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Nutritional values of green and white cucumber (*Cucumis sativus* L.) and African horned cucumber (*Cucumis metuliferus* E.)

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ABSTRACT

Purpose: Cucumbers play an immediate and crucial role in fighting against micronutrient deficiency and are often consumed crudely. This study aimed to assess the nutritional and phytochemical values of these three whole fruits of cucumber and the share of their different parts such as the epicarp, the mesocarp, and the endocarp. **Research method:** Fresh cucumber fruits were collected and their different parts were separated and crushed. Samples were analyzed to determine the proximate, the phytochemicals, the vitamins, and the minerals. **Findings:** The results show significant variation in nutritional and phytochemical content. White *Cucumis sativus* contained more sugars (704.57±124.79 mg/100g), total polyphenols (133.05±21.26 mg/100g), flavonoids (1.07±0.46 mg/100g), tannins (43.26±5.18 mg/100g), Sodium (28.52±1.37 mg/100g) and Potassium (286.58±25.40 mg/100g). Green *C. sativus* concentrated more protein (35.65±5.12 mg/100g) and Iron (4.22±5.44 mg/100g) while, non-bitter wild *C. metuliferus* was richer in acidity (6.5±1.45 meq/100g), vitamin C (275.07±44.23), Magnesium (47.87±10.53 mg/100g) and Calcium (21.25±25.40 mg/100g). According to the different parts, the endocarp concentrates more acidity (7.25±2.21 meq/100g), proteins (39.76±5.07 mg/100g), nitrogen (6.36±0.81 mg/100g), total polyphenol (104.12±28.67 mg/100g) and flavonoids (1.10±0.45 mg/100g). The Mesocarp has more sugars (663.50±12.10 mg/100g) while Epicarp concentrates more Tannin (40.19±1.99 mg/100g), Magnesium (56.51±2.94 mg/100g), Calcium (28.21±20.72 mg/100g), Sodium (25.05±5.28 mg/100g), Potassium (312.66±13.84 mg/100g) and Iron (4.79±4.98 mg/100g). Cucumbers are recognized as fruits and vegetables with multiple nutritional values. **Research limitations:** Further genotypic characterizations were required for a better understanding of the difference between cucumbers**. Originality/Value:** The knowledge of the nutritional value of each part of the fruit was necessary for better valorization and maximizing the nutrient supplies.

INTRODUCTION

Diets rich in vegetables, in all their many forms, ensure an adequate intake of most micronutrients, dietary fibers, and phytochemicals which can bring a much-needed measure of balance back to diets contributing to solving many of these nutrition problems. Increasing dietary diversity and the intake of vegetables and fruits is widely recognized as a key strategy to address the problem of macro and micronutrient deficiency (Hughes & Keatinge, 2012). The nutritional values derived from different plants, fruits, and vegetables have been studied to maintain food quality, food safety, and appeal, or as food additives or nutraceuticals to improve nutritional quality and support physiological functions (Šeregelj et al., 2021). Nutritional values refer to all compounds which are naturally present in foods that exert a specified biological effect on the human body. Recent studies reveal that numerous food wastes and non-edible parts are a good source of nutrients that can be extracted and reintroduced into the food chain as natural food additives (Vilas-Boas et al., 2021). This approach is supported by a circular economy that encompasses the valorization of waste, allowing for the extraction of novel ingredients by returning them to the supply chain and boosting the economy while reducing the environmental impact (Meléndez-Martínez et al., 2022).

Fruits and vegetables play a significant role in human nutrition by providing important nutrients including proteins, vitamins, minerals (zinc, calcium, potassium, and phosphorus), fiber, folacin, and riboflavin (Wargovich, 2000). The nutritional value varies greatly among fruits and vegetables (Prior & Cao, 2000). It is better to consume a variety of commodities rather than limit consumption to a few with the highest nutritional content. Cucumbers are the most important fruits and vegetables consumed and used for a salad a food. They are sources of nutrients required for human health (Sheela et al., 2004; Mukherjee, 2013; Deguine et al., 2015). The major species of cucumbers growing in Senegal are *Cucumis sativus* and *Cucumis metuliferus* (Diop et al., 2020). *Cucumis sativus* or the cucumber has many varieties, including green and white (Burkill, 1985). *Cucumis metuliferus*, horned melon, kiwano, and bitter or non-bitter wild cucumber have high economic and nutritional value that is yet to be fully exploited (Aliero & Gumi, 2012). It has many common names like jelly melon, Kiwano, Melano, and bitter or non-bitter wild cucumber (Vieira et al., 2020). It is often eaten raw, as a snack, but may also be used in cooking (Burkill, 1985).

The nutritional and phytochemical content of different parts of cucumber fruit is not well known. There is increasing evidence that the consumption of whole foods is better than isolated food components. This study aimed to assess the nutritional and phytochemical values of three fruits of cucumber varieties in Senegal and determine the content of parts of the fruit for optimal nutritional value.

MATERIALS AND METHODS

Vegetal material collection

The fruits of Green and White *C. sativus* and non-bitter *C. metuliferus* (Fig. 1) were collected at the market of Ziguinchor, Kadjinolle and Loudia Diola respectively (Fig. 2). The collection sites were located in Ziguinchor and Oussouye districts (Ziguinchor Province, Senegal). A total of ten fruits of each variety were collected.

Fig. 1. Fruit of Green (A) and White (B) *C. sativus* and Non-bitter *C. metuliferus* (C).

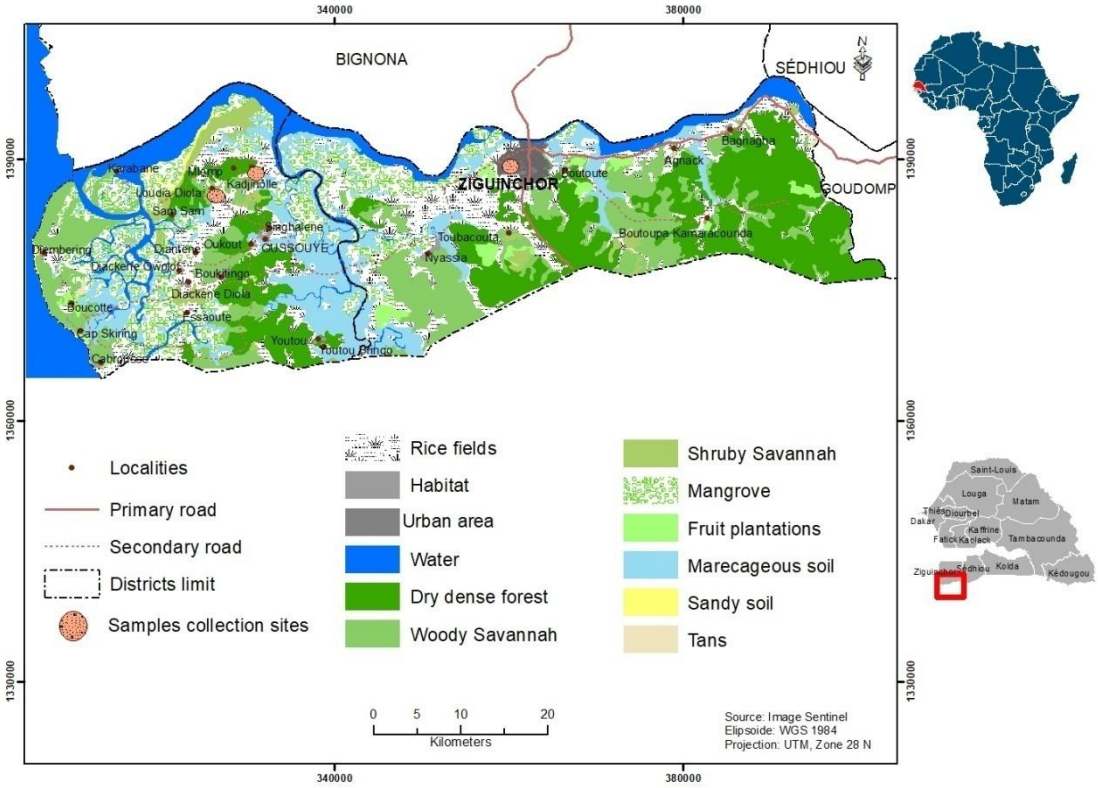

Fig. 2. Localization of collected samples.

Plant extract preparation

All the fruits were divided into three parts according to the Epicarp, Mesocarp, and Endocarp (Fig. 3). Each part was crushed for the proximate, phytochemical, vitamin, and mineral analyses.

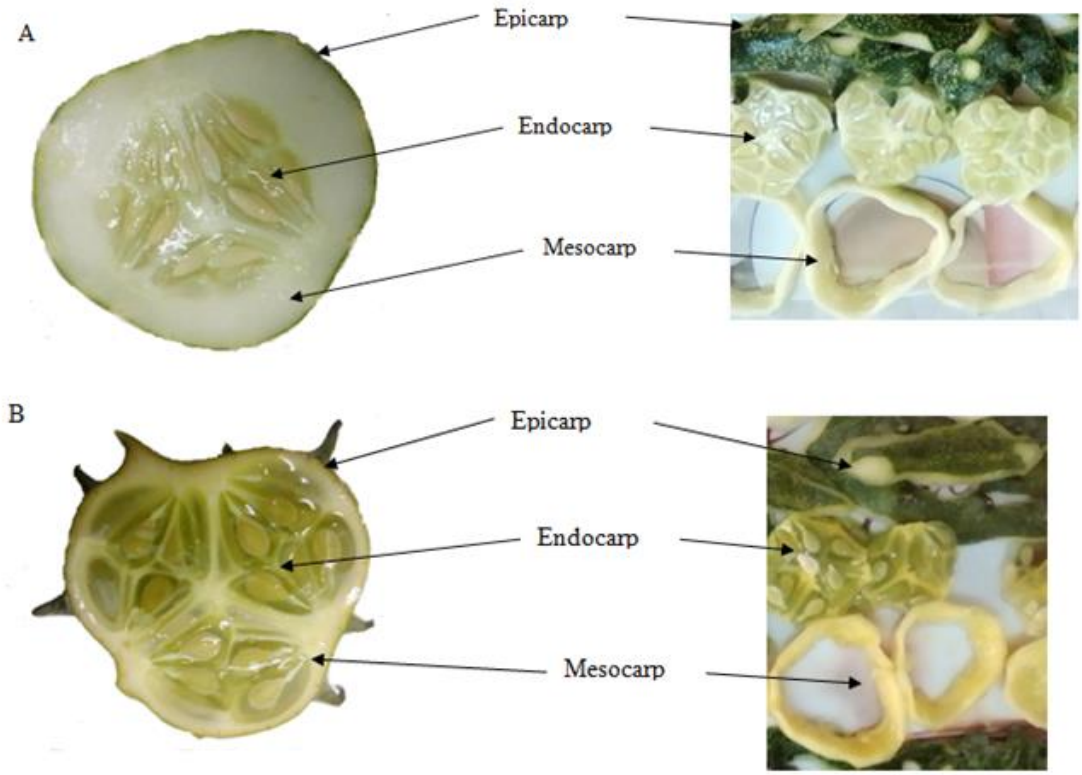

Fig. 3. Different parts of *C. sativus* (A) and *C. metuliferus* (B) fruit.

Proximate, Phytochemicals, vitamins, and minerals analysis

The different parts of fruit samples were analyzed to determine proximate (humidity, ash, sugars, proteins, and acidity), phytochemical properties (polyphenols, flavonoids, and tannins), vitamins (vitamin c), protein, and minerals (nitrogen, calcium, magnesium, sodium, potassium, and Iron).

Proximate

Samples were dried at 105^0C in an isothermal oven for three hours and the humidity was determined. To determine the ash, the samples were incinerated at 525 ± 25 °C for 4 hours using a muffle furnace. Total sugars were evaluated by acid hydrolysis (HCl) by the Luff-Schoorl method (Marrubini et al., 2017). Protein content was determined by the Kjeldahl method (Kirk, 1950; Sáez-Plaza et al., 2013). The acidity is the content of organic and mineral acids determined by titration according to the volumetric method.

Phytochemicals

Analytical methods were used to separate, identify and quantify nutritional components. Polyphenols, flavonoids, and tannins were analyzed by a separation technique using the Spectrophotometric method. 10 ml of the mixture of acetone and water (70/30) was added to 0.5 g of crushed sample and the mixture was agitated for 30mns to homogenize. In each extract of 50µl, 450 µl of distilled water and 2.5 ml of Folin-Ciocalteu were added. 2.5 ml of sodium carbonate is added to each mixture to extract the polyphenol, tannin, and flavonoid compounds. The homogenate mixture was filtered and the filtrates were incubated for 15 minutes at 50°C and subjected to absorbance at 760 nm. The method consists in oxidizing the oxidizable groups of phenols in the basic medium by the method of Folin-Ciocalteu

developed by Georgé et al (2005). Tannins were determined by the colorimetric method of Folin Denis, described by Joslyn (1970). The Flavonoid content of the extracts is determined using the colorimetric method described by Kim et al. (2003).

Vitamin C

One gram of the sample was mixed with 10 mL of distilled water and 50mg of oxalic acid. The mixture was titrated with 2.6-DCPIP (Dichlorophenol Indophenol) solution until a persistent pale pink color appears for 30 seconds to determine the vitamin C content (Nielsen, & Nielsen, 2017).

Minerals

Magnesium (Mg), Calcium (Ca), Sodium (Na), Potassium (K), and Iron (Fe) were analyzed by using Atomic Absorption Spectroscopy (AAS) and Inductively Coupled Plasma Emission (ICP). Nitrogen (N) content is determined by the Kjeldahl method (Kirk, 1950; Sáez-Plaza et al., 2013).

Statistical analysis

Data collected were subjected to a three-way analysis of variance (ANOVA) performed with R 4.1.3 (Team, 2015) to determine the main and interaction effects of the studied variables. When effects were significant, Tukey's test was used for multiple mean comparisons to detect the significant differences between the characteristics (varieties, parts, and varieties/parts of fruit). Statistical significance was fixed at 0.05. Considering the varieties, parts, and varieties/parts' nutritional content, all data are hence expressed as overall means ± SE.

RESULTS

Proximate and phytochemicals

Proximate and phytochemical screening on cucumber fruit samples is summarized in Table 1. The results showed a relatively high proportion of humidity (varying between 93.76±1.07 and 95.64±0.49%) and sugars (between 430.34±104.91 and 704.57±124.79 mg/100g), a moderate concentration of polyphenols, proteins and tannin (between 22.31±5.93 and 49.28±17.16 mg/100g) and a slightly present of ash and flavonoids (between 0.50±0.11 and 7.52±1.92 mg/100g). There was significant variation in proximate and phytochemical content between varieties. Comparing proximate and phytochemical parameters of the whole fruit of cucumber, the fruit of green *C. sativus* contained more humidity (95.64±0.49%) than the white variety (94.45±0.70%) and non-bitter *C. metuliferus* (93.76±1.07%). For ash, white *C. sativus* (7.35±0.78 mg/100g) and non-bitter *C. metuliferus* (7.52±1.92 mg/100g) had higher content than green *C. sativus*, while proteins content was significantly high in green *C. sativus* (35.65±5.12 mg/100g) and white *C. sativus* (31.96±2.60 mg/100g). The lower content of proteins was recorded in non-bitter *C. metuliferus* (27.72±5.88 mg/100g). White *C. sativus* contented significantly more polyphenols (133.05±21.26 mg/100g), flavonoids (1.07±0.46 mg/100g), tannin (43.26±5.18 mg/100g), and sugars (704.57±124.79 mg/100g) than non-bitter *C. metuliferus* and green *C. sativus* (Table 1). There was a significant difference in acidity between the fruits of cucumbers with higher acidity content recorded in non-bitter *C. metuliferus* fruit (6.5±1.45 meq/100g) (Fig. 4).

There was a significant difference in proximate and phytochemical values between and within different parts of cucumber fruit (Table 1, Fig. 4). Humidity (96.34±0.19%) and sugars (663.50±12.10 mg/100g) were higher in the mesocarp. For ash and tannins, a high content was found in epicarp. Endocarp contented more proteins (39.76±5.07 mg/100g), polyphenols

(104.12±28.67 mg/100g), flavonoids (1.10±0.45 mg/100g), and acidity (7.25±2.21 meq/100g). But the parts content of flavonoids and acidity depended on species. There were fewer flavonoids and acidity in the endocarp for green *C. sativus*, non-bitter *C. metuliferus*, and white *C. sativus* respectively (Table 1).

Table 1. Proximate and phytochemical content of cucumber fruit according to cucumber varieties, parts, and varieties/parts.

Parameters	Proximate				Phytochemicals		
	Humidity (%)	Ash (mg100g)	Proteins (mg/100g)	Sugars (mg/100g)	Polyphenols (mg/100g)	Flavonoids (mg/100g)	Tannins (mg/100g)
Varieties							
Green *C. Sativus*	95.64±0.49^{a}	4.82±0.97^{a}	35.65±5.12^{a}	430.34±104.91^{a}	28.39±5.89^{a}	0.50±0.11^{a}	22.31±5.93^{a}
White *C. Sativus*	94.45±0.70^{b}	7.35±0.78^{b}	31.96±2.60^{b}	704.57±124.79^{b}	133.05±21.26^{b}	1.07±0.46^{b}	43.26±5.18^{b}
Non-bitter C. Metuliferus	93.76±1.07^{c}	7.52±1.92^{b}	27.72±5.88^{c}	469.99±71.44^{c}	49.28±17.16^{c}	0.94±0.18^{b}	23.03±3.90^{a}
P value	*2.33e-05*	*0.018721*	*0.000116*	*2.70e-09*	*4.87e-08*	*0.000733*	*4.45e-08*
Parts							
Endocarp	94.62±0.61^{a}	4.63±0.64^{a}	39.76±5.07^{a}	491.99±187.07^{a}	104.12±28.67^{a}	1.10±0.45^{a}	27.78±9.10^{a}
Epicarp	92.88±0.80^{b}	10.20±1.22^{b}	23.38±4.12^{b}	449.41±13.80^{b}	55.33±25.91^{b}	0.83±0.21^{ab}	40.19±1.99^{b}
Mesocarp	96.34±0.19^{c}	4.850.54^{a}	32.19±2.07^{c}	663.50±12.10^{c}	51.28±13.01^{b}	0.57±0.13^{b}	20.63±2.78^{c}
P value	*1.45e-07*	*0.000139*	*2.75e-07*	*2.90e-08*	*1.11e-05*	*0.001577*	*2.22e-07*
Varieties/Parts							
Green *C. Sativus*							
Epicarp	94.14±0.24^{a}	7.76±0.93^{b}	23.89±0.00^{a}	469.43±5.39^{a}	34.66±0.58^{a}	0.85±0.00^{a}	40.99±0.05^{a}
Endocarp	96.05±0.01^{b}	3.20±0.17^{a}	50.98±0.00^{b}	125.53±3.95^{b}	40.06±4.39^{a}	0.26±0.02^{a}	11.64±0.21^{b}
Mesocarp	96.72±0.08^{b}	3.50±0.41^{a}	32.07±2.67^{c}	696.06±1.96^{c}	10.45±1.78^{b}	0.39±0.02^{a}	14.28±0.40^{b}
White *C. Sativus*							
Epicarp	94.08±0.47^{a}	9.72±0.74^{b}	34.37±1.11^{a}	407.33±6.04^{a}	133.89±10.52^{a}	0.30±0.01^{a}	44.38±3.99^{a}
Endocarp	92.77±0.00^{a}	6.42±0.00^{a}	24.22±0.00^{b}	1076.47±36.1^{b}	190.27±2.87^{b}	2.53±0.00^{b}	56.49±0.67^{b}
Mesocarp	96.48±0.26^{b}	5.89±0.00^{a}	37.29±2.48^{a}	629.90±0.00c	74.99±3.64^{c}	0.37±0.07^{a}	28.90±1.88^{c}
Non-bitter *C. Metuliferus*							
Epicarp	90.41±0.43^{a}	13.12±2.49^{a}	11.87±0.00^{a}	471.47±11.50^{a}	2.58±2.12^{a}	1.33±0.33^{a}	35.19±0.11^{a}
Endocarp	95.03±0.08^{b}	4.26±0.79^{b}	44.07±0.00^{b}	273.95±11.91^{b}	82.03±16.94^{b}	0.51±0.03^{b}	15.20±0.19^{b}
Mesocarp	95.82±0.03^{b}	5.16±1.07^{b}	27.22±0.00^{c}	664.54±0.00^{c}	68.38±0.57^{b}	0.96±0.11^{ab}	18.70±0.13^{b}
P value	*1.08e-05*	*9.86e-05*	*2.69e-07*	*1.08e-10*	*2.99e-05*	*2.22e-06*	*1.58e-06*

Results are expressed as mean ± SE, letters a, b and c are groups (groups with different letters are significantly different).

