures of *Coryphantha macromeris* originating from stem discs. The highest yield of callus biomass was attained after nine weeks of culture (Cabanas-García et al., 2021). In contrast, some other plant species, such as *Eysenhardtia polystachya* (Leguminosae), demonstrated maximum biomass accumulation at 12 days of culture (Bernabe-Antonio et al., 2017), while *Armeria maritima* (Plumbaginaceae) reached maximum biomass accumulation at day 14 (Gourguillon et al., 2018).

At the end of the culture, apart from A_1 and A_5T, the callus tissue remained healthy with compact characteristics, and only a few small brown points were observed. Similar results were observed in *C. macromeris* callus after nine weeks of culture, without any phenol exodation (Cabanas-García et al., 2021). In contrast to our findings, Adki et al. (2012) reported that in *Nopalea cochenillifera* (Cactaceae) cultures, the callus lost its vigorous characteristics and turned brown 40-50 days after inoculation. Interestingly, our results demonstrate that *E.pachanoii* callus, like *C. macromeris* callus, is a long-living culture, and cells can withstand water and nutrient deficits, similar to intact- plants of cacti species (Cabanas-García et al., 2021; Stahlschmidt et al., 2011).

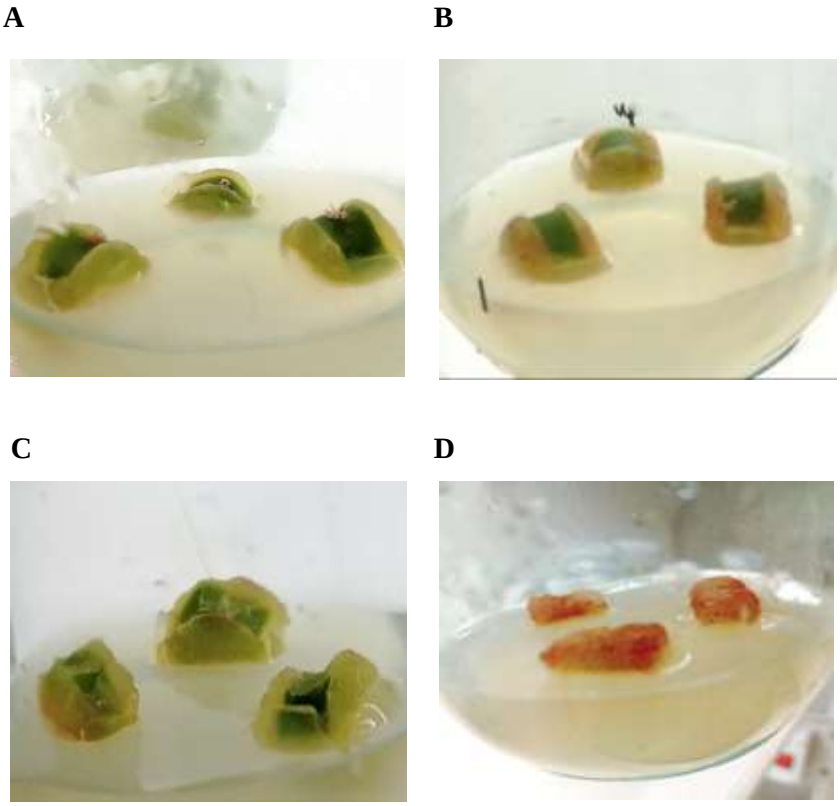

Fig. 1. Callus induction in four types of San Pedro cactus explants, 6 days after inoculation. A: explants containing an areola, B: explants without an areola, C: explants with a truncated areola, D: explants consisting of central tissue.

As far as callus color is concerned, initially the majority of calli colors were white, but as the experimental period progressed, they underwent various color changes, making it challenging to be reported as a statistically analyzed index. So, multiple colors, including white, cream, pale green, rich green, pale brown, and rich brown, were observed in all treatments, albeit in different proportions. This observation is consistent with the findings of Angulo-Bejarano and Paredes-Lopez (2011) on *Opuntia ficus-indica*, where they reported similar color changes during the development of a regeneration protocol through indirect organogenesis in the same cactus species.