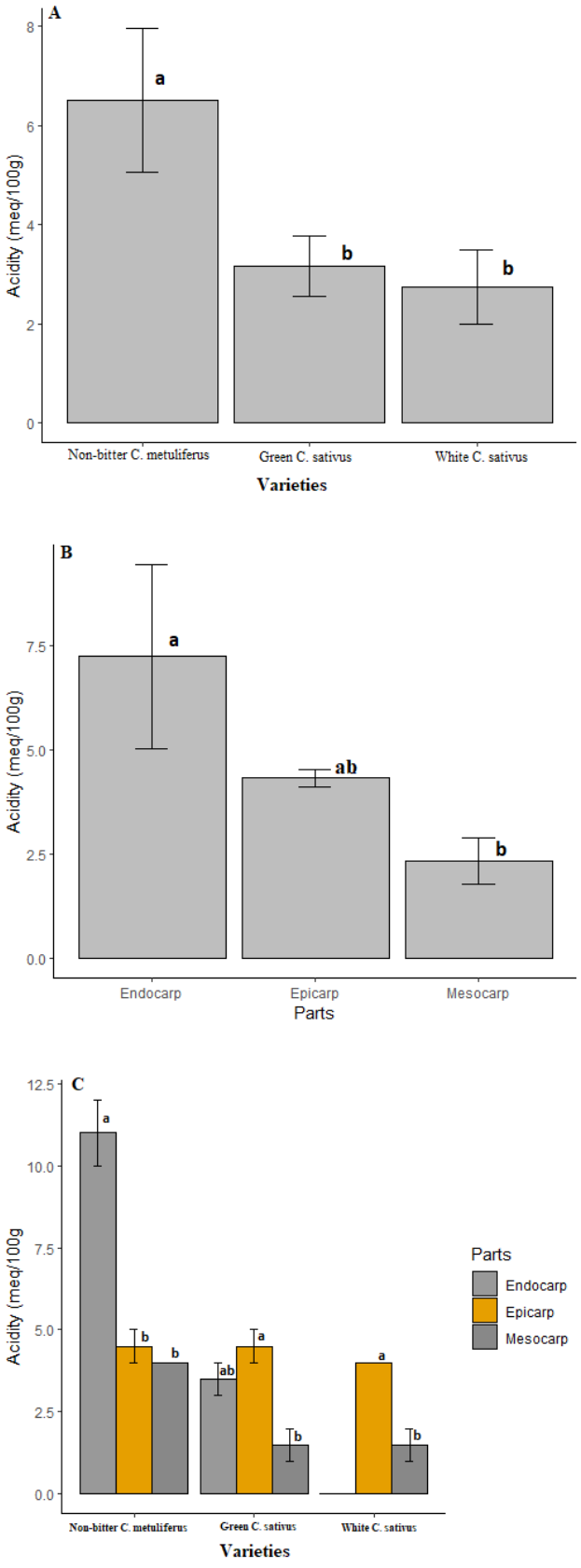

Fig. 4. Acidity content of cucumber fruit according to varieties (A), parts (B), and varieties/parts (C). Values are means ± SD; significant differences are indicated with different letters.

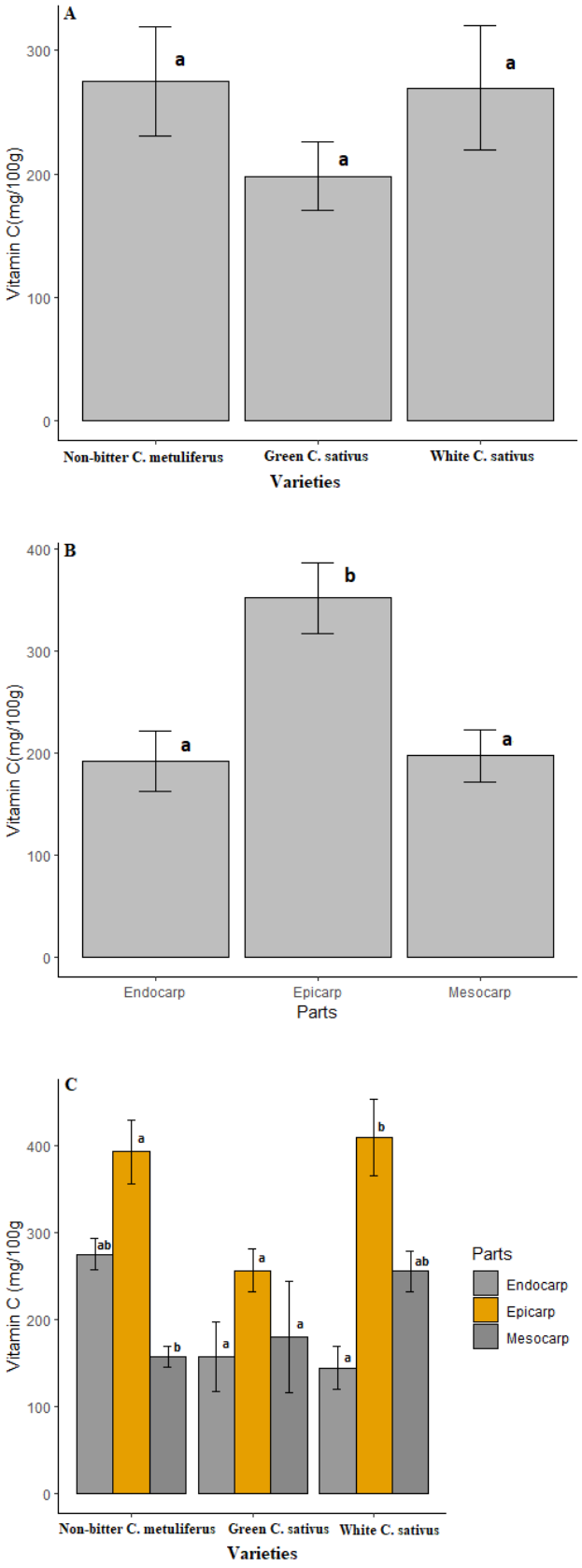

Fig. 5. Vitamin C content of cucumber fruit according to species (A), parts (B), and species/parts (C). Values are means ± SD; significant differences are indicated with different letters.

Vitamin C

Cucumber fruits were an important source of vitamin C varying between 198.05±27.91 and 275.07±44.23 mg/100g (Fig. 5). There was no significant difference in vitamin C content between species. However, there was more vitamin C in non-bitter *C. metuliferus* (275.07±44.23 mg/100g) and white *C. sativus* (269.57±50.52 mg/100g).

The repartition of vitamin C within different parts of fruit varied from 192.40±29.33 to 352.50±34.50 mg/100g (Fig. 5). Epicarp contained significantly more vitamin C (352.48±34.54 mg/100g) than mesocarp (197.81±25.83 mg/100g) and endocarp (192.40±29.33 mg/100g).

Minerals

No significant difference in Nitrogen (N), Magnesium (Mg), Calcium (Ca), Potassium (K), and iron (Fe) content between varieties was recorded (Table 2). In absolute value, non-bitter *C. metuliferus* fruit contented more Mg (47.87±10.53 mg/100g) and Ca (21.25±25.40 mg/100g) while higher K (286.58±25.40 mg/100g) and Fe (4224±5.44 mg/100g) were recorded respectively in white and green *C. sativus*. There was more Nitrogen in green *C. Sativus* (5.70±0.81%) followed by white *C. sativus* (5.11±0.41%) and non-bitter *C. metuliferus* (4.43±0.94%) (Fig. 6). Sodium (Na) content recorded in white (28.52±1.37 mg/100g) and green *C. Sativus* (19.89±5.98 mg/100g) were significantly higher than non-bitter *C. metuliferus* (17.02±2.51 mg/100g).

Comparing the mineral content of different parts of the fruit, there was no significant variation of Mg, Ca, Na and Fe. Epicarp contained more minerals (Mg, Ca, Na and Fe) than endocarp and mesocarp. While there was a significant difference between parts for N and K content. Endocarp (6.36±0.81%) contented more than mesocarp (5.15±0.33%) and epicarp (3.74±0.65%). But for white *C. sativus*, the endocarp (3.87±0.00%) contented less N than the mesocarp and Epicarp (Fig. 6). K content was significantly higher in epicarp (312.66±13.84 mg/100g) than in endocarp (222.84±57.40 mg/100g) and mesocarp (169.04±84.05 mg/100g) (Table 2).

Table 2. Minerals content of cucumber fruit according to species and parts.

Parameters	Mg (mg/100g)	Ca (mg/100g)	Na (mg/100g)	K (mg/100g)	Fe (mg/100g)
Varieties					
C. Sativus var. Green	43.72±10.22^{a}	6.74±2.48^{a}	19.89±5.98^{ab}	214.07±112.81^{a}	4.22±5.44^{a}
C. Sativus var. White	46.12±12.19^{a}	9.79±12.95^{a}	28.52±1.37^{b}	286.58±25.40^{a}	1.27±1.08^{a}
C. metuliferus	47.87±10.53^{a}	21.25±25.40^{a}	17.02±2.51^{a}	203.90±82.92^{a}	1.00±0.31^{a}
P value	*0.75519*	*0.362*	*0.0241*	*0.178*	*0.378*
Parts					
Endocarp	43.44±8.19^{a}	3.58±1.55^{a}	20.62±7.64^{a}	222.84±57.40^{ab}	0.82±0.14^{a}
Epicarp	56.51±2.94^{a}	28.21±20.72^{a}	25.05±5.28^{a}	312.66±13.84^{a}	4.79±4.98^{a}
Mesocarp	37.75±4.86^{a}	5.99±3.04^{a}	19.75±6.39^{a}	169.04±84.05^{b}	0.88±0.30^{a}
P value	*0.05629*	*0.105*	*0.2016*	*0.0483*	*0.247*

Results are expressed as mean ± SE, and letters a and b are groups (groups with different letters are significantly different).

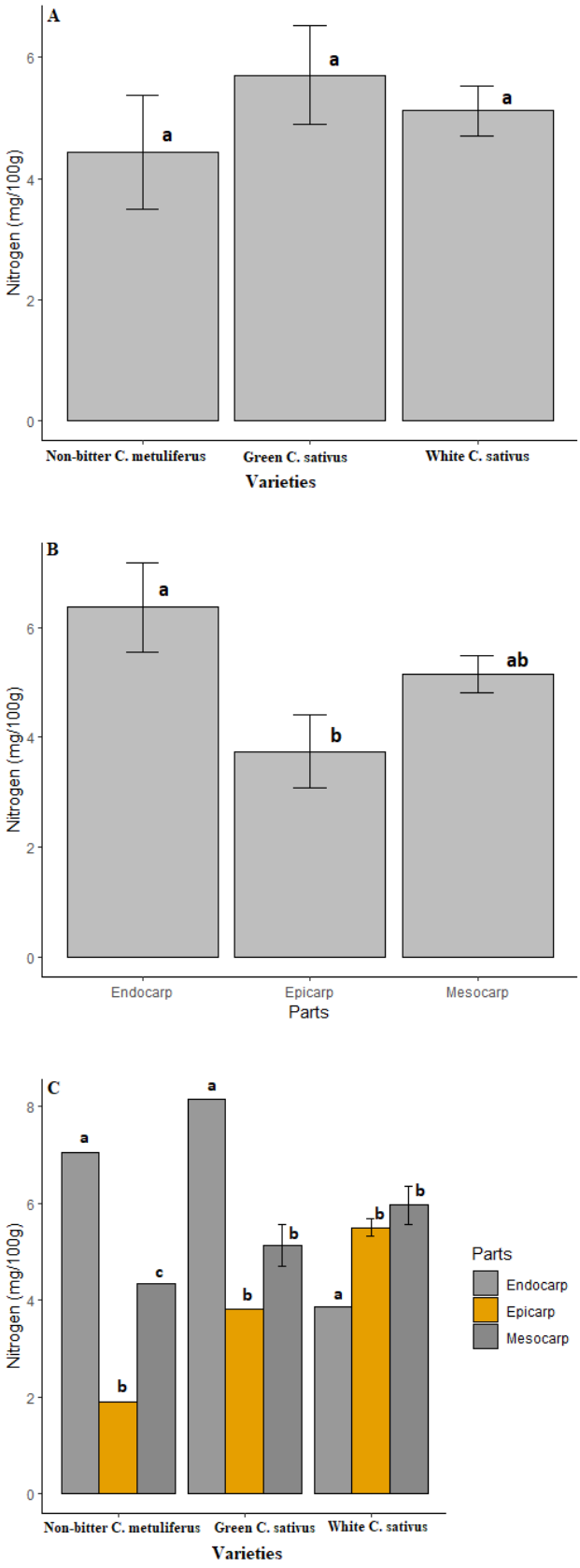

Fig. 6. Nitrogen content of cucumber fruit according to species (A), parts (B), and species/parts (C). Values are means ± SD; significant differences are indicated with different letters.

DISCUSSION

Proximate, physicochemical, vitamin C and minerals content of fruit

Proximate and phytochemical analysis on cucumber fruit samples showed a relatively high proportion of humidity and sugars, a moderate concentration of polyphenols, proteins, and tannins, and a slightly present of ash and flavonoids. For vitamins and minerals, high concentrations of N and K, and moderate and slight concentrations of Mg, Na, Ca, and Fe were recorded. The high humidity of the whole cucumber (*C. sativus* and *C. metuliferus*) fruit varying between 89 and 96.4% was recorded (Agatemor et al., 2018; Ferrara, 2006; Romero-Rodriguez et al., 1992; Mukherjee et al., 2013). Cucumber is a rich source of important nutrients and bioactive compounds; it has been used not only as food but also in therapeutic medicine and as an ornamental plant (Dixit & Kar, 2010; Kapoor, 2001; Uthpala et al., 2020). Cucumber considered a fruit and vegetable crop is rich in polyphenolics and other phytochemicals (Agatemor et al., 2018). Uthpala et al. (2018) have conducted a phytochemical screening on cucumber (*C. sativus*) homogenate samples and they have found that relatively higher amounts of steroids, terpenoids, glycosides, and resins are present in cucumber while moderate amounts of saponins, alkaloids, and flavonoids have been reported. Quantitative amounts of the proximate and phytochemicals found reducing sugars in the highest amount (574.4mg/g) relatively compared to other phytochemicals followed by polyphenols (8.51 mg/g), flavonoids (2.14 mg/g), and tannins (1.26 mg/g) were the lowest available phytochemicals (Agatemor et al., 2018). The analytical composition of *C. metuliferus* pulp showed that are present proteins, lipids, sugars, and minerals including magnesium with high concentration, calcium, potassium, and iron; vitamin C which concentration is four times higher in lemon (Usman et al., 2015; Hussein, 2009). The phytochemicals present in the fruit of *C. metuliferus* revealed the presence of useful secondary metabolites such as alkaloids, Flavonoids, saponins, tannins, steroids, and terpenoids (Jimam et al., 2011; Gotep, 2011; Usman et al., 2014).

Comparing the proximate and phytochemicals between varieties, green *C. sativus* contented more humidity and proteins than white *C. sativus* and non-bitter *C. metuliferus*. White *C. sativus* contained significantly more polyphenols, flavonoids, tannins, and sugars followed by non-bitter *C. metuliferus*. For the acidity and ash, the higher values were recorded in non-bitter *C. metuliferus*. For vitamin C and minerals (N, Mg, Ca, K and Fe), there was no significant variation between varieties. But more vitamin C, Mg, and Ca were recorded in non-bitter *C. metuliferus* followed by white *C. sativus* while higher K, Fe, and Na were recorded respectively in white and green *C. sativus*. The humidity of the whole fruit of cucumber varied from 89% for *C. metuliferus* and 96.4% for *C. sativus* (Agatemor et al., 2018; Ferrara, 2006; USDA, 2015; Romero-Rodriguez et al., 1992; Mukherjee et al., 2013). Higher ash (5 mg/g) of *C. metuliferus* (Romero-Rodriguez et al., 1992) and lower (0.94 mg/g) of *C. sativus* (Agatemor et al., 2018) were recorded. Agatemor et al. (2018) found high polyphenols (8.51 mg/g) and sugars (574.36 mg/g) content in *C. sativus* fruit than in *C. metuliferus* with respectively 0.89 mg/g (17) and 16.10 mg/g (Benzioni et al., 1993). Comparing the minerals content between species, Ferrara (2006) found higher Mg (40 mg/100g) and Fe (1.13 mg/100g) content of *C. metuliferus* than *C. sativus* with respectively 16 mg/100g and 0.70 mg/100 (Agatemor et al., 2018). These authors found higher content of K (249 mg/100g) and Ca (15 mg/100g) of *C. sativus* (Agatemor et al., 2018) than *C. metuliferus* with respectively 123 mg/100g and 13 mg/g (Ferrara, 2006).

Proximate, physicochemical, vitamin C and minerals content of parts of the fruit

The relative content of proximate and phytochemicals varied from epicarp to endocarp. The epicarp contained more ash and tannins while the high humidity and sugar content were recorded in the mesocarp. Proteins, polyphenols, flavonoids, and acidity content were found higher in the endocarp. For vitamin C and minerals, there was a slight difference in content between the parts of cucumber fruit. Epicarp contained more vitamin C, Mg, Ca, Na, K, and Fe. But endocarp was richer in N. There were significant differences among the samples. A comparison of the epicarp and other parts of cucumber fruit showed that epicarp had higher values for ash, proteins, fat, fiber, and carbohydrate while others were more concentrated in moisture content (Abulude et al., 2007). For minerals, epicarp had also a higher concentration of Mg, Fe, and Ca while endocarp and mesocarp contained more K and Na respectively (Abulude et al., 2007).

CONCLUSION

Cucumber fruits are a rich source of important nutrients and phytochemical compounds. The nutritional values of cucumber fruits varied according to the species and variety and the parts of the fruit. Based on the species and varieties, white cucumber was richer in sugars, polyphenols, flavonoids, tannins, sodium, and potassium. But other varieties are also rich in proteins and iron (green cucumber), acidity, vitamin C, magnesium, and calcium (non-bitter wild cucumber). Cucumbers are recognized as fruits and vegetables with multiple nutritional values including, proteins, polyphenols, flavonoids, tannin, sugars, vitamin C and minerals. The nutritional content varied significantly within the fruit parts. An important concentration of acidity, proteins, nitrogen, polyphenols, and flavonoids in the Endocarp, sugars in the Mesocarp, and Tannin in the Epicarp were recorded. But the Epicarp concentrated more minerals. In Senegal, cucumber fruit is consumed as a healthy food but most people in their habits consumed only the mesocarp. The knowledge of the nutritional value of each part of the fruit was necessary for better valorization. It is important to consume whole fruit to maximize the nutrient supply.

Conflict of interest

The authors declare no conflict of interest to report.

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Physical, plant growth regulators and TiO_2 nanoparticles priming treatments to improve seed germination of endangered asafoetida (*Ferula assafoetida* L.)

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ABSTRACT

Purpose: *Ferula assafoetida* (L.) is one of the most important medicinal plants with many applications in food, pharmaceutical and cosmetic industries. It has been endangered due to overharvesting from natural habitat and long period of seed dormancy. Knowledge of seed germination behavior leads to the development of its conservation and cultivation. **Research methods:** We conducted this research as a factorial experiment in Completely Randomized Design (CRD) to evaluate seed germination in response to low temperature, plant growth regulators (kinetin, gibberellin, carrageenan as plant bio-stimulant) and TiO_2 nanoparticles (TiO_2 NPs). The germination percentage and rate, mean germination time, and radicle elongation were measured. **Findings:** The results showed that the cold (4 °C), GA_3, carrageenan, kinetin and TiO_2 NPs increased seeds germination rate and percentage. Maximum seed germination percentage (86% or 23% more than control) and minimum mean germination time (26 days or 12.6 days shorter than control) obtained with seeds pretreated by kinetin soaking and TiO_2 NPs treatment at 4 °C. Furthermore, most treatments produced healthier and stronger radicles compared to the control which is vital for better establishment and growth. **Research limitations:** No limitations were found. **Originality/Value:** The price and demand of asafoetida products have been increased dramatically. The most important constrain to hinder reliable supply of the products is the shortage of plant or difficulty to access its products. Here, we showed the cost effective and environmentally friendly methods to provide high seeds germination with vigorous roots.

INTRODUCTION

Asafoetida (*Ferula assafoetida* L.) is a valuable perennial herbaceous medicinal plant indigenous to Iran, Tajikistan and Afghanistan. The main compound of the plant is an oleo-gum resin, called asafoetida (Anghozeh- in Persian), which is obtained by incision of the roots (Sadraei et al., 2003). Asafoetida has several medicinal properties such as antispasmodic, digestive, expectorant, sedative, laxative, analgesic (Abd El-Razek et al., 2001). Asafoetida has been widely used as spice or in traditional medicine mainly in west, central Asia and India. This plant belongs to the Apiaceae family with sweet and bitter types. Oleo gum resin is extracted from 4-7-year-old plants before flowering. Its chemical compositions contain 40-64% resin, 25% gum and 10-17% essential oil (Amalraj & Gopi, 2016). Demand for asafoetida has been increased due to the public acceptance of herbal medicines and its effective properties. The native habitats are being quickly diminished by human activities. Thus, there is an urgent need to develop the conservation strategies to restore its habitat and potential cultivation (Abd El-Razek et al., 2001). Asafoetida in its habitat propagates by dispersing its seeds through wind. This endemic plant is also endangered due to the weak seed germination. Seed germination of asafoetida is poor both in the native arid regions and under laboratory conditions (Nadjafi et al., 2006). The regeneration of this plant is possible only by seed, therefore, studies of breaking seed dormancy by chemical and physical factors are paramount importance.

The effects of prechilling at 4 °C for 10 to 90 days and gibberellic acid at 200 to 800 ppm and the combination of both treatments on *Ferula asafoetida* and *Ferula gummosa* seeds dormancy breaking was investigated. The results indicated that the higher the time of exposure to cold treatment and the higher concentration of GA_3, the better seed germination percent and rate, radicle length and plumule and seed vigor are become. The combination of cold for 70 days with GA_3 at 400 ppm was the best treatment (Sharifi et al., 2017). The effects of different temperature at 10, 15, 20 °C, gibberellic acid at 1000 ppm and kinetin on seed dormancy breaking of asafoetida seeds showed that pretreatment with kinetin at 250 ppm and 10 °C significantly improved seed germination parameters such as germination percentage and decreased germination time compared to the control (Malek et al., 2022). Plant growth regulators are found to play an important role in the germination process. It was reported that 0.1 µL of 24-epibrassinolide significantly enhanced gladiolus corm germination up to 300% compared to the control (Mollaei et al., 2018). It has been stated that other plant growth regulators, particularly those with cytokinin activity can be effective on breaking seed dormancy (Kabar, 1998). Carrageenans are the major polysaccharides exist in Rhodophyta red algae that may improve seed germination (Gonzàlez, 2013). It has been reported that TiO_2 NPs application can improve seed germination rate and percentage (Zheng et al., 2005; Castiglione et al., 2011). It is imperative to investigate the effects of natural and synthetic products on seed germination of each specific plant such as asafoetida.

The aims of this study were to evaluate the effects of low temperature, GA, kinetin, carrageenan and TiO_2 NPs treatments on asafoetida seed germination. Furthermore, to optimize the most effective combination of treatments suitable for seed germination of this rare plant.

MATERIALS AND METHODS

Seed collection and analysis

The seeds of Asafoetida [*Ferula assafoetida* (L.)] were collected from five healthy and robust mature eight-year-old plants in natural habitat which were labeled and separated by the fence. The asafoetida herbal parts and seeds that were collected from the natural habitats were taken to the Department of Botany at Shahid Bahonar University of Kerman, Iran and the samples were identified and confirmed by herbarium specialist. The seeds were mixed and randomly selected from composite samples for the experiments. The site is located at 25 km west of Chatrod city, Kerman province, Iran (between eastern longitudes of 30° 38´ to 30° 39´ and northern latitudes of 55° 0´ to 55° 2´) (Fig. 1). The experiments were conducted at the Faculty of Agriculture, Shahid Bahonar University of Kerman, Iran during 2021 for 6 months.

The seeds were stored in small cotton bag at room temperature (23 ± 2 °C) for six months before the experiments. To overcome seed dormancy, several treatments have been used: Temperature [4 °C (cold temperature), and -17 °C (freezing temperature)], plant growth regulators [Kinetin (5 mg L^{-1}, Duchefa Biochemie, Netherland), gibberellic acid 1500 mg L^{-1}, Merck, Germany) and kappa-carrageenan (0.5 g L^{-1}, Sigma-Aldrich, Germany) and TiO_2 NPs (10 mg L^{-1}, Sharif Co., Iran)].

All seeds were immersed in water and the blank seeds floated on top of the water were separated before the start of any treatments. Seeds were sterilized with 70% ethanol for two min and then washed with sterilized distilled water three times. The seeds were divided into two groups: The first group was soaked in 50 ml sterilized distilled water and placed in the refrigerator, which was replaced every 5 hours with fresh distilled water. The second group was soaked in 50 ml sterilized kinetin solution and placed in the refrigerator and was replaced with fresh kinetin solution every 5 hours. The soaking and leaching of two groups of seeds continued for 72 hours and then seeds were transferred for the next stages of germination test. For each treatment, five replications and in each replication 12 seeds were used and covered by film sheet. Seeds without drying were placed on double layered Whatman No.1 filter paper moistened with 5 ml of sterilized treatment solution [Water (control), GA_3 (1500 mg L^{-1}), carrageenan (0.5 g L^{-1})] and TiO_2 NPs (10 mg L^{-1}) in sterilized Petri dishes for ten days (Table 1) and 40 days in refrigerator, then 40 days more for germination tests had been performed up to 93rd day.

Fig. 1. The asafoetida (Yellow plants) natural habitats, the hillsides around Chatrod city (Kerman province, Iran).

Table 1. Treatment designations and conditions for asafoetida seed dormancy breaking.

Treatment Codes	Soaking and Leaching Treatments (72 h)	Temperature Treatments (24 h)	Treatment Solution (9 days in 4 °C)
0CW	(0) Water	4 °C (Cold)	Water
0FW	(0) Water	-17 °C (Freezing)	Water
0FCg	(0) Water	-17 °C (Freezing)	Carrageenan
0FG	(0) Water	-17 °C (Freezing)	Gibberellic acid
0FTi	(0) Water	-17 °C (Freezing)	TiO_2 NPs
0CCg	(0) Water	4 °C (Cold)	Carrageenan
0CG	(0) Water	4 °C (Cold)	Gibberellic acid
0CTi	(0) Water	4 °C (Cold)	TiO_2 NPs
1FW	(1) Kinetin	-17 °C (Freezing)	Water
1FCg	(1) Kinetin	-17 °C (Freezing)	Carrageenan
1FG	(1) Kinetin	-17 °C (Freezing)	Gibberellic acid
1FTi	(1) Kinetin	-17 °C (Freezing)	TiO_2 NPs
1CW	(1) Kinetin	4 °C (Cold)	Water
1CCg	(1) Kinetin	4 °C (Cold)	Carrageenan
1CG	(1) Kinetin	4 °C (Cold)	Gibberellic acid
1CTi	(1) Kinetin	4 °C (Cold)	TiO_2 NPs

0: Soak-leaching in water, 1: Soak-leaching in kinetin, C: Cold stratification, Cg: Carrageenan, F: Freezing stratification, G: GA_3 and Ti: TiO_2 NPs treatments, W: Water (control)

Cold stratification

Seeds were kept at 4 °C (refrigerator) in Petri dish for ten days. Freezing stratification: Seeds were kept at -17 °C (freezer) in Petri dish for one day, and then thawed and the seeds were placed in a refrigerator for nine days. After this treatment (ten days), seeds were washed with sterilized water and transferred to a new Petri dish containing filter paper and sterilized distilled water and stored for 40 days in a refrigerator at 4 °C, filter papers were kept moist with sterilized distilled water during the experiments.

Germination analysis

Germinated seeds were counted every 24 h for 40 days. A seed was considered as germinated when the radicle tip had grown free of its coat (when the radicle showed at least 2 mm in length). Counting was continued until the cumulative value of the germinated seeds reached a constant level.

Germination percentage was calculated with the following formula (1) (Nadjafi et al., 2006).

$$GP = N/Nt \times 100 \qquad (1)$$

Where N = Number of germinated seeds, and Nt = Total number of seeds.

Mean germination time (MGT) was measured for seed germination of time period, after applying each treatment (2) (Ellis & Robert, 1981).

$$MGT = \Sigma n \times x/\Sigma n \qquad (2)$$

Where n is the number of seeds newly germinated at the day of x, x is the number of days from sowing. Σn is the total number of germinated seeds during the test.

Germination rate (GR) is the number of germinated seeds per day, which was determined according to Yang et al. (2007) (3).

$$GR = \sum_1^i ni/di \qquad (3)$$

Where ni is the number of germinated seeds after i days from the start of imbibition (di).

Statistical analysis

This research was carried out as a factorial experiment in Completely Randomized Design (CRD) with five replications. All experiments were conducted twice and the mean results of the experiments were presented. To detect significant differences among means, the data were statistically calculated according to the analysis of variance (ANOVA) by Duncan's multiple range test ($P \leq 0.05$) using the SPSS 23 software (IBM, Armonk, NY, USA). The results were shown as mean ± SE (standard error of the mean).

RESULTS AND DISCUSSION

Asafoetida seed germination percentage under different treatments showed positive correlations with germination rate. In other words, when germination rate of seeds increased, their germination percentage also significantly increased. Therefore, fast seed germination was associated with high germination percentage (Fig. 2).

We observed that the cold stratifications stimulated the germination of asafoetida seeds. The cold treatment was effective to overcome seed dormancy and germination percentage. In the control (0CW: soaking in water under 4 °C) these results were observed: seed germination percentage (63.3%), germination rate (no/day; 0.23), mean germination time (day; 38.5) and root elongation (cm; 3.3).

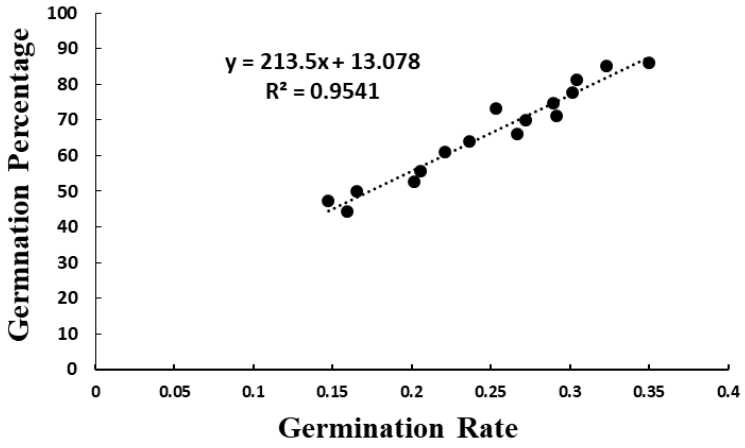

Fig. 2. Correlation between asafoe tida seed germination percentage and rate.

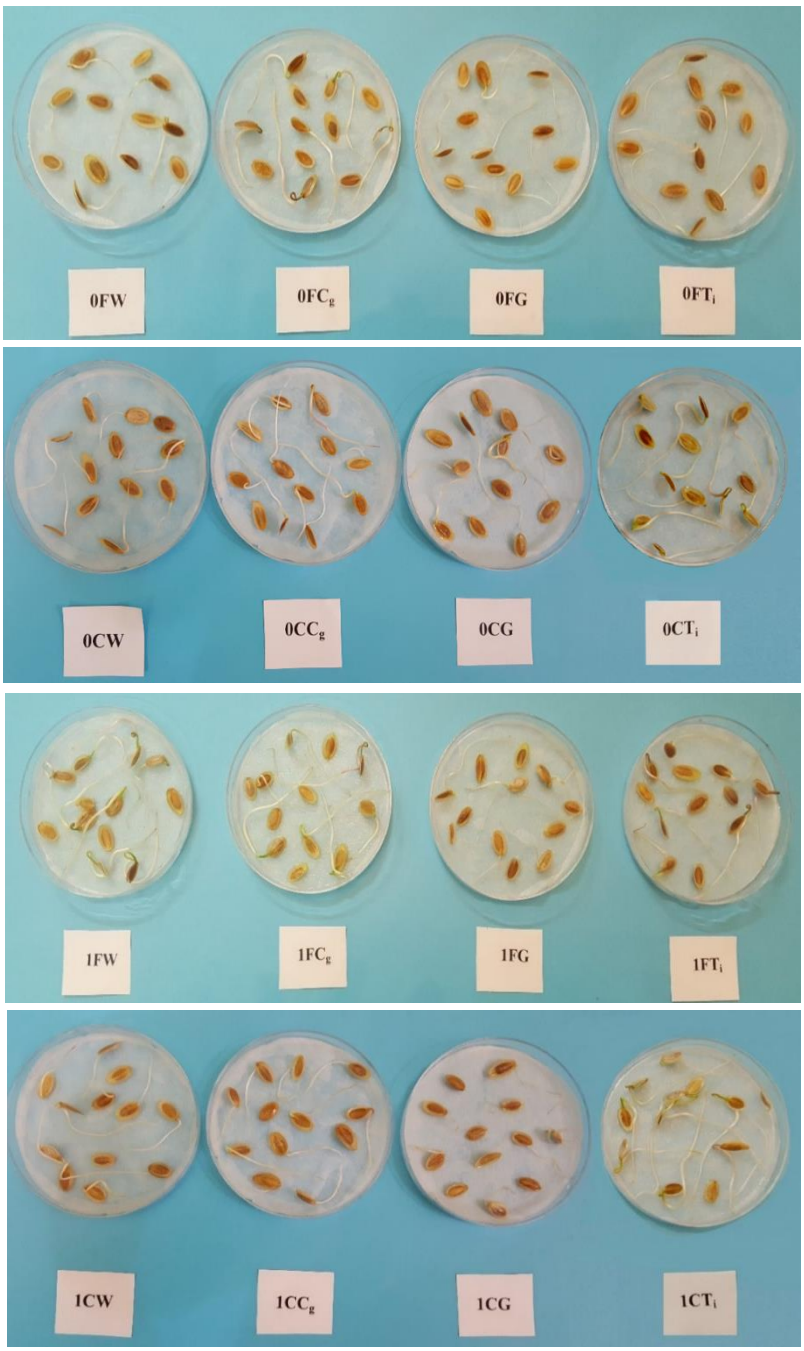

Fig. 3. Effect of seed germination treatments on radicle emergence of germinated seedlings.
Seed treatments are abbreviated as follows:
0: Soak-leaching in water, 1: Soak-leaching in kinetin, C: Cold stratification, Cg: Carrageenan,
F: Freezing stratification, G: GA_3 and Ti: TiO_2 NPs treatments, W: Water (control).

The seeds that were soaked in kinetin and exposed to cold temperatures improved germination percentage and rate (69.9% and 0.27, respectively), and reduced mean germination time (32.7 days, Table 2). In the present study, pre-treatment and soaking of the asafoetida seeds by kinetin solution (under freezing or cold treatments) increased root length (5.1 cm, Table 2). Results showed that soaking of the seeds in kinetin solution which were under freezing temperature did not improve seed germination percentage and rate notably although, the root growth significantly improved (63.9%, 0.24 and 5.5 cm, respectively).

Table 2. Seed germination of asafoetida under physical, plant growth regulators and TiO_2 NPs treatments.

Treatments	Germination percentage (%)	Germination rate (n/day)	Mean germination time (day)	Root elongation (cm)
0CW	63.3±2.3^{fg}	0.23±0.008^{fg}	38.5±1.02^{a}	3.3±0.09^{g}
0FW	44.4±3.9^{k}	0.16±0.001^{j}	32.3±0.57^{ef}	3.6±0.12^{g}
0FCg	52.8±3.9^{ij}	0.20±0.008^{i}	29.9±0.42^{g}	4.8+0.12^{d}
0FG	50±0.4^{ijk}	0.16±0.007^{j}	34.8±0.58^{bc}	4.4±0.15^{e}
0FTi	47.2±3.9^{jk}	0.15±0.016^{j}	38.1±0.66^{a}	4.0±0.14^{f}
0CCg	81.2±2.9^{abc}	0.30±0.003^{bc}	36.3±0.54^{b}	5.3±0.12^{bc}
0CG	71.2±3.8^{def}	0.29±0.01^{cd}	30.2±0.62^{g}	3.6±0.09^{g}
0CTi	77.8±3.9^{bcd}	0.30±0.008^{bc}	30.7±0.51^{fg}	5.3±0.14^{bc}
1FW	63.9±3.9^{fg}	0.24±0.16^{gh}	36.1±0.39^{b}	5.5±0.13^{ab}
1FCg	66.2±0.9^{efg}	0.27±0.12^{ef}	29.7±1.14^{g}	5.8±0.17^{a}
1FG	55.6±3.9^{hi}	0.21±0.15^{i}	34.4±1.2^{cd}	4.0±0.15^{f}
1FTi	61.1±3.9^{gh}	0.22±0.007^{hi}	29.8±0.85^{g}	4.8±0.13^{d}
1CW	69.9±4.9^{def}	0.27±0.005^{def}	32.7±1.2^{de}	4.1±0.1^{f}
1CCg	85±4.9^{ab}	0.32±0.005^{b}	31.2±1.2^{efg}	5.1±0.08^{cd}
1CG	74.7±4.5^{cd}	0.29±0.16^{cde}	32.6±0.87^{de}	2.8±0.09^{h}
1CTi	86.1±3.9^{a}	0.41±0.009^{a}	25.9±0.81^{h}	5.1±0.11^{cd}

0: Soak-leaching in water, **1**: Soak-leaching in kinetin, **C**: Cold stratification, **Cg**: Carrageenan, **F**: Freezing stratification, **G**: GA_3 and **Ti**: TiO_2 NPs treatments, **W**: Water (control).
Mean values ± standard error; different letters in the same column indicate significant differences among treatments ($p \leq 0.05$).