The analysis of variance revealed that experimental factor, hormonal composition, has a significant impact on all studied traits ($P < 0.01$). Explant type has similar impact on studied traits ($P < 0.01$), except for total phenols and callus dry weight ($P < 0.05$) (Table 2). In Table 3, the impact of different concentrations of BA and 2,4-D on various callus morphological and biochemical growth parameters is presented. The data clearly indicates that the A_2 treatment shows the best performance in traits related to callus morphological growth, including callus fresh weight index, callus volume, and callus moisture percentage (Fig. 2). Among the experimental treatments, A_1, contained 2 mg/L 2,4-D, similar to the control (A_5), exhibited the lowest rank in fresh weight and callus volume. Additionally, the callus in this treatment displayed a brown color and had a loose and crumbling texture. Consequently, in the assessment of callus tissue firmness, treatment A_1 demonstrated the lowest value among all the experimental treatments, even lower than the control treatment. This contrasts with the findings of Teodora et al. (2015), who reported enhanced callus formation and size in *Echinopsis* (zucc) *chamaecerus* with the presence of 2.5 mg/L 2,4-D in the culture medium, compared to the control.

Table 2. The influence of explant type and combination of growth regulators on phenol, flavonoid, antioxidant activity and certain callus characteristics of San Pedro cactus.

Sources of variation	df	(Mean square)							
		Callus firmness	Fresh weight mg/ explant	Dry weight mg/ explant	Callus moisture %	Callus volume cm^3/ explant	Total phenols mg/g fw	Total flavonoids mg/g fw	Antioxidant activity %
Hormonal composition	4	13.27**	91.66**	0.21**	1458.65**	91.32**	0.58**	0.05**	512.00**
Type of explant	3	20.66**	31.55**	0.008*	1690.55**	37.31**	0.04*	0.03**	529.28**
Hormonal composition × Type of explant	19	1.87**	5.46**	0.033*	1253.02**	5.25**	0.19**	0.10**	1073.37**
Error	40	0.13	15.94	0.024	25.34	11.33	0.02	0.33	156.96
CV		10.53	17.06	8.58	5.60	14.01	20.40	19.56	20.72

ns, *, and **: No significant, significant at 5 and 1% probability, respectively.

Table 3. The effect of different concentrations of BA and 2,4-D on some callus morphological and biochemical characteristics of San Pedro cactus.

Growth regulators concentration	Callus firmness	Fresh weight mg/explant	Dry weight mg/explant	Callus moisture %	Callus volume cm^3/explant	Total phenols mg/g fw	Total flavonoids mg/g fw	Antioxidant activity %
A_1	1.91^{c}	0.83^{c}	0.04^{b}	94.82^{a}	0.88^{d}	0.70^{b}	0.44^{b}	58.45^{b}
A_2	4.16^{a}	6.34^{a}	0.27^{a}	95.62^{a}	6.50^{a}	1.01^{a}	0.55^{a}	56.47^{b}
A_3	4.08^{a}	5.43^{b}	0.22^{a}	95.72^{a}	5.83^{b}	0.96^{a}	0.48^{ab}	70.41^{a}
A_4	4.33^{a}	5.30^{b}	0.31^{a}	92.61^{a}	4.98^{c}	0.94^{a}	0.46^{b}	63.12^{ab}
A_5	2.83^{b}	0.57^{c}	0.03^{b}	70.20^{b}	0.77^{d}	0.49^{c}	0.37^{c}	53.78^{b}

Means, in each column, followed by same letter are not significantly different at the 5% probability level, using Duncan test.
A1: 0 mg/L BA + 2 mg/ l 2,4-D ،A2: 2 mg/ l BA + 2 mg/ l 2,4-D ،A3: 3 mg/ l BA + 3 mg/ l 2,4-D ،A4: 4 mg/ l BA + 4 mg/ l 2,4-D ; A5: 0 mg/ l BA + 0 mg/ l 2,4-D.

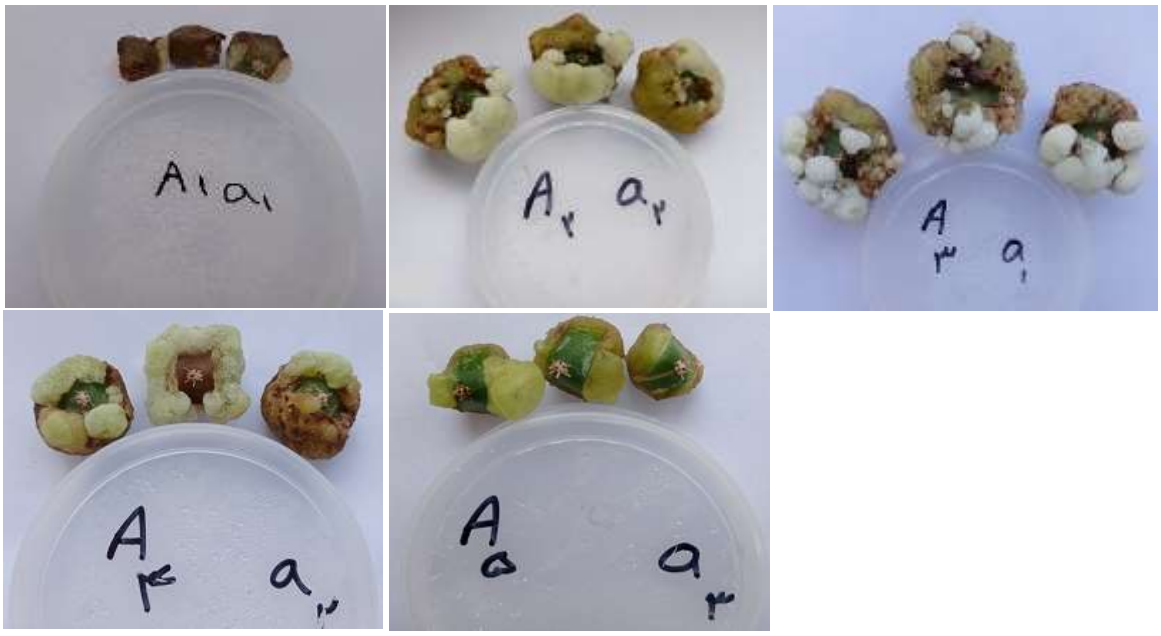

Fig. 2. Comparative callus growth of San Pedro cactus in five different concentrations of BA and 2,4-D, using explants containing areoles. A1: 0 mg/L BA + 2 mg/L 2,4-D, A2: 2 mg/L BA + 2 mg/L 2,4-D, A3: 3 mg/L BA + 3 mg/L 2,4-D, A4: 4 mg/L BA + 4 mg/L 2,4-D, A5: 0 mg/L BA + 0 mg/L 2,4-D.

The application of 2,4-D alone typically induces and promotes callus growth in most plants. However, in the case of the San Pedro cactus, the application of this growth regulator has an inhibitory effect on callus induction and growth, as evidenced by the color and tissue type of the explant. Given the reported effects associated with this growth regulator at different concentrations, it appears that cactus tissue is sensitive to 2,4-D. Therefore, it may be necessary to utilize concentrations lower than those employed in the present research. Fiedler et al. (2022) reported that the utilization of 2,4-D at moderate concentrations (2 mg/L) allowed the obtaining of a vigorous callus of *Ariocarpus retusus*, while 3 mg/L inhibited the callogenesis process.

The optimal combination of growth regulators appears to have a significant impact on increasing cell size within the callus mass. This resulted in the A_2 treatment showing higher fresh weight and volume of the callus compared to other treatments. When comparing the experimental treatments involving different concentrations of growth regulators (A_1, A_2, A_3, and A_4) to the control treatment (A_5), there was a noticeable increase in the percentage of moisture. A higher moisture content is often associated with higher cell viability (Tan et al., 2010), suggesting that growth regulators may play a crucial role in facilitating water and nutrient absorption, as well as promoting cell wall expansion within the callus mass. This observation is consistent with previous research, such as the high callus moisture content of *Grewia tenax* obtained with BA+NAA calli compared to NAA, which can be attributed to the presence of cytokinin in the culture medium (Daffalla et al., 2019). Research has shown that application of cytokinin can stimulate the growth of unorganized cultures (Luczkiewicz et al., 2014). These findings support the notion that growth regulators can have a significant impact on callus morphology and biochemical parameters.