Seed germination and dormancy play important roles in plant reproduction. Seed germination is the process, in which physiological and morphological changes culminated in the activation of embryo. If the seed dormancy is broken, the expansion and development of embryo will occur successfully. The mechanism of seed germination is completed when the radicle has grown out of the seed coat (Hermann et al., 2007). Most of the medicinal plants suffer from low seed germination including those in Apiaceae family that possess morpho-physiological seed dormancy (Hassani et al., 2010). There are many environmental factors that may affect seed germination and dormancy: light, temperature, water, plant growth regulators, smoke, oxygen and carbon dioxide (Taiz et al., 2015). Many seeds respond to more than one environmental factor. Different dormancy breaking treatments can either substitute for each other or work in combinational treatments (Taiz et al., 2015). Asafoetida is originated in cold climate; therefore, stratification is helpful to break seed dormancy. In this study, we observed that the cold stratification improved the germination of asafoetida seeds. Temperature is the most important environmental factor for breaking seed dormancy among medicinal plant seeds from the temperate or cold regions (Baskin & Baskin, 1998). The balance between inhibitors and stimulators determines the status of embryo dormancy. The most important inhibitor of seeds germination is abscisic acid (ABA) (Taiz et al., 2015). Before seed to germinate, these inhibitors must be removed; therefore, seeds need to have enough moisture to wash out these inhibitors. Seed soaking and leaching treatment releases the inhibitors and improves germination. The action of low temperatures in breaking dormancy may be due to the reduction of inhibitors level, and/or the increasing level of stimulator hormones. Cold stratification of *Acer morrisonense* seeds for 12 weeks showed the substantial changes in biochemicals and ultrastructure traits of embryo. ABA content reduced up to 3.3-fold, proteins and lipids decreased significantly but total soluble sugars and amino acids increased markedly. Sucrose increased considerably during the stratification period. However, after radicle emergence it catabolized significantly. It was suggested that hydrolyzed lipids, sugars and oligopeptides and amino acids supply needed energy for cell division and seed germination. Large vacuoles formed in cotyledons and true leaves of germinated seeds indicated that the depleted lipid and protein bodies provide space to absorb more water in organelles for growth and development (Chen et al., 2015).

Freezing acts as the abiotic stress and probably produced chemicals such as osmolytes and oxidants that impede seed germination. Although we did not measure these compounds, it seems that freezing especially in combination with other treatments works as drought stress that led to the longer roots. The most important functions of plant growth regulators are controlling and coordinating cell division, growth, differentiation and dormancy breaking (Majumdar & Kar, 2021). Cytokinins are plant growth regulators that control wide range of plant processes including seed germination. They are active in all germination steps (Riefler et al., 2006). Asafoetida seed has a rudimentary embryo, cytokinins assist the growth and development of the embryo and therefore, the seeds were soaked in kinetin solution. The results showed that in most cases, soaking of asafoetida seed with kinetin solution under cold was more effective than freezing temperatures to enhanced seed germination rate and percentage. Also, seeds soaking in kinetin at cold temperatures significantly reduced mean germination time. Soaking and leaching of asafoetida seeds in kinetin solution improved root growth. Cytokinin regulates different processes related to the seed physiology and development such as the development of embryo by affecting the cell division, seed size and germination, radicle growth, hypocotyl and shoot growth, and minerals uptake (Heyl et al., 2012). Santner et al. (2009) reported that the cytokinins improve seed germination by increasing the activities of meristemic cells in both radicle and plumule.

In our study, it was observed that GA_3 treatment in water-soaked seeds under cold treatment, significantly increased germination percentage and decreased mean germination time (71.2% or 30.2 days). GA_3 treatment in the seeds that were soaked in water and were under freezing treatment decreased germination percentage and rate but increased root length (50%, 0.16 and 4.4 cm). GA_3 treatment in the seeds that were soaked in kinetin and were under freezing temperature did not increase percentage of seed germinations (55.6%, Table 2). In this study, GA_3 treatment in combination with cold and kinetin improved asafetida seed germination and rate but not with freezing treatment.

It has been shown that stratification causes an increase in internal GA concentration. GA stimulates hydrolytic enzyme activity and concentration (Apipinis et al., 2012). It probably induces respiratory systems contain citric acid cycle, glycolysis, and pentose phosphate pathway which provide enough ATP for germination process (Norastehnia et al., 2007). It was reported that GA_{4+7} at the concentration of 100 ppm and 500 ppm significantly increased seed germination and establishment of pennycress. Both treatments as soaking in GA or seed pelleting with GA showed very promising results compared to the control (Koirala et al., 2022). It seems that exogenous GA_3 application enhanced seed germination by inhibiting ABA activity. It is caused by the activation of hydrolase enzymes and inhibition of the related biosynthesis pathways for ABA, which decreases seed ABA content (Atia et al., 2009). Moreover, GA stimulates the production of proteins and catabolizing enzymes (hydrolases), especially amylase, glucanases and proteases resulting in seed germination (Atia et al., 2009, Kavandi et al., 2018; Yamaguchi, 2008). Proteins involve in the conversion of cell wall like expansins and xylo glucanendo trans glycosylase/hydrolases can improve embryo growth (Voegele et al., 2011). The study of the effects of GA and moist prechilling on asafoetida seed dormancy showed that the simultaneous application of GA and stratification significantly improved seed germination. However, GA treatment alone at 400 ppm failed to improve seed dormancy noticeably. It was suggested that although one function of cold treatment is to stimulate GA production, moist stratification must have other mechanisms irrelevant to GA increase in asafoetida for seed dormancy breaking (Sharifi et al., 2017).

In our experiments, carrageenan application on the asafoetida seeds that were exposed to cold treatment increased the germination percentage and rate (81.2% and 0.3). Also, carrageenan in seeds that were soaked in kinetin and were treated under cold temperature

significantly improved both the germination percentage and root elongation (85% and 5.1 cm, Table 2). Carrageenan in seeds that treated with freezing temperature and kinetin produced the longest roots (5.8 cm, Fig. 3). Carrageenan treatment increased root growth in almost all groups of the asafoetida seeds. In the seeds that were soaked in water and exposed to freezing temperature and carrageenan both the germination percentage and rate were low (52.8% and 0.2, respectively) but roots were longer compared to the control (4.4 cm). We observed that in seeds that were soaked in water and exposed to freezing temperature, carrageenan treatment was not significant.

The marine red algae oligosaccharide trigger seed germination by increasing the metabolic activity (Hu et al., 2004). The kappa, iota and lambda-carrageenan increased plants growth by enhancing photosynthesis, rubisco and glutamate dehydrogenase activity, which is involved in cell proliferation, nitrogen assimilation and basal metabolism (Vera et al., 2012). Ahmadi Mousavi et al. (2017 and 2018) reported that the application of kappa-carrageenan induced beneficial effects in plants such as increasing ions uptake, regulation of water absorption, growth and antioxidant enzyme activities.

TiO_2 NPs treatment (10 mg L^{-1}) on the asafoetida seeds that were soaked in water and were under cold temperature, increased germination percentage and rate (77.8% and 0.3, respectively; Table 2). Nevertheless, TiO_2 NPs treatment in the seeds that were soaked in water and under freezing temperature showed one of the lowest seed germination percentage and rate (47.2% and 0.15, respectively) (Table 2). TiO_2 NPs treatment of the seeds that were soaked and leached with kinetin solution and under freezing temperature did not significantly improved seed germination percentage, rate and mean germination time (61.1%, 0.22 and 29.8 days). The highest percentage of germination (86%) was observed in kinetin pre-treated seeds under cold and TiO_2 NPs. Moreover, the mean germination time of these seeds decreased up to 25 days. TiO_2 NPs treatment increased the root length of asafoetida seeds under all treatments (Table 2, Fig. 3).

TiO_2 NPs treatment stimulates seed germination and seedlings growth. Lu et al. (2002) showed that TiO_2 NPs could increase soybean (*Glycine max*) abilities to absorb water and fertilizers which accelerate the seed germination and growth (Lu et al., 2002). The engineered nanoparticles could sequester nutrients on their surfaces and serve as a nutrient stock to the organisms, particularly those with high specific surface area (Navarro et al., 2008). It has been stated that the germination rate of spinach (*Spinacia oleracea*) was enhanced with TiO_2 NPs treatments (Zheng et al., 2005). It seemed that TiO_2 NPs increase embryo growth, rubisco activity, chlorophyll formation, and the photosynthetic rate. It was reported that in treated *S. oleracea* by TiO_2 NPs, rubisco activity was 2.67 times higher than the control (Gao et al., 2006).

CONCLUSION

The results of this study showed that the seed of asafoetida is temperature dependent. To break seed dormancy in asafoetida, usually low temperature is used. Although, this technique is simple and cheap, it takes long time and produce weak roots. Here, we report the combinational treatments that are cost effective and need less time to improve seed germination. It is concluded that seed soaking in water or kinetin then carrageenan or TiO_2 NPs treatments under cold temperature at 4 °C were the most effective treatments to break asafoetida seed dormancy to improve seed germination percentage, rate and root growth. These treatments also produce stronger roots.

Conflict of interest

The authors declare no conflict of interest to report.

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Effects of electromagnetic treated saline water on potatoes (*Solanum tuberosum* L.) physiological and nutritional characteristics

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ABSTRACT

Purpose: This work was designed to assess the physiological response of three potatoes cultivars to saline water irrigation, and the role of the electromagnetic treatment on alleviating salinity impacts on potatoes crops. **Research Method:** The experiment was conducted under factorial RCBD with potato varieties Spunta, Bellini and Alaska under three irrigation treatments; ground water (C), saline water (SW) and electromagnetic saline water (EMSW). We analyzed soil proprieties, minerals and water usage efficiency, plant water status, chlorophyll pigments, absiscic acid (ABA) content, and expression of *StNCED* gene. **Findings:** Data showed that EMSW promote salt leaching from soil, decrease soil salinity and improve the efficiency of water and minerals use. Leaf area, leaf dry weight, stem thickness, tuber weight, water potential, water content and water use efficiency were more disturbed under SW. The ABA content in Alaska leaves was associated with *StNCED* expression level. The cultivar Alaska displayed highest leaf size, SPAD and minerals use efficiency. Spunta had upmost correlation between LA and WC_{ap} and highest water use efficiency. Contrarily, Bellini manifested less water potential and ABA content. **Research limitations:** No limitations were found. **Originality/value:** Thus, Spunta and Alaska revealed better physiological and metabolic capacity to tackle salinity. The results advance knowledge on potatoes response to salinity and could improve management of saline water.

INTRODUCTION

Saline water is becoming a foremost factor hampering the productivity of cultivated soils. About 20% (45 million ha) of irrigated lands, bringing one-third of the world's food, are salt-affected. Soil salinization results in more than US$12billion annual losses in the world due to reduced crop productivity (Jägermeyr & Frieler, 2018). These proportions may be increased by climate change, saline irrigation and poor drainage. In Tunisia, a growing volume of saline water is being used in irrigation and plenty of irrigated areas showed a rising trend to salinization (Boughdiri et al., 2018). Scarcity of rainfall and high evaporation, principally during dry months of the year caused salts to move up and accumulate in the upper layers causing compact soil structure. This may offset root expansion, water absorption and numerous physiological and metabolic functions (Akrimi et al., 2021).

Potatoes, commonly cultivated in arid and semi-arid areas, are known for their low salt requirements (1.1 dS m^{-1} EC of irrigation water and 1.7 dS m^{-1} in the soil) (Machado & Serralheiro, 2017). Among the known implications of using saline water on potatoes are perturbations of ions homeostasis, photosynthesis and reduction in chlorophyll content (Bhargava et al., 1995). Phytohormone levels are also affected by changes in nutrient elements (Zhang et al., 2016). In this line, Marrush et al. (1998), observed a decrease of ABA concentrations in potassium-limited sorghum plants. In contrast, elevated ABA concentrations have been reported in potato (Bhargava et al., 1995). Plant water status regulation with ABA, is another fundamental physiological process induced by genes encoding enzymes and other proteins involved in cellular dehydration (Zhang et al., 2016).

The great challenge of producing more food crops with poor water quality resources like saline water, have focused many researches on assessment of the reliability of saline water treatment options. It is acknowledged that magnetic treatment of water is very favored at industrial level, specially, to avoid scale formation in pipes. However, magnetic water remains a contentious topic in agriculture. Effects of magnetic fields on water characteristics are linked to changes on dissolved ions, hydrogen bonds, clustering of water molecules, flow rate, viscosity etc. (Amor et al., 2017). It has been reported that magnetic water increases soil aeration and infiltration, which contribute to better soil moisture and reduction of salt buildup in the root zone (Hamza, 2019). The aptitude of electromagnetic water to improve soil structure and ions availability (Akrimi et al., 2021; Zhou et al., 2021), allows suggesting that electromagnetic saline water can enhance the efficiency of water and nutrient usage. In this work, we aim to establish the role of electromagnetic saline water (irrigation and drainage) on potatoes physiological behavior. We hypothesized that electromagnetic saline water may enhance soil properties, salt leaching and therefore plant water relations and ions usage. It is our aim to appraise the role of ABA signaling on physiological and metabolic modulations of potato varieties. The response patterns of leaf area, chlorophyll content, water potential and tuber weight, to water status of aerial parts were evaluated.

MATERIALS AND METHODS

Field experiments and applied treatments

The experiment was conducted at the Regional Center of Agricultural Research of Sidi Bouzid (CRRA-Sidi Bouzid), Tunisia, during two growing seasons (October-February) 2015/2016 and 2016/2017. The mean annual precipitation was 200 mm. The mean evapotranspiration was 1200 mm. The soil texture was sandy-clay, with alkaline pH (7.7), 2.9 mS cm^{-1} electrical conductivity (EC), 7% moisture content and 0.7 sodium absorption ratio (SAR). A factorial design was used with two factors: i) Varieties (Spunta, Bellini and

Alaska); ii) Irrigation treatments (C: ground water with 2.2 mS cm^{-1} EC, EMSW: electromagnetic saline water with 8.5 mS cm^{-1} EC and SW: saline water with 8.5 mS cm^{-1}EC), on the basis of a randomized complete block design (RCBD). Each plot consisted of 9 rows, 2.5 m long, with 100 cm space between rows and 40 cm distance between plants on the rows. Electromagnetic treatment of water was done with Aqua-4D® 60E series device, that serves to circulate water through an electro magnet tube 60E, designed for transmitting electromagnetic signals into water and connected to an electro magnet box (Command 60E Pro) pre-programmed to generate electromagnetic signals. The amount of irrigation water was calculated using crop evapotranspiration (ETc) that represent the reference evapotranspiration (ET0) multiplied by the potato crop coefficient (Kc). Values of Kc were 0.5; 1.15 and 0.75 for the beginning (Kcini), the middle (Kcmid) and the end (Kcend) of potatoes growth cycle.

Water and soil analyses

Samples of percolate water, originating from the three types of irrigation treatments (C, SW and EMSW), were taken monthly (Month 1: following one month of irrigation; Month 2: following two months of irrigation; Month 3: following three months of irrigation). Water and soil analysis were carried out according to Sparks (1996) protocols. Soil moisture content (Ɵs) was determined by gravimetric method. Extractable ions were measured in three depths (D1: 0-30 cm, D2: 30-60 cm and D3: 60-90 cm). Sodium (Na^+) and potassium (K^+) contents were determined with flame photometer (Model No. PFP7 JENWAY). Bicarbonate (HCO_3^-), calcium (Ca^{2+}), magnesium (Mg^{2+}) and chloride (Cl^-) contents were quantified by titration method. Sulfate (SO_4^{2-}) concentration was measured by colorimetric method. In fact, 5 g of air dried and sieved soil samples were added to 50 ml of distilled water. Then, the suspension was filtered and 50 ml of the filtrate was taken and added to the mixture (10 ml NaCl-HCl, 10 ml Glycerol-ethanol and 0.15 g barium chloride). Afterward, samples were stirred for about 1h and the absorbance was measured at 420 nm with spectrophotometer (Jenway 6300), using distilled water as blank. The calibration curve was drawn using standard sulphate solutions (Saxena, 2001). The pH and the electrical conductivity (EC) were measured using a pH meter "MP 22, Mettler Toledo, Switzerland" and conductivimeter "Hanna, HI8424, Canada" respectively.

Plant analysis

The efficiency of ions usage (UE) was calculated by dividing the plant dry weight (PDW) by the concentration of specific leaf element ([element]) (1):

$$UE = PDW/[element] \quad (1)$$

The content of Na^+, K^+ and Ca^{2+}were measured using flame photometer (Model No. PFP7 JENWAY) and expressed in meq l^{-1} (Akrimi et al., 2021). Regarding chloride (Cl^-) determination, powdered dry leaves were incubated overnight in a 0.1M HNO_3 and 10% glacial acetic acid solution. After centrifuging, Cl^- concentration was measured with chloridometer (SLAMED) (Gilliam, 1971). Nitrate content was determined using Nitrachek/Merkoquant® method. The stem thickness (ST) was calculated using the formula (2) adopted by Wang et al. (2017):

$$ST = (ST30 - ST0)/30 \quad (2)$$

Where ST30 and ST0 are respectively the stem thickness at day 30 after saline treatments and the stem thickness before saline treatments.

For leaf pigment determination, leaves were digested in acetone 80%, and the extracts were centrifuged for 5 min at 1.500×g. The pellet was re-extracted and centrifuged again, until the supernatant become colorless. The obtained supernatants were pooled and the absorbance was recorded at 452, 644 and 663 nm. Concentrations of leaf pigments were calculated using the following equations (3-6) (Lin et al. 2006):

$$\text{Chla}\ (\mu g\ g-1\ FW) = (10.3\ A663 - 0.918\ A644) \times V/1000 \times FW \quad (3)$$

$$\text{Chlb}\ (\mu g\ g-1\ FW) = (19.7\ A644 - 3.87A663) \times V/1000 \times FW \quad (4)$$

$$\text{Carotenoids}\ (\mu g\ g-1\ FW) = 4.2\ A452 \times \frac{V}{1000} \times FW - (0.026\ \text{chla} + 0.42\text{chlb}) \quad (5)$$

$$\text{Porphyrine}\ (\mu g\ g-1\ FW) = [\text{Chla}] + [\text{Chlb}] + [\text{Carotenoids}] \quad (6)$$

Were A is the absorbance, V: final volume of leaf extract, FW: leaf fresh weight. Leaf electrolyte leakage (EL) was calculated with the following formula (7):

$$EL = (\frac{EC1}{EC2}) \times 100 \quad (7)$$

Where EC1 and EC2 are the EC measured after incubation of leaves in a 32º C water bath for 2 h, and the EC after autoclaving at 121ºC for 20 min respectively (Daneshmand et al., 2010).

For ABA determination, powdered leaves were extracted in distilled autoclaved water with constant shaking at 4°C. The supernatant was collected after centrifugation (10.000 × g for 10 min) and diluted with TBS buffer. Then ABA was analyzed using Phytodetek ABA test kit (Agdia, Elkhart, IN, USA) by indirect enzyme-linked assay (ELISA). Color absorbance following reaction with substrate was determined at 405 nm using a plate autoreader (1420 Multilabel Counter Victor3TM, PerkinElmer) (Ruggiero et al., 2019). Leaf area (LA) was determined using leaf area meter (ADC-Biosciebtific Ltd, Hoddesdon, UK).

Total chlorophyll content (SPAD) was determined with chlorophyll meter (SPAD-502, Minolta, Japan). Water use efficiency (WUE) was calculated (8) as the ratio of potato yield (Y) to total water received (TWR):

$$WUE = (\frac{Y}{TWR}) \quad \text{(Akrimi et al., 2021)} \quad (8)$$

Water content of aerial parts (Leaves and stems) (WC_{ap}) was calculated according to the following formula (9):

$$WCap(\%) = [(FW - DW)/FW] \times 100 \quad (9)$$

Where FW and DW are the fresh and dry weight of leaves and stems (Trifilò et al., 2022). Leaf water potential (Ψ_w), was measured with pressure chamber (*Scholander* model M-1000).

Tuber dry weight was determined after drying in an oven at 75 ºC until weight stabilization.

The RNA was extracted from potato leaves using Trizol method and quantified by ND-1000 spectrophotometer (NanoDropTechnologies). The cDNA was synthesized from 1 µg RNA using Superscript reverse transcriptase (invitrogen) in a 20 µl reaction volume. The program for quantitative real-time PCR (qPCR) was set to 35 cycles of 5 minutes at 94 °C, 30

seconds at 94 °C, 30 seconds at 58 °C, 45s at 72 °C and 10 minutes at 72 °C with ABI 7900 HT (Applied Biosystems, Foster City, CA, USA). PCR reactions were carried out in 96-well optical reaction plates. Reaction included 4.5 µl of 1:20 diluted cDNA, 4.28 µM of primer (Forward primer: CATAATCGAAAACCCGGATG; Reverse primer: AACTTTTGGCCATGGTTCAG) of the *StNCED* gene and 6.25 µl Sybr Green (Akrimi et al., 2021).

Statistics

Statistical analysis was performed using SPSS 20.0. Variables were subjected to two way analysis of variance (ANOVA). Means were compared using LSD's test at $p \leq 0.05$. Linear regression and curve estimation were analyzed using a probability value of 0.05 as the benchmark of significance.

RESULTS

Soil characteristics

The soil EC in the three depths was set in the following decreasing trend $Soil_{SW} > Soil_{EMSW} > Soil_{C}$ (Table 2). The ANOVA results revealed that changes in soil EC were significantly ($p \leq .001$) dependent on irrigation treatments (IT) and soil depth (D), as least values were ascribed to C treatment and deep layer (D3: 60-90 cm). Given that, intermediate and highest values of EC were registered under EMSW and SW respectively. Meanwhile, pH was not influenced by D and IT (Table 1). Regarding soil moisture (Θs), registered proportions were significantly higher under EMSW compared to SW, though C treatment maintains highest Θs level. The Θs was always maintained above 5% within C and EMSW ensuring better soil moisture conditions. The ANOVA results depicted in table 1 revealed that soil Θs was not affected by depth. Moreover, IT influenced significantly soil ions content except HCO_3^-. The highest values of Na^+, SO_4^{2-} and Cl^- were recorded for soil irrigated with SW. Though, K^+, Ca^{2+} and Mg^{2+} concentrations were higher in soil irrigated with EMSW. The ANOVA results revealed that all analyzed ions were significantly influenced by depth (Table 1).

Drained water characteristics

Results in Table 4 revealed that the EC and concentrations of Na^+, Mg^{2+}, SO_4^{2-} and Cl^-were higher in water percolated from EMSW irrigated soil. Additionally, Na^+, K^+ and HCO_3^- concentrations were more important after the third month of irrigation. ANOVA results (Table 3) demonstrated that pH, Cl^-, SO_4^{2-} and Mg^{2+} values were significantly affected by M×IT interaction. In effect, registered values were in the following order EMSW>SW>C for the three months of study. Meanwhile, K^+ and HCO_3^- contents were not changed by IT.

Table 1. Analysis of variance for the effect of depth (D), irrigation treatment (IT) and their interactions on soil proprieties.

	Θs	pH	EC	Na^+	K^+	Ca^{2+} and Mg^{2+}	SO_4^{2-}	HCO_3^-	Cl^-
D	0.89^{ns}	0.22^{ns}	32.73^{***}	33.14^{***}	11.86^{***}	14.48^{***}	17.75^{***}	8.34^{***}	26.56^{***}
IT	5.78^{**}	0.15^{ns}	220.43^{***}	54.92^{***}	63.32^{***}	1.80^{***}	9.97^{***}	2.34^{ns}	28.41^{***}
D×IT	0.78^{ns}	0.049^{ns}	1.66^{ns}	0.84^{ns}	1.69^{ns}	0.70^{ns}	3^{*}	0.26^{ns}	4.12^{**}

ns: not significant, $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq .001$.

Table 2. Physico-chemical proprieties of soil irrigated with ground water ($Soil_C$), saline water ($Soil_{SW}$) and electromagnetic saline water ($Soil_{EMSW}$) after three months of irrigation in three depths (D1: 0-30 cm, D2: 30-60 cm and D3: 60-90 cm).

		$Soil_C$	$Soil_{SW}$	$Soil_{EMSW}$
Θs (%)	D1	7.61±0.33^a	4.08±0.44^c	5.53±0.08^b
	D2	5.59±0.41^a	4.08±0.63^b	5.81±0.21^a
	D3	5.64±0.33^a	4.09±0.45^b	5.06±0.08^a
EC (dSm^{-1})	D1	4.57±0.00^c	8.45±0.02^a	7.22±0.41^b
	D2	3.51±0.28^c	7.64±0.15^a	6.44±0.01^b
	D3	3.49±0.28^c	7.31±0.15^a	6.24±0.01^b
pH	D1	8.14±0.10^a	8.29±0.01^a	8.14±0.00^a
	D2	8.30±0.02^a	8.35±0.02^a	8.25±0.00^a
	D3	8.33±0.02^b	8.35±0.02^a	8.35±0.00^a
Na^+ ($meql^{-1}$)	D1	3.03±0.10^c	4.50±0.21^a	3.36±0.22^b
	D2	1.93±0.10^c	3.63±0.24^a	2.70±0.30^b	
	D3	1.80±0.07^b	3.40±0.06^a	1.94±0.46^b
K^+ (meq l^{-1})	D1	2.53±0.14^a	1.30±0.07^c	2.03±0.10^{ab}
	D2	1.96±0.09^a	1.10±0.18^b	1.73±0.04^a
	D3	1.87±0.04^a	1.13±0.07^b	1.77±0.04^a
Ca^{2+} and Mg^{2+} (meq l^{-1})	D1	11.07±0.81^a	8.53±0.91^b	9.73±0.59^b
	D2	10.27±0.65^a	7.60±0.28^c	10.40±0.37^b
	D3	7.60±0.37^a	6.00±0.49^b	7.87±0.39^a
SO_4^{2-} (meq l^{-1})	D1	12.06±0.94^c	15.77±1.20^a	13.10±0.55^b
	D2	7.69±1.29^c	13.68±0.47^a	8.17±0.54^b
	D3	8.32±0.76^b	10.00±0.60^a	9.98±0.15^a
HCO_3^- (meq l^{-1})	D1	10.66±0.81^a	9.33±1.16^a	10.66±1.14^a
	D2	8.66±0.81^a	6.00±0.70^a	7.33±0.81^a
	D3	8.00±0.70^a	6.00±0.70^a	6.10 ±0.81^a
Cl^-(meq l^{-1})	D1	53.33±4.08^c	76.66±4.08^a	63.44 ±3.22^b
	D2	33.33±4.08^c	63.33±4.08^a	53.33±3.33^b
	D3	33.33±5.40^c	53.33±3.33^a	36.66±3.33^b

Means ± SE (n = 4). Different letters indicate significant differences between irrigation treatments, in the same depth, according LSD's test.

Table 3. Analysis of variance of the effect of month of irrigation (M), irrigation treatment (IT) and their interactions on percolating water characteristics.

	pH	EC	Na^+	K^+	Ca^{2+}	Mg^{2+}	SO_4^{2-}	HCO_3^-	Cl^-
M	7.17***	18.02***	3.20^{ns}	48.10***	17.33***	18.79***	32.96***	16.88***	7.81***
IT	1.51^{ns}	99.26***	161.63***	3.24^{ns}	15.99***	15.30***	314.77***	0.55^{ns}	51.26***
M×IT	9.03***	1.86^{ns}	0.53^{ns}	2.15^{ns}	2.28^{ns}	3.59*	27.95***	0.55^{ns}	8.40***

ns: not significant, $^*p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq .001$.

Ions and water usage efficiency

From Table 5 and 7, ions usage efficiency, in response to irrigation treatments, differed among assessed varieties, as indicates the significant effect of irrigation treatment, variety and their interactions. Alaska had highest ions UE. The UE of Na^+, Ca^{2+}, Cl^- and NO_3^- were least under SW. However, K^+UE was enhanced with EMSW only in Alaska. There is also a clear variation in ions UE between studied elements. In fact, Na^+ UE was higher than other studied elements; NO_3^- UE was higher than Cl^-UE while K^+, Cl^-, and Ca^{2+} had comparable values. The antagonism between Cl^- and NO_3^- accumulation may reflect the osmoregulatory role of Cl^- in potato leaves, whereas NO_3^- may be allocated to plant organs. ANOVA analysis revealed that, WUE was significantly ($p \leq 0.05$) least under SW and in Bellini variety. Indeed, Spunta had highest WUE.

Table 4. Characteristics of drained water after three months of irrigation (Month 1, 2, and 3).

		C	SW	EMSW
EC (dSm^{-1})	Month 1	5.41 ± 0.47^{c}	9.66 ± 0.34^{b}	13.07 ± 1.50^{a}
	Month 2	6.78 ± 0.62^{b}	10.89 ± 1.12^{a}	13.27 ± 1.95^{a}
	Month 3	7.05 ± 1.24^{c}	14.57 ± 0.89^{b}	17.59 ± 0.07^{a}
pH	Month 1	7.56 ± 0.00^{a}	7.46 ± 0.00^{b}	7.05 ± 0.00^{c}
	Month 2	7.11 ± 0.08^{a}	6.99 ± 0.19^{a}	7.18 ± 0.02^{a}
	Month 3	6.67 ± 0.27^{b}	7.31 ± 0.13^{a}	7.23 ± 0.01^{a}
Na^{+}($meql^{-1}$)	Month 1	8.46 ± 0.21^{c}	39.33 ± 0.81^{b}	49.66 ± 4.72^{a}
	Month 2	12.23 ± 3.43^{c}	39.23 ± 5.04^{b}	48.66 ± 1.72^{a}
	Month 3	13.56 ± 3.45^{b}	48.66 ± 2.04^{a}	53.00 ± 3.53^{a}
K^{+}($meql^{-1}$)	Month 1	1.55 ± 0.21^{b}	2.94 ± 0.07^{a}	3.00 ± 0.07^{b}
	Month 2	7.14 ± 0.28^{a}	6.23 ± 0.37^{a}	6.86 ± 0.80^{a}
	Month 3	9.00±0.70^{b}	8.66±1.35^{b}	13.15 ± 1.08^{a}
Ca^{2+}($meql^{-1}$)	Month 1	20.13 ± 2.47^{a}	15.20 ± 3.18^{a}	22.13 ± 4.71^{a}
	Month 2	37.33 ± 4.32^{a}	22.26 ± 3.11^{b}	39.20 ± 5.16^{a}
	Month 3	38.80 ± 4.82^{a}	24.26 ± 3.46^{b}	32.93 ± 3.40^{a}
Mg^{2+}($meql^{-1}$)	Month 1	15.40± 3.20^{b}	15.33 ± 1.77^{a}	18.53 ± 2.16^{a}
	Month 2	10.80 ± 2.31^{b}	23.13 ± 1.75^{a}	28.53 ± 1.31^{a}
	Month 3	6.06 ± 1.68^{c}	22.00 ± 5.65^{b}	38.00 ± 4.67^{a}
SO_4^{2-}($meql^{-1}$)	Month 1	4.45±0.55^{c}	25.83±0.52^{b}	39.96±4.20^{a}
	Month 2	4.14±0.75^{c}	25.53±1.65^{b}	43.94±0.29^{a}
	Month 3	5.20±0.35^{c}	23.23±2.21^{b}	22.31 ±1.59^{a}
HCO_3^{-}($meql^{-1}$)	Month 1	8.66 ± 0.81^{a}	8.00 ± 1.41^{a}	8.00 ± 1.41^{a}
	Month 2	9.33 ± 0.81^{a}	8.00 ± 1.41^{a}	10.66 ± 1.60^{a}
	Month 3	12.66 ± 1.63^{a}	13.33 ± 0.81^{a}	14.00 ± 1.41^{a}
Cl^{-}($meql^{-1}$)	Month 1	17.33 ± 0.81^{c}	62.00 ± 1.41^{b}	115.33 ± 2.94^{a}
	Month 2	36.66 ± 10.61^{c}	86.00 ± 11.37^{b}	94.66 ± 2.94^{a}
	Month 3	59.33 ± 13.06^{c}	105.33 ± 9.14^{b}	134.66 ± 2.16^{a}

Means ± SE (n = 4). Different letters indicate significant differences between irrigation treatments in the same month according to LSD's test ($p \leq 0.05$).
C: Ground water; EMSW: Electromagnetic saline water; SW: Saline water.

Table 5. Sodium usage efficiency (Na^{+}UE), potassium usage efficiency (K^{+}UE), calcium usage efficiency (Ca^{2+}UE), chloride usage efficiency (Cl^{-}UE) and water use efficiency (WUE) of potato varieties.

		Spunta	Bellini	Alaska
Na^{+}UE (g meq l^{-1})	C	261.00 ± 29.04^{a}	77.97 ± 25.09^{a}	900.71 ± 250.59^{a}
	SW	12.78 ± 1.01^{c}	9.97 ± 2.54^{c}	36.42 ± 5.94^{c}
	EMSW	42.44 ± 0.20^{b}	28.74 ± 0.31^{b}	126.97 ± 0.54^{b}
K^{+}UE (g meq l^{-1})	C	17.35 ± 0.69^{a}	6.86 ± 1.11^{a}	20.57 ± 2.03^{a}
	SW	8.99 ± 0.84^{b}	3.28 ± 1.01^{b}	12.25 ± 0.62^{c}
	EMSW	11.44 ± 0.36^{b}	3.86 ± 6.58^{b}	14.06 ± 0.80^{b}
Ca^{2+}UE (g meq l^{-1})	C	9.70 ± 0.51^{a}	7.31 ± 0.93^{a}	16.69 ± 1.64^{a}
	SW	4.33 ± 0.28^{c}	3.48 ± 0.70^{c}	7.93 ± 0.63^{c}
	EMSW	5.90 ± 0.29^{b}	5.03 ± 0.71^{b}	10.77 ± 0.40^{b}
Cl^{-}UE (g $mmoll^{-1}$)	C	12.07 ± 0.63^{a}	9.16 ± 0.35^{a}	24.02 ± 1.55^{a}
	SW	5.42 ± 0.32^{c}	5.65 ± 1.72^{c}	14.23 ± 1.27^{b}
	EMSW	9.13 ± 0.47^{b}	7.73 ± 0.71^{b}	20.82 ± 0.40^{a}
NO_3^{-} (g ppm^{-1})	C	197.68 ± 18.17^{a}	69.08 ± 26.17^{a}	639.53 ± 139.82^{a}
	SW	12.24 ± 3.21^{c}	7.28 ± 1.70^{c}	33.36 ± 6.88^{c}
	EMSW	38.61 ± 1.44^{b}	22.72 ± 2.41^{b}	113.91 ± 6.03^{b}
WUE (Kg m^{-3})	C	5.31 ± 0.16^{a}	3.82 ± 0.15^{a}	4.99 ± 0.18^{a}
	SW	4.20 ± 0.19^{b}	2.63 ± 0.35^{b}	3.08 ± 0.15^{ab}
	EMSW	4.94 ± 0.14^{c}	2.81 ± 0.23^{b}	3.51 ± 0.16^{b}

Means ± SE (n = 4). Different letters in the same row indicate significant differences between irrigation treatments according to LSD's test at ($p < 0.05$).

Stem thickness, leaf pigment content and electrolyte leakage

From Table 6 and ANOVA results summarized in Table 7, the ST did not show any significant differences among saline treatments (SW and EMSW) in Spunta and Bellini. However, Alaska had its ST 63%higher under EMSW than SW. Concentrations of chlorophyll b, carotenoids and porphyrin did not show any significant difference between water treatments. In opposition, lowest chlorophyll a concentration was registered under SW. Already; SPAD behaved similar response to IT, as least values were recorded for plants grown under SW (Figure 1). The ANOVA results on Chla showed significant effect of water treatments and variety. Thus highest concentrations were recorded for C treatment and Alaska variety. Leaf EL varied substantially among irrigation treatments in Spunta and Alaska with highest and least values were recorded for C and SW respectively. Comparable level of EL was found in Bellini plants irrigated with SW and EMSW.

Table 6. Leaf pigment content, electrolyte leakage (EL) and stem thickness (ST) of potato varieties.

		Spunta	Bellini	Alaska
ST (cm)	C	0.07±0.01^{a}	0.04±0.01^{a}	0.17±0.01^{a}
	SW	0.04±0.00^{b}	0.05±0.02^{a}	0.11±0.01^{b}
	EMSW	0.04±0.00^{b}	0.04±0.01^{a}	0.18±0.03^{a}
Chl a (µg g^{-1} FW)	C	2154.98±213.84^{a}	1564.08±253.63^{a}	1810.73±604.21^{a}
	SW	1261.90±154.47^{c}	862.53±32.00^{c}	1348.13±256.83^{c}
	EMSW	1917.30±253.75^{b}	1112.18±161.43^{b}	1541.57±170.69^{b}
Chl b (µg g^{-1} FW)	C	1101.09±195.63^{a}	1405.23±176.29^{a}	1499.50±72.80^{a}
	SW	953.61±167.80^{a}	792.49±56.90^{c}	1121.44±203.58^{ab}
	EMSW	1013.55±204.04^{a}	1164.88±172.72^{b}	1343.16±210.99^{a}
Carotenoid (µg g^{-1} FW)	C	762.64±75.27^{a}	303.26±456.90^{a}	1111.82±469.45^{a}
	SW	558.39±126.49^{a}	240.64±163.63^{a}	409.93±172.34^{c}
	EMSW	424.75±104.89^{a}	362.74±0.00^{a}	740.06±240.45^{b}
Porphyrin (µg g^{-1} FW)	C	3680.83±504.28^{a}	3580.33±681.41^{a}	4422.06±61.85^{a}
	SW	3429.30±538.48^{a}	3209.97±591.05^{a}	2879.51±567.30^{b}
	EMSW	3038.09±261.10^{a}	2017.79±20.37^{b}	3624.79±621.54^{a}
EL (%)	C	63.91±4.78^{a}	69.17±9.23^{a}	83.93±4.33^{a}
	SW	60.31±6.60^{b}	70.44±3.25^{a}	72.57±4.12^{b}
	EMSW	64.42±5.16^{a}	65.13±6.27^{a}	80.07±3.61^{a}

Means ± SE (n = 4). Different letters in the same row indicate significant differences between irrigation treatments according to LSD's test (p <0 .05).

Table 7. Analysis of variance of the effect of irrigation of treatment (IT), variety (V) and their interactions on stem thickness (ST), leaf pigments, electrolyte leakage (EL), water (WUE) and ions use efficiency (UE).

	ST	Chla	Chlb	Carotenoid	Porphyrin	EL
V	56.65**	5.45*	3.60*	0.54^{ns}	2.41^{ns}	12.43***
IT	3.04^{ns}	4.38*	2.25^{ns}	0.16^{ns}	3.03^{ns}	7.50**
IT×V	3.19^{ns}	1.69^{ns}	2.42^{ns}	5.66**	2.79*	2.17^{ns}

ns: not significant, $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq .001$.

Table 7. (Continued).

	WUE	Na^{+}UE	K^{+}UE	Ca^{2+}UE	Cl^{-}UE	NO_3^{-} UE
V	81.40***	17.43**	142**	89.09**	211.77**	28.48**
IT	55.44***	29.09**	57.24**	69.93**	53.58**	46.36**
IT×V	3.51*	11.16**	2.82*	4.27*	4.22*	15.83**

ns: not significant, $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq .001$.