There were significant differences in total phenols and flavonoid contents among the treatments. A_2 treatment has shown most total phenol content, however it had no significant differences with A_3 and A_4 treatments, but all of them had significant differences with A_1 and

A_5 treatments, respectively. A_2 had most total flavonoids content too. Also, notable variations were observed in antioxidant activity, with treatment A_3 exhibiting the highest level, followed by treatment A_4, and then other treatments. Importantly, the control treatment (A_5) displayed lower antioxidant activity compared to the experimental treatments containing growth regulators. Results showed that antioxidant activity in San pedro cactus may increase with higher concentrations of plant growth regulators.

Remarkably, previous studies have also shown the influence of specific growth regulator combinations on antioxidant activity in plant callus cultures. For instance, in *Salvia moorcroftiana* callus, the maximum DPPH scavenging activity was achieved using 2,4-D and BAP at 1mg/L each, along with 1.5 mg/L melatonin (Bano et al., 2022). Similarly, in *Ocimum basilicum* L. callus, the highest levels of antioxidant content were achieved with 0.5 mg/L 2,4-D (Wongsen et al., 2015). Furthermore, callus cultured on a medium supplemented with 2.0 mg/L 2,4-D and 1.0 mg/L BAP showed the greatest antiradical activities against DPPH in *Crataegus azarolus* callus (Chaâbani et al., 2015). These findings are consistent with research on *S. tebesana* callus culture, which revealed the highest antioxidant activity in callus extracts derived from shoot apical meristems on a medium with 0.5 mg/L 2,4-D and 1 mg/L BAP (Hemmati et al., 2020). Similarly, maximum DPPH free radical scavenging activity in *Cnidium officinale* was reported in callus grown on a medium supplemented with 2.3 µM 2,4-D and 2.2 µM BA (Adil et al., 2018). These collective results, along with numerous other studies, underscore the role of plant growth regulators in stimulating and enhancing antioxidant activities in plant callus cultures.

Analysis of the impact of four distinct explant types on callus induction and growth traits in Table 4 reveals that the explant prepared from central tissues, exhibits the lowest amount among all studied traits, except total phenol, significantly differing from the other three explant types. Furthermore, the comparison of biochemical traits indicates that the central tissue explant displayed lower antioxidant activity as compared to the other explants (Fig. 3).

Previous research by Elias et al. (2014) has demonstrated that skin removal or wounding on *Echinocereus cinerascens* explants leads to induce callus production. In present experiment, no difference was observed among explants containing areole and those with a truncated areole. The callus formation mainly occurred in the basal part of the explants, consistent with findings reported by Cabanas-Garcia et al. (2021) in *C. Macromeris*. However, studies by Karimi et al. (2010) on *C. jamacaru* and *C. hildmannianus*, as well as Mondragón-Jacobo and Chessa (2010) on pear cactus, suggest that the areole was not effective for callus induction. In contrast to these results, the explants containing an areole did not show any diffrence evidence of callus induction in *E. pachanoi*. Callus tissues produced in these explant types were more compact and denser than others.

Fig. 3. The *in vitro* performance of different explants of San Pedro cactus inoculated on A_2 medium 60 days after inoculation.

Table 4. The effect of explant types on some morphological and biochemical parameters of callus in San Pedro cactus.

Explant type	Callus firmness	Fresh weight mg/explant	Dry weight mg/explant	Callus moisture %	Callus volume cm^3/explant	Total phenols mg/g fw	Total flavonoids mg/g fw	Antioxidant activity %
A	4.33^{a}	4.37^{a}	0.19^{a}	95.14^{a}	4.50^{a}	0.89^{a}	0.43^{b}	60.91^{ab}
WA	3.93^{b}	4.30^{a}	0.18^{a}	95.30^{a}	4.46^{a}	0.84^{ab}	0.47^{ab}	66.89^{a}
CA	3.86^{b}	4.58^{a}	0.19^{a}	94.85^{a}	4.57^{a}	0.79^{ab}	0.42^{b}	61.48^{ab}
T	1.73^{c}	1.53^{b}	0.14^{b}	73.87^{b}	1.43^{b}	0.76^{b}	0.52^{a}	52.50^{b}

Means, in each column, followed by same letter are not significantly different at the 5% probability level, using Duncan Test. A : explants containing areola /WA: without areola / CA: cut areola & T :central tissue.

Table 5. The interaction effect of growth regulators and the explant type on some callus morphological characteristics in San Pedro cactus.

Growth regulators	Explant type	Callus firmness	Callus volume cm^3/explant	Callus moisture %	Dry weight mg/explant	Fresh weight mg/explant
0 mg/L BA + 2 mg/L 2,4-D	A	2.00^{e}	0.88^{i}	94.38^{a}	0.04^{efg}	0.81^{fg}
	WA	2.00^{e}	0.88^{i}	94.99^{a}	0.04^{efg}	0.80^{fg}
	CA	2.00^{e}	0.88^{i}	94.42^{a}	0.04^{efg}	0.84^{fg}
	T	1.66^{e}	0.88^{i}	95.48^{a}	0.03^{efg}	0.87^{fg}
2 mg/L BA + 2 mg/L 2,4-D	A	5.00^{a}	7.66^{b}	95.43^{a}	0.33^{abc}	7.24^{b}
	WA	5.00^{a}	7.55^{bc}	95.69^{a}	0.31^{abcd}	7.28^{b}
	CA	5.00^{a}	8.66^{a}	95.73^{a}	0.37^{ab}	8.84^{a}
	T	1.66^{e}	2.11^{g}	95.63^{a}	0.08^{cdefg}	2.00^{e}
3 mg/L BA + 3 mg/L 2,4-D	A	5.00^{a}	7.33^{bcd}	95.81^{a}	0.29^{abcde}	7.00^{b}
	WA	4.66^{ab}	7.22^{bcd}	95.75^{a}	0.28^{abcdef}	6.81^{bc}
	CA	4.33^{b}	6.77^{cd}	95.88^{a}	0.25^{bcdef}	6.25^{bc}
	T	2.33^{d}	2.00^{gh}	95.44^{a}	0.07^{cdefg}	1.65^{ef}
4 mg/L BA + 4 mg/L 2,4-D	A	4.66^{ab}	5.44^{f}	96.10^{a}	0.22^{bcdefg}	5.78^{c}
	WA	5.00^{a}	6.55^{de}	95.67^{a}	0.25^{bcdefg}	5.84^{c}
	CA	4.66^{ab}	5.77^{ef}	95.85^{a}	0.26^{bcdef}	6.46^{bc}
	T	3.00^{c}	2.16^{g}	82.80^{b}	0.52^{a}	3.11^{d}
0 mg/L BA + 0 mg/L 2,4-D	A	5.00^{a}	1.16^{hi}	94.00^{a}	0.06^{defg}	1.03^{efg}
	WA	3.00^{c}	1.16^{hi}	94.40^{a}	0.04^{efg}	0.76^{fg}
	CA	3.33^{c}	0.77^{ij}	92.39^{a}	0.36^{fg}	0.49^{g}
	T	0.00^{f}	0.00^{j}	0.00^{c}	0.00^{g}	0.00^{g}

Means, in each column, followed by same letter are not significantly different at the 5% probability level, using Duncan Test. A : explants containing areola /WA: without areola / CA: cut areola & T :central tissue.