ABA content and *StNCED* expression level

From Figure 1 A, ABA level in potato leaves was significantly increased under SW in Bellini and Alaska. However, ABA synthesis remained nearly constant in Spunta, under the three types of irrigation treatments. The least values of ABA content in Bellini leaves suggested a less in ABA metabolism. The increase in ABA synthesis of Alaska under saline treatments was associated to *StNCED* over expression. In Spunta, the marked increase of *StNCED* expression (2 fold) under EMSW was not consistent with ABA level.

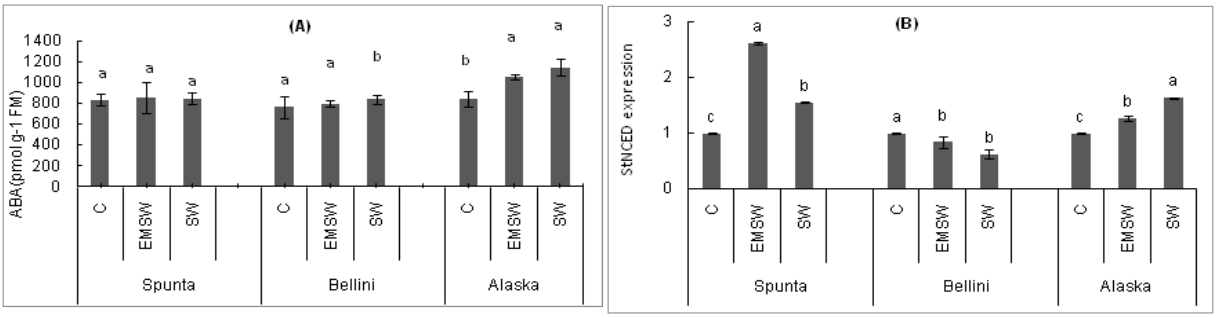

Fig. 1. Changes of leaf absiscic acid (ABA) content (A) and relative expression pattern of *StNCED* gene (B) in potatoes.

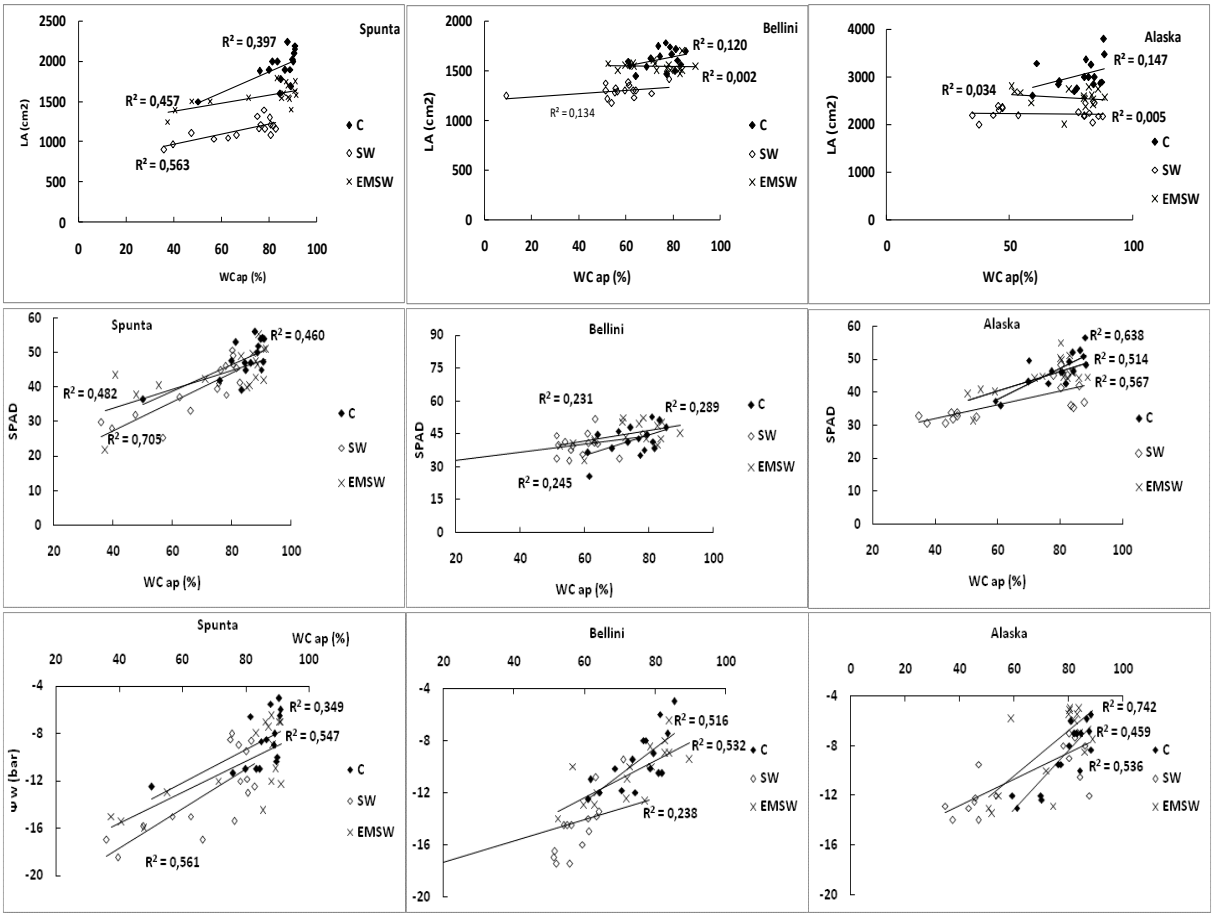

Fig. 2. Relationship between leaf area (LA) and water content of aerial part (WCap); SPAD and water content of aerial part (WCap); leaf water potential (Ψw) and water content of aerial part (WCap).

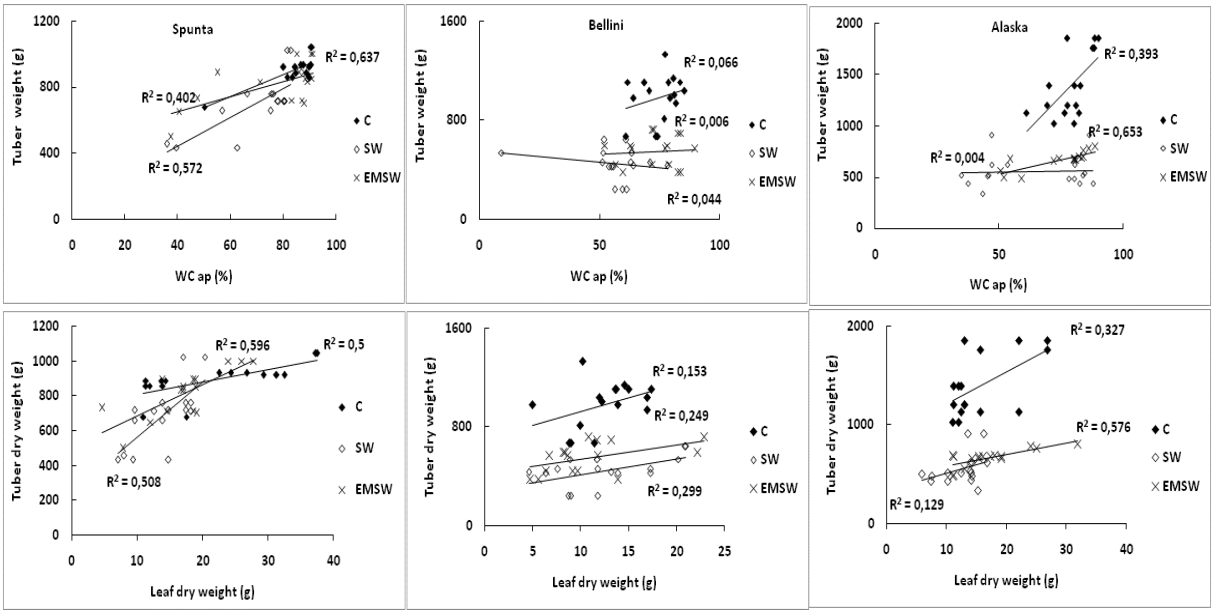

Fig. 3. Relationship between tuber dry weight and water content of aerial part (WCap); tuber dry weight and leaf dry weight.

Leaf area, SPAD and water status

Leaf area (LA) of Spunta have globally an increased trend as WCap increased, reaching 2245 cm^2 by 87% WCap (R^2=0.39) in plants grown under C treatment; 1132 cm^2 by 75%WCap (R^2=0.56) under SW and 1750 cm^2 by 88% WCap (R^2=0.47) under EMSW (Fig. 2). Low correlation coefficients (R^2) were found between LA and WCap in Bellini and Alaska. The linear relationship between SPAD and WCap was clearer and showed higher R^2 coefficients. Concomitantly to WCap, SPAD was more elevated under C and EMSW. In spite, Bellini showed a similarity in SPAD values between SW and EMSW. Nevertheless, Alaska had the highest SPAD and LA. In the other hand, it seems that Bellini irrigated with SW was less efficient in water usage due to the inability to sustain high Ψw, that ranged from -9.5 to -18 under SW facing values within the range -8 to -17 in Spunta and -7 to -14 in Alaska.

Tuber weight and water status of aerial part

Tuber weight of Spunta was increased favorably with the increase of WCap. Similar relationship between tuber weight and WCap was found in Alaska plants grown under C and EMSW (Fig. 3). This was most probably due to the increased production and mobilization of photoassimilates to tubers. Additionally, tuber biomass was positively correlated to leaf dry biomass, in Spunta (under C, SW and EMSW) and Alaska (under C and EMSW). The positive relationship between leaf dry biomass and tuber weight seems to consent to better underground biomass accumulation.

DISCUSSION

Avoidance of soil salinization and ionic metabolism disturbance are of prime importance under saline irrigation. We expected that EMSW can improve nutrient availability and uptake. Our assumption was based on early findings that EMSW enhanced soil proprieties and plant physiological behavior (Akrimi et al., 2021). These results are referred to changes on soil structure, making easier for water to flow and to be absorbed by plants. The enhancement of soil moisture under EMSW is likely linked to better macro-aggregate structure (Zhou et al., 2021). One of the reasons of reduction of sodium and sulfate, in soil watered with EMSW, is

probably the higher leaching rate that occurs due to higher water flow rate (Mostafazadeh-Fardet al., 2012).

The less K^+UE in Alaska plants grown under SW, suggest that K^+ was used as an osmoticum in leaf tissues, under SW, and allocated to plant organs under EMSW. This implies that Alaska was more efficient user of potassium than Spunta and Bellini. Therefore, greater leaf expansion of Alaska can be attributed to potassium nutrition. Data also revealed that highest ions UE in Alaska, along with salt stress, can be one of main reasons for enhanced resistance to salinity. Likewise, elevated Ca^{2+}UE of Alaska may be a key criterion for cell membrane integrity maintains, thereby reducing Na^+ and Cl^- toxicity (Grattan & Grieve, 1999). Moreover, enhanced NO_3^- UE, in plants grown under EMSW, may diminish its use as osmolyte, facilitating its assimilation by the plant and therefore increasing the dry biomass of leaves and tubers. In this way, Juan et al. (2015) proposed that NO_3^- usage may be increased when Cl^- is not sufficiently available in the soil. This assumption also supports the high Cl^- leaching in soil irrigated with EMSW.

The less ABA content in Bellini leaves may be attributed to diminished stress signals. Like so, Ma et al. (2017) stated that when stress signals are diminished ABA is metabolized into inactive products. Decreased ABA concentrations have been also attributed to limited potassium absorption (Marrush et al., 1998). This in part agrees our results, since Bellini was less efficient in mineral use. Further, although *StNCED* over expression mirrored changes in ABA content in Alaska, the high abundance of this transcript in Spunta, watered with EMSW, confuses assessment of its role in ABA biosynthesis. Similar explanation was devoted by Destefano-Beltran et al. (2006) where *StNCED* genes have been identified to exhibit either tissue-specific or developmentally regulated expression in potato tubers. Meanwhile, in the case of Alaska the constitutive over expression of *StNCED* may be a key feature in minimizing water loss and therefore increasing growth and production.

The prominent decrease in SPAD under SW may be credited to high Na^+ accumulation in leaf tissues. In fact, the plant absorb high amounts of sodium instead of potassium and calcium, and this leads to calcium and potassium deficiency, decrease and even acceleration of pigment degradation and early senescence (Naheed et al., 2021). The decline in SPAD and chlorophyll pigments is also another reason of low photosynthetic activity and therefore growth weakening in salt stressed plants. In the other hand, it appears that Alaska was more salt tolerant as it had high SPAD and LA. Meanwhile, Bellini presents a resemblance in SPAD between SW and EMSW treatments. The difference between varieties was possibly owed to differential regulation of growth at metabolic level. Furthermore, the higher correlation between SPAD and WCap in Spunta may illustrate that this variety possibly, prevented early senescence through an adequate water status (Dahal et al., 2019). Positive correlations between i) LA and WCap, ii) SPAD and WCap suggest that leaf size and chlorophyll content may be enhanced by adequate water content of aboveground part. Overall, the reduction of soil salinity under EMSW contributes mainly to an increase in Ψw. In this concern, it seems that Bellini was more susceptible to salinity as it sustains least Ψw and WUE.

CONCLUSION

Results showed an enhancement of salt leaching from soil, reduction of soil salinity and decline of Na^+ accumulation in potato leaves under EMSW. The increased ability to minerals use and ABA metabolism promoted water status and growth especially in Alaska. In view of the less correlation between tuber weight and WCap, less efficiency in ions usage and ABA content, we suggested that Bellini was more sensitive to salinity. Inversely, high correlation

between tuber weight-WCap and leaf area-WCap in Spunta extended the ability to maintain growth and yield under EMSW. These results suggest that EMSW may provide opportunity for alleviation of salinity in potatoes, while depending in variety.

Conflict of interest

Authors declare no conflict of interest.

Author Contribution Statement

R.A. performed the experiments, analyzed data and wrote the first draft of the manuscript. H.H. conceived the research and revised the manuscript.

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Evaluation of the growth and status of some nutrients in pistachio seedlings treated with phosphorus under different levels of irrigation water salinity

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ABSTRACT

Purpose: Irrigation with saline water and poor quality and fertility of the soil are the most important factors limiting the growth, establishment, and yield of pistachio trees in many pistachio farming areas of Iran. In addition, phosphorus plays an important role in plant growth, especially under environmental stress conditions. Thus, the purpose of this experiment is to investigate the role of P use in improving the growth of pistachio seedlings at different levels of irrigation water salinity. **Research method:** A greenhouse study was conducted as a factorial combination based on a completely randomized design with three replications. The treatments include two levels of P [Control (P_0) and 30 mg kg^{-1} soil (P_1) as triple superphosphate] and three levels of irrigation water salinity (0, 5, and 10 dS m^{-1}). **Findings:** Irrigation with saline water (10 $dS.m^{-1}$) significantly decreased the shoot dry weight (94%), root dry weight (64%), leaf area (62%), plant height (35%), shoot and root P content (41% and 52%), shoot K content (40%) and shoot and root K/Na (85% and 28%) of pistachio seedlings. However, P application increased the growth parameters and the concentration of P and K elements in the pistachio seedlings shoot and root under water salinity stress. **Research limitations:** No limitations were encountered. **Originality/Value:** According to the results of this experiment, phosphorus application increased the growth of pistachio seedlings in saline condition. Therefore, according to soil and water salinity in pistachio farming areas of Iran, optimal nutrition with nutrients such as P can increase the tolerance of pistachio seedlings to salinity stress and their establishment.

INTRODUCTION

Soil salinity has become one of the most critical environmental and socio-economic problems in the world, and climate change has intensified the process of soil salinization (Hassani et al., 2021). According to estimates, about 20 percent or 62 million hectares of irrigated agricultural lands in the world are facing different degrees of salinity, and their extent is continuously increasing due to climate changes and human activities (Arora, 2019). With the current trend, about 50% of agricultural land will be affected by salinity by 2050 (Hasanuzzaman et al., 2014). Fertilization, irrigation with saline water, salt build-up soils, and scarcity of rainfall are the main reasons for increasing the salinity of agricultural soils (Kumar et al., 2022). Under the saline conditions, due to high osmotic stress and changes in the availability of nutrients in the soil or the physiological destruction of some plant organs involved in the absorption of nutrients, the uptake and transport of nutrients in different parts of the plant are disturbed and nutritional imbalance occurs in the plant (Farooq et al., 2015). Despite the destruction of chlorophyll, the reduction of the rate of photosynthesis, the stomatal closure, and the increase of oxidative damage in plants exposed to salinity, plants deal with it in various ways, such as the production of osmoprotectants and phytohormones and the increase of antioxidant activities (Arif et al., 2020). Soil and water salinity is increasing significantly in irrigated agricultural lands of arid and semi-arid regions (Hu & Schmidhalter, 2005). It has been proven that the decrease of P availability in the soil is intensified by salinity. Due to high ratios of Na^+/K^+, Na^+/Ca^{2+} and Cl^-/NO_3^- in the solution of saline soils, the activity of nutrient ions such as P is low (Bidalia et al., 2019). Plants grown in saline soils usually face phosphorus (P) deficiency, the main reason of which is the decrease in the availability of P in the soil due to precipitation with other cations such as Ca^{2+}, Mg^{2+} and Zn^{2+} depending on the soil pH (Evelin et al., 2019; Azarmi-Atajan & Sayyari-Zohan, 2022). Therefore, it is necessary to maintain and improve the fertility and status of nutrients, including P, in agricultural soils affected by salinity for the growth and optimal performance of agricultural products.

Phosphorus is one of the essential macronutrients for the optimal growth of plants, and after nitrogen, it is the second nutrient limiting the growth and yield of plants. This nutrient plays a vital role in different cellular processes, including high-energy molecules formation, membrane structures maintenance, cell division, biomolecules synthesis and activation/inactivation of enzymes (Razaq et al., 2017). Phosphorus is an essential and fundamental element for all stages of plant growth and stimulates seed germination, root development, stem strength, flower and fruit formation, and increases crop quantity and quality (Malhotra et al., 2018). Despite the high amount of total P in agricultural soils, the amount of soluble P in the soil is meager and the plants grown in these soils often show symptoms of P deficiency. Generally, water-soluble P in soil is often less than 1 mg kg^{-1} (Rodriguez & Fraga, 1999). Abbas et al. (2018) reported that P deficiency (10 µM) had a more negative effect on the growth of the shoot and root of two wheat cultivars compared to moderate salt stress (100mM NaCl) under soil culture conditions. It was also described in the study that the application of P improved the growth and content of some nutrients in pistachio seedlings under saline conditions (Shahriaripour et al., 2011).

Pistachio (*Pistachia vera* L.) is one of the most important agricultural products of Iran and most pistachio orchards in Iran are located in arid and semi-arid areas with salty soils. Despite the relatively high tolerance of pistachio trees to salinity stress, the increase in soil and water salinity in pistachio farming areas of Iran - especially in recent years - has reduced their growth and yield (Azarmi et al., 2016). In addition to salinity stress, other factors such as high pH value and poor fertility of soil have also affected the growth and yield of pistachio

trees. Considering soil and water salinity in pistachio farming areas of Iran and the lack of available P in most soils of pistachio orchards in Iran, the purpose of this research is to investigate the application of P on the growth and amount of nutrients of pistachio seedlings at different levels of irrigation water salinity.