The interaction effects of explant type and hormonal combination on callus were shown in Tables 5 and 6. The control treatment A_5 (0 mg/L BA + 0 mg/L 2,4-D) with the central tissue explant has been normalized to zero across all investigated parameters. Notably, after 4 weeks from the initial cultivation date, when the explants were expected to be thriving, all the explants in this experimental treatment were observed to be dark brown in color, turning black, and ultimately resulting in complete tissue death and destruction. Consequently, the cultivation of these explants was discontinued during the experiment. This occurrence suggests that the presence of growth regulators in the central tissue explant medium is crucial for sustained growth.

The results indicate that noticeable differences were observed in all growth parameters at a 5% probability level. Table 5 suggests that the central tissue explant type yielded the least favorable outcomes across all the experimental treatments. Additionally, the application of 2 mg/L 2,4-D (treatment A_1) did not have a positive effect on callus growth, resulting in a very loose, fragile, and weak callus texture, even weaker than the control treatment. This indicates that the use of the growth regulator 2,4-D in the absence of BA may induce toxicity symptoms in San Pedro cactus explants. It is worth noting that the efficiency of different combinations and concentrations of plant growth regulators to stimulate callus induction could be different depends on plant species and type of the tissue (Dawa et al., 2017).

Table 6. The interaction effect of BA and 2,4-D and the type of explants on some biochemical traits of callus in San Pedro cactus.

Growth regulators	Explant type	Antioxidant activity %	Total flavonoids mg/g fw	Total phenols mg/g fw
0 mg/L BA + 2 mg/L 2,4-D	A	46.89^{c}	0.36^{efg}	0.68^{defgh}
	WA	77.13^{a}	0.68^{ab}	0.87^{cdefg}
	CA	45.59^{c}	0.33^{g}	0.59^{h}
	T	64.21^{abc}	0.41^{defg}	0.66^{efgh}
2 mg/L BA + 2 mg/L 2,4-D	A	78.66^{a}	0.82 ^{a}	1.48^{a}
	WA	53.74^{bc}	0.52^{cd}	0.74^{cdefgh}
	CA	60.71^{abc}	0.43^{defg}	0.85^{cdefgh}
	T	60.71^{abc}	0.42^{defg}	0.97^{bc}
3 mg/L BA + 3 mg/L 2,4-D	A	69.83^{ab}	0.35^{fg}	0.95^{bcd}
	WA	65.03^{abc}	0.42^{defg}	0.94^{bcde}
	CA	69.20^{ab}	0.63^{bc}	0.98^{bc}
	T	77.58^{a}	0.51^{cde}	0.98^{bc}
4 mg/L BA + 4 mg/L 2,4-D	A	68.94^{ab}	0.50^{cdef}	1.18^{b}
	WA	59.89^{abc}	0.44^{defg}	0.95^{bcd}
	CA	63.65^{abc}	0.31^{g}	0.92^{bcdef}
	T	60.04^{abc}	0.61^{bc}	0.69^{defgh}
0 mg/L BA + 0 mg/L 2,4-D	A	68.19^{ab}	0.51^{cde}	0.65^{fgh}
	WA	50.73^{bc}	0.54^{cde}	0.69^{defgh}
	CA	68.27^{ab}	0.43^{defg}	0.62^{gh}
	T	0.00^{d}	0.00^{h}	0.00^{i}

Means, in each column, followed by same letter are not significantly different at the 5% probability level, using Duncan Test. A : explants containing areola /WA: without areola / CA: cut areola & T :central tissue.

The comparative analysis of the three A_2, A_3, and A_4 treatments and control (A_5), revealed that they yielded better results in terms of fresh weight, volume, and firmness of the callus tissue. Among these treatments, A_2 was found to be the most effective.

The color of callus tissue can provide valuable insights into its quality and physiological status. As it was already noted, when the callus tissue color was transitioned from dark brown to black, it was ultimately leading to tissue death and destruction. This observation underscores the potential link between callus color and its overall quality and viability. In a study on Taxus callus cultures, Wickremesinhe and Arteea (1993) reported that the callus color gradually changed from pale yellow to dark brown over a six-week period, with a subsequent decrease in the mitotic index. The absence of mitotic figures in the dark brown callus cells further supports the association between callus color and physiological changes.

In plant tissue culture, the color of callus tissue can reflect various physiological and biochemical processes within the cells. Darkening or browning of callus tissue is often associated with the accumulation of phenolic compounds, oxidative stress, and cell death. These color changes are attributed to the oxidation of phenolic compounds by enzymes such as polyphenol oxidase, resulting in the formation of dark pigments (Taghizadeh & Dastjerdi, 2020). Phenolic compounds are released as a natural defense response against plant injuries, potentially leading to damage or cell death (Amente & Chimdessa, 2021). Hesami et al. (2018) emphasized that tissue browning in tissue culturing occurs due to the accumulation and oxidation of phenolic compounds.

Furthermore, research on divided pigeon orchid (*Dendrobium crumenatum* Swartz) callus revealed that dark brown callus had significantly higher polyphenol oxidase (PPO) activity and total phenolic content as compared to green callus (Kaewubon et al., 2014). Similar findings were observed in peony tree (*Paeonia suffruticosa* Andr.) roots (Fu et al., 2011), *Pinus virginiana* Mill callus (Tang & Newton 2004), and the browning of bamboo shoots

(Huang et al., 2002). Ultrastructural disorganization involving the nucleus, mitochondria, and chloroplasts serves as indicators of enzymatic oxidative browning. Therefore, levels of PPO and total phenolics can serve as biochemical markers when selecting suitable callus (Kaewubon et al., 2014). These collective findings underscore the importance of considering callus color as a reflection of underlying physiological and biochemical changes in plant tissue culture.

The presence of dark-colored callus may indicate an imbalance in the tissue culture environment, potentially signifying stress, nutrient deficiencies, or the accumulation of toxic compounds. In an experiment on peony tree callus culture, the evidence indicated that the browning of callus is influenced by various factors, including the composition of the medium, such as macro elements of Murashige and Skoog (MS salts) and iron salt (Fe^{2+}), pH, agar, and specific plant growth regulators like 6-benzylaminopurine (6-BA), 1-naphthaleneacetic acid (NAA), and kinetin (KT). The optimal medium for preventing callus browning was found to be 1/2 MS medium supplemented with 6.95 mg/L Fe^{2+}, 0.3 mg/L KT, 0.5 mg/L NAA, 6.0 g/l agar, at a pH of 6.5 (Zhou et al., 2016).

Additionally, other research has shown that exposure of green callus of castor bean (*Ricinus communis* L.) to certain concentrations of $CuSO_4$ resulted in the callus turning brownish and eventually partly dark brown (Huang et al., 2016). Consequently, the quality of callus tissue can be compromised, affecting its suitability for subsequent stages of tissue culture, such as regeneration or organogenesis. Furthermore, the morphological characteristics and cell viability of coffee plant callus have revealed that yellow callus exhibits higher cell viability, potentially contributing to a greater potential for embryogenesis (Pádua et al., 2014). These findings underscore the importance of considering callus color as an indicator of underlying physiological and biochemical changes in plant tissue cultures, as well as its impact on subsequent developmental processes.

On the other hand, healthy and high-quality callus tissue often displays a light, creamy, or greenish coloration, indicating active growth and physiological balance. This type of callus is more likely to possess the desired characteristics for further developmental processes, such as somatic embryogenesis, shoot organogenesis, or the extraction of secondary metabolites. For instance, in the case of *Taxus*, it has been observed that pale-yellow-colored callus was selected for subculture to enhance Taxol production, while brown-colored callus was discarded due to its eventual progression to callus death (Wickremesinhe & Arteea, 1993).

Furthermore, previous research by Ashokhan et al. (2019; 2020) demonstrated that green callus contains the highest content of bioactive compounds, antioxidant and cytotoxic potentials, as well as the highest amount of Azadirachtin, an essential biopesticide in *Azadirachta indica*, compared to brown callus. These findings emphasize the significance of callus color as an indicator of its potential for further applications, such as the production of bioactive compounds and essential secondary metabolites.