MATERIALS AND METHODS

The experiment was carried out as a factorial combination based on a completely randomized design with three replications under greenhouse conditions with the relative humidity of 45% and temperature of 28±2/19±2 ºC on day/ night. The treatments include two levels of P [Control (P_0), and 30 mg kg^{-1} soil (P_1) as triple superphosphate] and three levels of irrigation water salinity (0, 5 and 10 $dS.m^{-1}$). A non-saline soil sample was collected from the agricultural fields of South Khorasan province, and after air drying; it was crushed, passed through a 4-mm sieve to remove gravels and mixed thoroughly. Selected physicochemical properties of the soil used in this study were measured based on standard methods (Sparks, 1996), and are shown in Table 1. After adding nutrients (N, K, Fe, and Zn) to the soil based on the soil analysis and mixing them thoroughly and uniformly with the soil, the plastic pots were filled with 2 kg of homogeneous soil. Phosphorus was also added to the pots before planting based on experimental treatments. For planting, first, 5 germinated pistachio seeds (*P. vera* L. cv. Badami) were planted in each pot and irrigated with non-saline water (EC=1.2 $dS.m^{-1}$) for 4 weeks. Then the number of seedlings in each pot was thinned to 2 and irrigation was done based on experimental treatments with prepared saline water to keep the soil at 80% of field capacity until the end of the growth period (twenty weeks).

Measurements

At the end of the experiment, the pistachio seedlings were collected and measurements were done. The height of the seedlings was measured from the soil surface using a ruler. After cutting the seedlings from the soil surface, the roots were carefully separated from the soil and washed to remove the soil particles the adhered to them. To determine the dry weight of the shoot and root, the plant samples were placed in an oven at a temperature of 70 ºC for 48 hours, weighed, and then ground into powder for chemical analysis. The powdered plant samples were dry-ashed at 500 °C and digested with 2N hydrochloric acid and made to volume with hot distilled water. Total P concentration in the shoot and root of pistachio seedlings were determined the method described by Chapman and Pratt (1961). Also, the content of K and Na in the samples was recorded by flame photometer.

Statistical analysis

The experimental data were used in analysis of variance (ANOVA) by SAS software, and the difference between the mean values was evaluated using the least squares difference (LSD) test at the 5% level of probability.

Table 1. Some characteristics of the soil used in the experiment.

Texture	pH	ECe	OM	SP	P_{av}	K_{av}	Na_s	Ca_s	Mg_s	SAR
		($dS\ m^{-1}$)	%	%	($mg\ kg^{-1}$)		($mEq\ L^{-1}$)			$(MEq\ L^{-1})^{0.5}$
Sandy Loam	7.50	1.40	0.57	30	7.12	179	7.30	5.60	7.58	2.85

ECe: Electrical Conductivity, OM: Organic Matter, SP: Saturation Percentage, av: Available forms of element in the soil, s: Soluble forms of element in the saturated soil solution, SAR: Sodium Adsorption Ratio.

RESULTS AND DISCUSSION

Shoot and root dry weight

The analysis of variance revealed that the main and interaction effect of P and irrigation water salinity had a significant effect ($p \leq 0.05$) on the shoot and root dry weight of pistachio seedlings (Table 2).

According to the result, the increase of irrigation water salinity to 5 and 10 $dS.m^{-1}$ reduced shoot dry weight by 20% and 94%, and root dry weight by 19% and 64% in comparison with the control, respectively (Table 3). Salinity stresses primarily affect the roots. Murshed et al. (2015) reported that as the salinity level increases, the diameter, length and number of roots decrease. The decrease in the dry weight of seedlings with increasing salinity can be related to the decrease in the number of leaves, smaller leaf surface area and also the decrease in their height. However, treatment with P had a positive effect on the shoot and root dry weight of pistachio seedlings in both saline and non-saline conditions. Application of 30 mg P kg^{-1} at salinity levels of 0, 5 and 10 $dS.m^{-1}$ increased shoot dry weight by 43%, 60% and 61% and root dry weight by 60%, 48% and 96%, respectively, at the same salinity levels (Table 3). Phosphorus plays an essential role in processes such as respiration, photosynthesis, membrane stability and energy production (Marschner, 1995). The increase in dry weight of pistachio seedlings with the application of mineral P has also been reported by Shahriaripour et al. (2011) and Fekri et al. (2015).

Table 2. Analysis of variance (mean of square) for measured traits.

SOV	df	ShDW	RDW	Leaf Area	Plant Height	Shoot P	Root P
Phosphorus (P)	1	0.811**	0.692**	1014**	125**	0.049**	0.009**
Salinity (S)	2	0.492**	0.704**	1489**	43.1**	0.014**	0.010**
P × S	2	0.009*	0.021*	13.9*	1.52^{ns}	0.0007*	0.0004*
Error	12	0.002	0.004	5.16	1.51	0.0002	0.0001
CV		4.75	7.78	4.59	9.92	7.64	5.95

SOV: Source of Variation; ShDW: Shoot Dry Weight; RDW: Root Dry Weight.
**, * and ns: significant at $p \leq 0.01$, significant at $p \leq 0.05$ and non-significant, respectively.

Table 2. (Continued**).**

SOV	df	Shoot K	Root K	Shoot Na	Root Na	Shoot K/Na	Root K/Na
Phosphorus (P)	1	0.084**	0.072**	0.027**	0.039**	3.26*	0.15**
Salinity (S)	2	0.53**	0.074**	0.30**	0.086**	196**	0.70**
P × S	2	0.027*	0.004*	0.004*	0.002*	0.668^{ns}	0.11*
Error	12	0.006	0.001	0.001	0.0005	0.630	0.020
CV		5.73	4.86	8.25	6.24	12.5	6.92

SOV: Source of Variation; ShDW: Shoot Dry Weight; RDW: Root Dry Weight.
**, * and ns: significant at $p \leq 0.01$, significant at $p \leq 0.05$ and non-significant, respectively.

Table 3. The interaction effect of phosphorus × salinity of irrigation water on the dry weight (DW) and phosphorus (P) content of shoot and root of pistachio seedlings.

Phosphorus (mg kg^{-1})	Salinity of irrigation water (dS m^{-1})			Salinity of irrigation water (dS m^{-1})		
	0	5	10	0	5	10
	Shoot DW (g $plant^{-1}$)			Root DW (g $plant^{-1}$)		
0	1.05 ± 0.03 c	0.82 ± 0.04 d	0.54 ± 0.03 e	0.89 ± 0.04 c	0.72 ± 0.02 d	0.32 ± 0.02 e
30	1.50 ± 0.02 a	1.31 ± 0.02 b	0.87 ± 0.03 d	1.42 ± 0.05 a	1.07 ± 0.04 b	0.63 ± 0.03 d
	Shoot P Concentration (%)			Root P Concentration (%)		
0	0.12 ± 0.009 d	0.15 ± 0.007 c	0.07 ± 0.005 e	0.19 ± 0.006 b	0.14 ± 0.005 c	0.09 ± 0.007 d
30	0.23 ± 0.008 b	0.26 ± 0.009 a	0.15 ± 0.006 c	0.22 ± 0.003 a	0.19 ± 0.006 b	0.15 ± 0.005 c

For each parameter, values followed by the same letter are not significantly different ($p \leq 0.05$) according to LSD test.

Leaf area and plant height

The leaf area of seedlings was significantly influenced ($p \leq 0.05$) by the P, salinity and P× salinity interaction. Also, only the main effects of P and salinity on the height of pistachio seedlings were significant (Table 2).

The results indicated that the leaf area of seedlings showed 19% and 62% decrease at the 5 and 10 $dS.m^{-1}$ salinity levels relative to the control. Salinity stress in the initial stages causes the stomata to close and the partial pressure of CO_2 between the cells decreases, and as a result, the growth of the leaves is limited by reducing the rate of photosynthesis. Also, salinity causes the premature senescence of leaves and a severe reduction in plant growth (Arif et al., 2020). Although the application of P in both saline and non-saline conditions increased the leaf area of seedlings, but its effect was more significant in high salinity (10 $dS.m^{-1}$) than other salinity levels. Treatment with P at salinity levels of 10 $dS.m^{-1}$ increased this parameter by 75% in comparison with the same salinity level (Fig. 1). Use of 30 mg P kg^{-1} increased plant height by 54% compared to the control (Fig. 2a). Considering the low amount of P in the studied soil, the application of P has improved various growth indicators of pistachio seedlings such as leaf area and height. The response of plants to the supply of P is different depending on the concentration and plant species (Ben hamed et al., 2019). On the other hands, when the salinity of irrigation water increased from 0 to 5 and 10 $dS.m^{-1}$, the seedling height decreased by 21% and 35%, respectively (Fig. 2b). One of the most apparent effects of salinity on the plant is to reduce its growth and development. The decrease in plant growth under salinity stress is mainly due to the increase in osmotic pressure, the decrease in water availability in the root environment, the accumulation and toxicity of sodium and chlorine ions, and the loss of nutritional balance in the plant (Munns, 2002).

Shoot and root P content

The analysis of variance showed that the main and interaction effect of P and irrigation water salinity had a significant effect ($p \leq 0.05$) on the shoot and root concentration of P in pistachio seedlings (Table 2).

Based on the results, irrigation with water with low salinity level (5 $dS.m^{-1}$) increased (25%), but with high salinity level (10 $dS.m^{-1}$) decreased (41%) the P content in the shoot of pistachio seedlings. But both salinity levels caused a significant decrease in P concentration in the root of seedlings. Application of P at salinity levels of 0, 5 and 10 $dS.m^{-1}$ increased shoot P content by 91%, 73% and 114%, and root P content by 15%, 35% and 61%, respectively, relative to the same salinity levels (Table 3). In general, the relationship between salinity and the content of P in plants is complex, and the concentration of plant P varies depending on the plant species, the growth stage of the plant, and the amount of P in the culture medium (Loupassaki et al., 2002). The reduction of P uptake in leaves and roots of pistachio seedlings under saline conditions was reported by Eskandari and Mozafari (2014).

Table 4. The interaction effect of phosphorus × salinity of irrigation water on the shoot and root potassium (K) and sodium (Na) content of pistachio seedlings.

Phosphorus ($mg\ kg^{-1}$)	Salinity of irrigation water ($dS\ m^{-1}$)			Salinity of irrigation water ($dS\ m^{-1}$)		
	0	5	10	0	5	10
	Shoot K Concentration (%)			Root K Concentration (%)		
0	1.78 ± 0.06 a	1.42 ± 0.04 bc	1.06 ± 0.03 d	0.62 ± 0.02 c	0.68 ± 0.02 b	0.87 ± 0.03 a
30	1.49 ± 0.06 b	1.35 ± 0.04 c	1.02 ± 0.03 d	0.51 ± 0.02 d	0.60 ± 0.01 c	0.69 ± 0.02 b
	Shoot Na Concentration (%)			Root Na Concentration (%)		
0	0.15 ± 0.01 e	0.34 ± 0.02 c	0.64 ± 0.03 a	0.27 ± 0.007 d	0.36 ± 0.01 c	0.54 ± 0.02 a
30	0.11 ± 0.01 e	0.28 ± 0.01 d	0.51 ± 0.02 b	0.22 ± 0.006 e	0.25 ± 0.01 de	0.42 ± 0.01 b

For each parameter, values followed by the same letter are not significantly different ($p \leq 0.05$) according to LSD test.

Shoot and root K and Na content

Based on the results, the content of K and Na in the shoot and root of pistachio seedlings was significantly influenced ($p \leq 0.05$) by the P, salinity and P × salinity interaction (Table 2).

The results revealed that with the increase of irrigation water salinity level, the content of K reduced in the shoot but increased in the root. In comparison with the control, the shoot K concentration decreased by 20% and 39% at the salinity levels of 5 and 10 $dS.m^{-1}$, respectively. On the other hands, at these salinity levels, the content of root K increased by 10% and 40% compared to the control, respectively (Table 4). Despite the relatively high amount of K in the soils of arid and semi-arid regions, the availability of this element for the plant can decrease in different conditions, including salinity. The most important factor limiting the availability of K for the plant in saline conditions is the high concentration of ions that cause salinity, including Na, and also the reduction of root growth. Shahriaripour et al. (2010) showed that the increase of NaCl decreased the concentration of K in the shoot and increased its concentration in the roots of pistachio seedlings. Their results are consistent with the results of this research. The increase in K concentration in the roots with the application of salt water is related to the decrease in plant growth and the dilution effect. However, an increase in K concentration in the leaves of pistachio seedlings with increasing salinity has also been reported (Hojjatnooghi et al., 2014). The use of P only in non-saline conditions was effective on the content of shoot K, which caused it to decrease by 16% compared to the control at same salinity levels. Furthermore, the application of P at salinity levels of 0, 5 and 10 $dS.m^{-1}$ reduced the content of root K by 17%, 11% and 21% relative to the control, respectively (Table 4). Phosphorus plays a role in the process of cell division and thus causes the development of the growth of plant shoot and roots and increases the availability of nutrients in the soil (Razaq et al., 2017).

According to the results, an increase in the irrigation water salinity levels caused an increase in the concentration of Na in the shoot and root of the pistachio seedlings, although the accumulation of this element was higher in the shoot than in the root. For example, with application of high water salinity level (10 $dS.m^{-1}$), the content of Na in the shoot and root increased by 4.2 times and 2.0 times compared to the control, respectively. One of the main effects of salinity stress on plants is the accumulation of toxic ions in their various tissues, especially leaves. Studies have shown that one of the mechanisms of plants resistance to salinity stress is keeping Na concentration low in leaves and reducing its transfer from roots to aerial parts (Chen et al., 2010). Although the resistance of the pistachio plant to salinity stress is relatively high, but with the increase of Na concentration in the root growth environment, the ability of the root to control and accumulate Na is reduced and as a result, its transfer to aerial organs increases. In general, due to the accumulation of toxic elements such as Na and Cl in the shoot of plants, leaves are more vulnerable to these elements than root. Based on the studies of Behzadi Rad et al. (2021), salinity increased the concentration of Na in the shoots and roots of three cultivars of pistachio seedlings. However, the accumulation of Na in the shoot of all three cultivars was more than in the root. The application of P (30 $mg.kg^{-1}$) caused a decrease in the accumulation of Na in the shoot and root of seedlings, especially in saline conditions (Table 4). Considering the role of P in rooting and increasing the root growth of plants, the decrease in Na concentration in pistachio seedlings with the application of P can be attributed to the increase in root growth and, as a result, the increase in the absorption of water and nutrients such as Ca and K. Optimum application of P in saline conditions can improve various soil properties, increase K use efficiency, increase tolerance to salinity stress, and as a result, increase plant yield (Sadji-Ait Kaci et al., 2022).

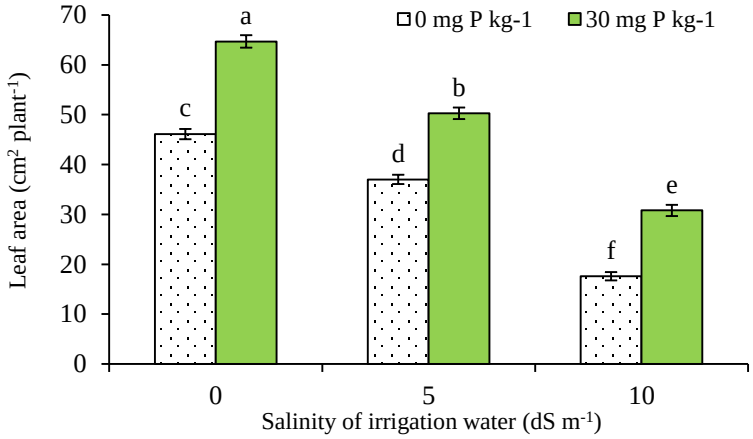

Fig. 1. The effect of phosphorus × salinity of irrigation water on the leaf area of pistachio seedlings. Within each graph, values followed by the same letter are not significantly different (p≤0.05) according to LSD test. The error bars in the graphs are standard errors.

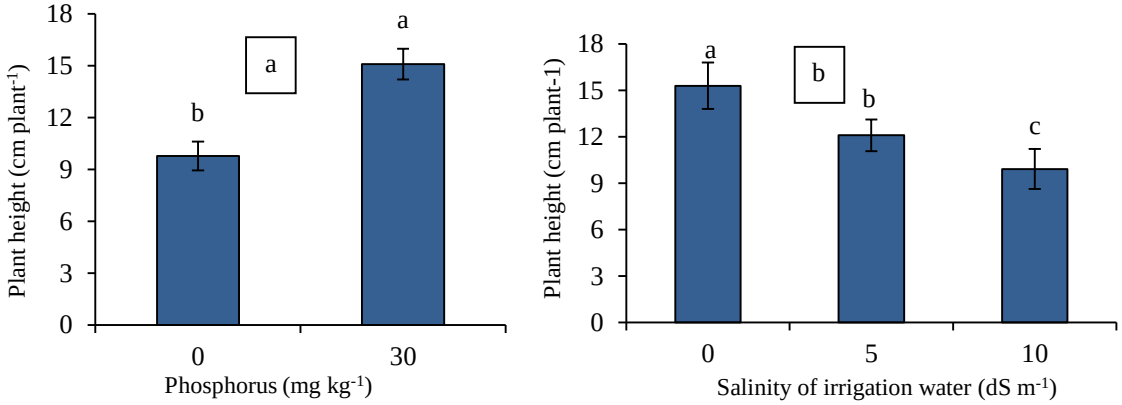

Fig. 2. Main effect of phosphorus (a) and irrigation water salinity (b) on the height of pistachio seedlings. Within each graph, values followed by the same letter are not significantly different (p≤0.05) according to LSD test. The error bars in the graphs are standard errors.

Shoot and root K/Na

The analysis of variance showed that only the main effects of P and salinity on the shoot K/Na of pistachio seedlings were significant. Also, the root K/Na of seedlings was significantly influenced (p≤0.05) by the P, salinity and P× salinity interaction (Table 2).

The results indicated that use of P significantly increased shoot K/Na by 14% in comparison with the control (Fig. 3a). Also, the ratio of shoot K/Na showed 64% and 85% decrease at water salinity levels of 5 and 10 dS.m^{-1} compared to the control (Fig. 3b). According to the results, the root K/Na significantly reduced with rising irrigation water salinity. Salinity levels of 5 and 10 dS.m^{-1} decreased root K/Na by 16% and 28% relative to the control. In the plant under salt stress, the outflow of K from the plant increases and as a result the K/Na decreases (Qi & Spalding, 2004). Also, the competition of Na with other nutrients such as K causes nutritional imbalances (Mohamed & Gomaa, 2012). The application of P was effective only at low salinity level (5 dS.m^{-1}) on the ratio of root K/Na, which caused it to increase by 26% compared to the control (Fig. 4). The increase in K/Na with the application of P can be related to the improvement of root growth, the plant's access to water and nutrients, and the increase in uptake of nutrients.

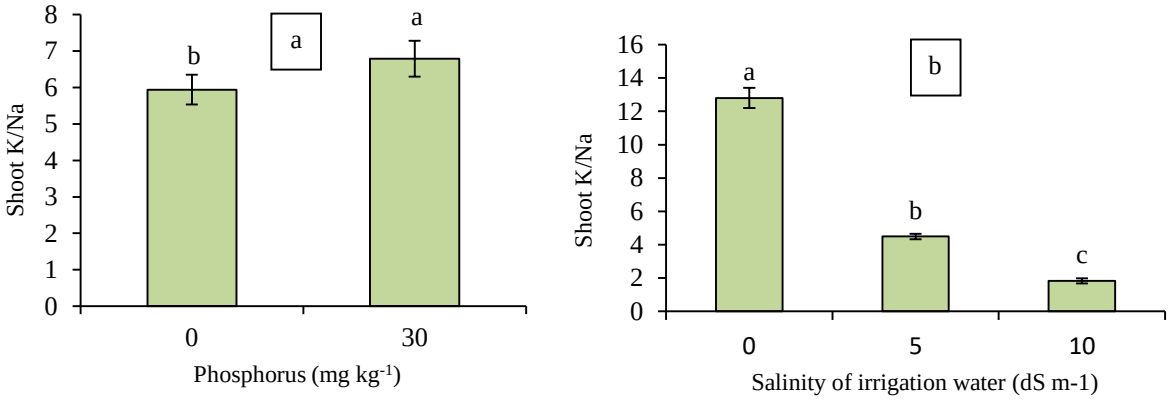

Fig. 3. Main effect of phosphorus (a) and irrigation water salinity (b) on the shoot K/Na of pistachio seedlings. Within each graph, values followed by the same letter are not significantly different (p≤0.05) according to LSD test. The error bars in the graphs are standard errors.