Table 6 illustrates the interaction between different combinations of plant regulators and explant types on various biochemical traits of callus. Phenolic acids are molecules that contain at least one carboxylic substituent bonded to an aromatic ring (Robbins, 2003). Phenolic metabolites have garnered significant attention due to their pharmacological and functional properties, such as antioxidant, anticarcinogenic, and anti-inflammatory effects (Martinez-Valverde et al., 2000). The highest total phenol content was observed in A_2 treatment with areola explant and A_4 treatment with areola explant.

Flavonoids are polyphenolic metabolites that have drawn attention due to their health-promoting effects in diseases such as cancer, Alzheimer, and others, as well as their functional applications in cosmetic, pharmaceutical, and medicinal industries (Panche et al., 2016). In terms of the flavonoid index, A_2-A exhibited the highest flavonoids content at the

5% probability level compared to all treatments, followed by A_1-Wa, A_3-CA and A_4-T. Regarding antioxidant activity, the treatments A_1-WA, A_2-A and A_3-T demonstrated the highest levels of antioxidant activity. Conversely, the treatments A_1-A, A_1-CA, and A_5-T displayed the lowest levels of antioxidant activity, respectively.

Based on the findings, there are correlations that can be inferred regarding the quality of callus and the presence of phytochemicals under different treatments. The text highlights the impact of interactions between various combinations of plant regulators and explant types on the biochemical traits of callus. Specifically, the effects of treatments on total phenol content, flavonoids, and antioxidant activity are discussed. The highest total phenol content was observed in the A_2 treatment with areola explant and the A_3 treatment with areola explant. This suggests that specific treatments have the potential to enhance the accumulation of phenolic compounds, which are important phytochemicals associated with antioxidant and other beneficial properties. This means that higher phenolic content may indicate better callus quality in terms of its potential health-promoting properties, as phenolic compounds are generally present in healthy plant tissues (Amente & Chimdessa, 2021).

The results indicate that treatments A2-A and A_2-T exhibited the highest flavonoid accumulation. Known for their health-promoting effects, the increased presence of flavonoids may suggest improved callus quality for potential functional applications in industries such as cosmetics, pharmaceuticals, and medicine (Jedinak & Maliar, 2004). Additionally, treatments A_1-WA, A_2-A, A_3-CA and A_3-T demonstrated the highest levels of antioxidant activity. Antioxidants play a crucial role in protecting cells from damage caused by free radicals, and higher antioxidant activity in callus may indicate better quality with potential health benefits (Raj et al., 2020; Nishchal et al., 2018). It has also been observed that antioxidant compounds can influence callus growth (Huh et al., 2017). In summary, the different treatments had a significant impact on the phytochemical composition of callus, and there are correlations between the presence of specific phytochemicals and the quality of callus. These findings are valuable for understanding how different treatments can influence the accumulation of bioactive compounds in callus and may provide insights into optimizing tissue culture protocols to enhance the production of high-quality callus with desirable phytochemical profiles.

CONCLUSION

In conclusion, the results demonstrate that the A_2-A treatment yields callus with superior weight and volume, indicating its effectiveness in promoting callus growth. Moreover, the resulting callus exhibits exceptional quality characterized by its consistency and firmness, making it highly suitable for further research related to San Pedro cactus regeneration and the induction and production of hairy roots from callus. The callus obtained from this treatment displays a color spectrum ranging from white to cream and light green, further highlighting its potential for future applications. The favorable quality of the callus generated through the A_2-A treatment recommends its use in callus generation for other valuable cacti species. These findings provide valuable insights into the optimal combinations of plant regulators and explant types for callus induction in San Pedro cactus. This data can significantly contribute to the development of efficient and sustainable methods for the propagation and conservation of this valuable plant species. Furthermore, the study's findings may serve as a valuable resource for future research related to cell tissue culture and the elicitation of secondary metabolites in *Echinopsis* spp. and other cacti species. By understanding the effects of different treatments on callus quality and phytochemical composition, researchers can advance the development of innovative approaches for enhancing the production of high-

quality callus with desirable phytochemical profiles, thereby contributing to the broader field of plant tissue culture and conservation.

Conflict of interest

The authors declare that there is no conflict of interest.

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