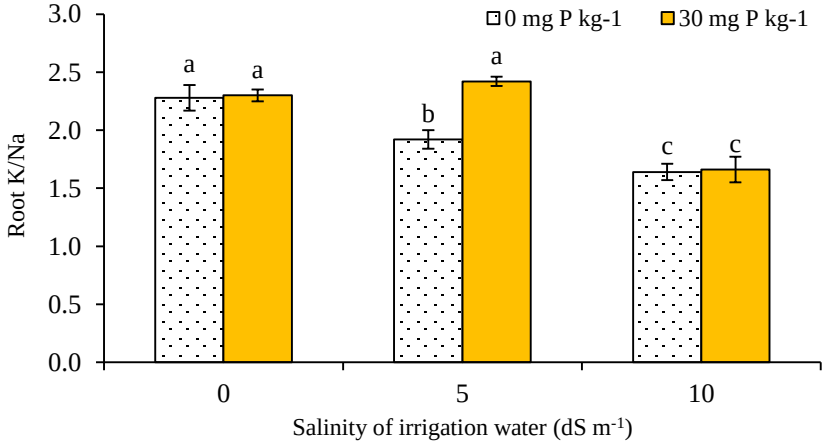

Fig. 4. The effect of phosphorus × salinity of irrigation water on the root K/Na of pistachio seedlings. Within each graph, values followed by the same letter are not significantly different (p≤0.05) according to LSD test. The error bars in the graphs are standard errors.

CONCLUSION

Irrigation with saline water is one of the most important factors that reduce the quality and fertility of the soil and as a result the growth, establishment and yield of pistachio trees in a large part of pistachio orchards in Iran. According to the results of the present experiment, irrigation with saline water (especially salinity level 10 dS.m^{-1}) decreased the shoot and root dry weight, leaf area, plant height, shoot and root P content, shoot K content and shoot and root K/Na of pistachio seedlings. On the other hand, with increasing salinity of irrigation water the concentration of shoot and root Na and root K in seedlings was increased. However, the application of P significantly increased the growth parameters such as dry weight and nutrient concentration in pistachio seedlings under various salinity conditions. Also, the use of P decreased the content of Na in the shoot and root of pistachio seedlings. Considering the lack of available P in the soil and the role of this element in many plant processes including root growth and development, the application of P was able to increase the growth of the roots and, as a result, increase the access to water and nutrients, and the growth of pistachio seedlings at different levels of water salinity. Therefore, the use of P can help to develop the root system and ultimately increase the establishment of pistachio seedlings in regions with saline soil and water.

Conflict of interest

The authors declare no conflict of interest.

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Agro-physiological responses of the pistachio (*Pistacia vera* L., cv. Mateur) to partial root drying (PRD) irrigation

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ABSTRACT

Purpose: Water irrigation regimes strongly influence the agrophysiological parameters in pistachio. This study aims to investigate the impact of the partial root drying on the yield, vegetative growth, physiological parameters, water status and biochemical traits of the pistachio cv. Mateur budded on *P. atlantica* rootstocks during the growing season (2021). **Research Method:** The agro-physiological responses of the pistachio trees located in the experimental orchard of the Regional Center of Agriculture Research (CRRA, Sidi Bouzid, Tunisia), were studied. Three water treatments were applied; T0: 100% Partial root drying (PRD) during all the season, T1; 75% PRD during all the season and T2; 50% PRD during all the growing season. The leaf gas exchange parameters were determined using a portable photosynthesis system (CI-340 handheld photosynthesis system, USA). **Findings:** Results showed the stomatal conductance (gS) of pistachio leaves ranged from 320 to 760 mmol H2O m-2s-1 in the 100% PRD treatment whereas the water regimes 75% PRD and 50% PRD presented a clear decrease in this parameter. The proline and the soluble sugar content reached its maximum value (2.10 µmol g−1 FW and 275.60 µg g−1 FW, respectively) under the 50 % PRD treatment during the month of August. **Research limitations:** No limitations were found. **Originality/Value:** The 75% PRD treatment was the most efficient as it did not show significant differences with the 100% PRD treatment while 25% of the irrigation water was saved. The partial root drying strategy can be used in pistachio orchards under semi-arid conditions.

INTRODUCTION

The pistachio (*Pistacia vera* L.) is the most economically important species in the genus *Pistacia*. Pistachio world production reached 915.7 thousand tons in a harvested area of around 817 thousand ha in 2021 (FAOSTAT, 2023). The main pistachio producing countries are Iran, USA, Turkey, China and Syria contributing over 90% of the world production (FAOSTAT, 2023). In Tunisia, pistachio production reached 3123 tons with harvested area of around 27810 ha in 2021 (FAOSTAT, 2023). The most productive zones are Kasserine, Sidi Bouzid, Gafsa and Sfax presenting more than 80% of the national production. Among the most cultivated varieties, the cv. Mateur is the most important showing a high production, a high pomological nut quality traits and adaptability to the soil and climatic conditions.

Stressful environmental factors such as water deficiency represents a major constraint limiting crop growth and yield worldwide (Abboud et al., 2021). Pistachio tree has the reputation of being drought tolerant and saline-resistant species cultivated under rainfed conditions in its region of origin (Rieger, 1995). In pistachio, the nut development is characterized by three different periods (Goldhamer, 1995): stage I starts at the beginning of the nut growth and ends when its maximum size is reached; during stage II the shell hardening takes place and finally, the stage III is the period of kernel growth. The impact of drought stress on tree agrophysiological parameters is a complex process (Fereres et al., 2012). Hence, plants have evolved morphological, anatomical, physiological, biochemical and molecular adaptive responses that enable them to adapt to drought under water shortage conditions (Abboud et al., 2021). The primary effects of drought are the reduction of plant stomatal conductance, water potential, leaf area and leaf gas exchange (Abboud et al., 2021; Jiménez et al., 2013). Goldhamer and Beede (2004) reported that water deficiency in pistachio decreases tree growth, nut yield, affects nut quality by decreasing the proportion of split nuts and increases the alternate bearing intensity. Others responses of pistachio tree to the water deficiency is relative water content decrease (Sajjadinia et al., 2010), proline accumulation (Anjum et al., 2011), soluble sugars accumulation in leaves (Kempa et al., 2008). Galindo et al. (2017) reported that pistachio tree exposed to water stress also developed stress avoidance and stress tolerance mechanisms. Hence, during pistachio fruit stages I and II, when the soil water content is quite high and the evaporative demand of the atmosphere is low, the tree showed higher net photosynthesis and leaf conductance values. In contrast, during fruit stage III, at which the evaporative demand of the atmosphere is higher, the pistachio plants showed lower net photosynthesis and leaf conductance values (Memmi et al., 2016b).

In pistachio trees, irrigation increases the yield and improves the nut quality (Kanber et al., 1993). However due to the prolonged drought periods, there are scarce water resources for crop irrigation (Giorgi & Lionello, 2008). The implementation of irrigation strategies that improve the water use efficiency (WUE), without yield and quality reduction is of a great interest (Fereres & Soriano, 2007). Partial root drying (PRD) consists in alternate the irrigation periodically in the two parts of the root zone (Dry et al., 1996). The PRD has been successfully applied to a large number of crops taking into consideration variety-rootstock interaction, type and characteristics of soil, agricultural practice, and specific agro-climatic conditions, demonstrating that the main benefit of PRD irrigation is the reduced use of water for irrigation (Jovanovic et al., 2017). The fully hydrated roots maintain a favorable plant water status, while the dried ones send chemical signals (abscisic acid) to the shoots to induce partial stomatal closure and thus reduce the relevant water demand (Abboud et al., 2019). Several studies reported that the PRD practice induced a decrease in leaf water potential in olive (Centritto et al., 2005), a slight decrease in the average shoot length and yield as

compared to the full-irrigated treatment (Wahbi et al., 2005), a decrease in stomatal conductance and subsequently leaf water potential (Abboud et al., 2019).

With recurring decrease of precipitation and prolonged drought periods in major pistachio orchard under semi-arid areas, water deficit become a critical factor for irrigated high-density pistachio plantations. Therefore, the use of water-saving irrigation strategies and the selection of the best adapted cultivars are crucial for a sustainable production and an efficient water use under water scare conditions. The aim of this study was to evaluate the agro-physiological and biochemical responses of the pistachio cultivar Mateur conducted with fewer than three partial root drying water regimes (100% PRD, 75% PRD, and 50% PRD).

MATERIALS AND METHODS

Plant material and Experimental design

The trial was carried out in the experimental orchard of the Regional Center of Agriculture Research (CRRA, Sidi Bouzid) in west central Tunisia (9º43'E, 35º01'N; altitude 354 m). Fifteen-years-old pistachio trees cultivar Mateur grafted on *P. atlantica* rootstock were studied. Trees were planted at a spacing of 6×6 m and grown under standard conditions of fertilization, pruning, pollination and pest and disease control. The surveyed trees were selected for uniform trunk and canopy size. The experiment was designed as a complete randomized block design with 9 trees (8 females and 1 male) per experimental plot. The production area is the semi-arid Mediterranean climate with a low annual rainfall of 200 mm irregularly distributed over the growing season and a reference evapotranspiration (ETo) of more than 1300 mm (Fig. 1).

Treatments for partial root drying

The trees were conducted under the partial root drying (PRD) irrigation strategy. During the experiment period, trees had only one side of their root zones irrigated and irrigation was alternated every two weeks. The pistachio trees were grown under three water regimes (100% PRD, 75% PRD, and 50% PRD). In the control treatment (100% PRD), trees received water equivalent of 100% of crop evapotranspiration (ETc). For the treatment 75% PRD, trees received 75% of crop evapotranspiration. In the treatment 50%PRD, trees received 50% of crop evapotranspiration. Two drip lines were employed for each tree row one on each side with drippers located at 60 cm from the trunk and discharged an average flow rate of 2.0 L/h. The irrigation scheduling was applied from March to September with a frequency of 3 times per week. The amount of water provided (Table 1) was calculated on the basis of the crop evapotranspiration and the crop coefficient according to the FAO method demand using the following formula (Allen et al., 1998).

$$ETc = ETo \times Kc \times Kr \quad (1)$$

With ETc: crop evapotranspiration, Kc: crop coefficient values (0.4; 1.06 and 1.14 during the stage I, II and III respectively (Fereres & Goldhamer, 1990).

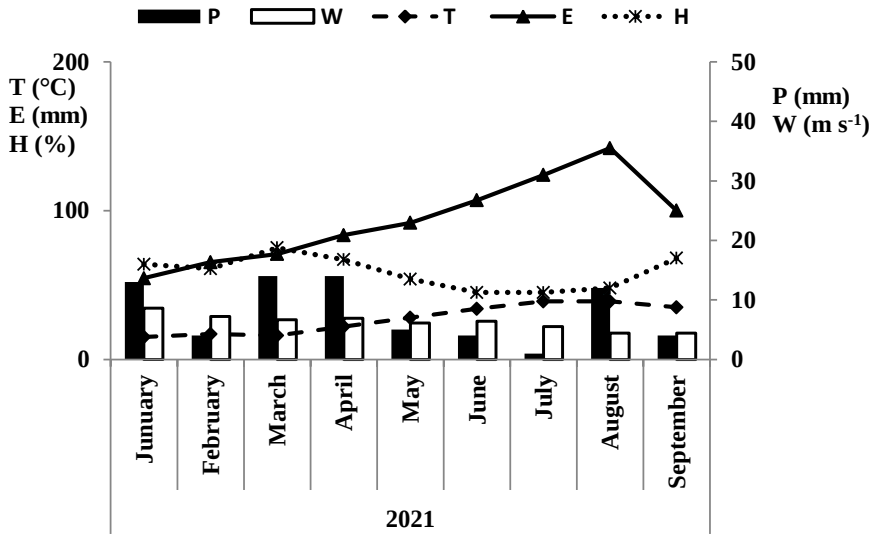

Fig 1. Climatic conditions during pistachio nut development. Abbreviations: T: temperature; P: precipitation; E: evapotranspiration; W: wind; H: humidity; m: meter; mm: millimeter; s: second.

Table 1. Crop evapotranspiration rate (ETc) and water applied to the three water regimes (T0=100 % PRD, T1=75 % PRD and T2=50 % PRD).

Year	ET0 (mm)	P (mm)	Water regime (mm)		
			100% PRD	75% PRD	50% PRD
2021	1350	186	650	487.5	325

Abbreviations: ET0= reference evapotranspitation; T0= 100% PRD; T1= 75% PRD; T3= 50% PRD; P= precipitation.

Agronomical measurements

Trees vigor

The trees vigor parameters were assessed in the three water regimes during the growing season 2021 as reported in Abidi et al. (2023). The tree height, canopy and the trunk cross-sectional area (TCSA) were measured during the dormant season at 30 cm above the graft union. The yield (Kg/tree), cumulative yield per tree, and yield efficiency (cumulative yield in kilograms per final TCSA) were determined.

Phenological traits

The initial blooming (5% of flowers are opened), full blooming, final blooming, harvesting dates (when hull separates easily from the shell) and nut development period were recorded during the growing season (2021) according to International Plant Genetic Resources Institute (IPGRI, 1997) descriptors for pistachio.

Vegetative growth

The vegetative growth parameters under the applied treatments were assessed as described in Abboud et al. (2019). In each tree, four shoots were selected in four orientations around the canopy. Then the shoot length, shoot diameter, internode number and leaf area were recorded each month during the growing season, from April to August on the tagged shoots.

Leaf gas exchange parameters

The leaf gas exchange parameters were assessed in the trees of the cv. Mateur under the three water regimes as described in Abboud et al. (2019). The photosynthesis rate (P_n), stomatal conductance (gs), transpiration rate (E) were measured using a portable photosynthesis system

(CI-340 handheld photosynthesis system, USA) with a flow rate of 0.2 l min^{-1} and leaf temperature within 2–3 °C of ambient air temperature (25–35 °C). Water use efficiency (WUEi) was calculated was calculated as the ratio of net photosynthesis to stomatal conductance and expressed in µmol m^{-2} s^{-1}/mol m^{-2} s^{-1}. All Measurements were made from the tip of the youngest fully expanded leaf (usually the third or fourth leaf from the apex) between 11:00 and 12:00 h every month from April to August. Three leaves per tree were monitored in three experimental trees for each treatment.

Water status

Leaf relative water content

Fully mature and healthy leaves were used to determine the leaf relative water content (RWC). Leaves of similar age were collected from three trees for each per experimental plot and the fresh weight (FW) was immediately determined. Then leaves were placed in similar volumes of distilled water for 24 hours to re-hydrate (Cameron et al., 1999). After that leaf turgid weight (TW) was measured and then leaves were dried at 80°C for 48 hours to obtain the dry weight (DW). The leaf relative water content (RWC) was calculated using the formula: LRWC = 100x (FW-DW) / (TW-DW) as described in Yamazaki and Dillenburg (1999).

Midday leaf water potential

The midday leaf water potential (Ψmd) was measured following the methodology described in Abboud et al. (2019). The Ψ_{md} was determined at 10 to 12 am under clear sky every 4 weeks from April to August. The measurements were performed on current-year shoot from the mid canopy of the trees that had been enclosed in plastic bags covered with aluminum foil at least 2 hours before measurements in order to reduce leaf transpiration (Shackel et al., 1997) and to equilibrate foliar and stem water potential. Shoots were then detached, and Ψstem measurements were performed using a pressure chamber (Soil Moisture Equip., Santa Barbara, CA, USA).

Biochemical measurement

Proline content

Proline contents were determined using the ninhydrin method described by Troll and Lindsley (1955). Three 200 mg samples were extracted for 30 min in 5 ml 40% (v/v) methanol heated to 80°C in hermetically sealed tubes. A 1 ml aliquot of extract was mixed with 2 ml glacial acetic acid, 1 ml 25 mg ml^{-1}ninhydrin solution and 2 ml of a mixture consisting of 24% (v/v) distilled water, 60% (v/v) glacial acetic acid and 16% (v/v) orthophosphoric acid. The mixture was boiled for 30 min then cooled on ice and 3 ml toluene added before shaking vigorously. Two phases were obtained. The upper phase was saved and dehydrated with anhydrous Na_2SO_4. The extracts were kept in the dark for a minimum of 2 h before their absorbance was measured at 528 nm and free proline concentration was calculated from a calibration curve using proline as a standard (Sigma-Aldrich). Free proline content was reported as mg g^{-1}FW.

Total soluble sugar

Three samples (200 mg) of leaves were extracted in 5 ml 80% (v/v) methanol and heated to 70 °C for 30 min. The extract was then centrifuged at 5,000 rpm for 15 min and the supernatant was assayed for soluble sugars using the phenol-sulphuric acid method (Robyt & White, 1987). One ml of extract was shaken with1 ml 5% phenol and 5 ml concentrated sulphuric acid. Once the extract had cooled, its absorbance was determined at 640 nm with spectrophotometer (Jenway 6300).

Statistical analysis

Analysis of variance (ANOVA) was performed to test the effect of PRD irrigation regimes on phonological traits, agro-physiological parameters, water status, and biochemical traits, using SPSS Statistics 20.0 for Windows. Posthoc analysis was performed using the Scheffe test.

RESULTS AND DISCUSSION

Phenological traits

The dates of initial blooming, full blooming, end blooming, harvesting dates and the fruit development period were shown in Table 2. The cv. Mateur presented an initial blooming in March, 24 whereas the full blooming date was in April, 06 and the end of blooming was in April, 13. The harvesting date was inAugust16 and the fruit development period was of 145 days. The male tree presented a blooming date from 15 to 20 March. The full blooming dates of the male and female trees were not in total synchronization. Results showed that the cv. Mateur showed similar blooming and harvesting dates under the three applied water regimes. In this line, Vargas et al. (1995) reported that 'Mateur' cultivar was among the earliest female cultivars evaluated, with the mean blooming period from March, 30 to April, 13. In another study, Monastra et al. (1998) reported that the flowering process development in irrigated trees was two years earlier than in non-irrigated. The authors reported that the important branch growth with presence or absence of inflorescences reduced the alternate bearing that is especially higher in non-irrigated trees.

Agronomical traits

The tree height, tree canopy and the trunk cross sectional area were shown in Table 3. The tree height varied from 2.2 to 2.9 m. The tree canopy was in the range of 3.0 to 3.2 m. The trunk cross sectional area varied from 60.6 cm^2 in the treatment T2 (50% PRD) to 66.5 cm^2 in the treatment T1 (75% PRD). The water regime has no effect on the tree vigor parameters. In this line, Monastra et al. (1998) indicated that irrigation with 50% of evaporative demand could support trunk growth equal to that in the fully irrigated trees. Our findings are not in accordance with the study of Egea et al. (2010) showing a negative impact of regulated deficit irrigation on trunk growth.

The analysis of the impact of the three irrigation regimes on yield and fruit weight (FW) showed significant difference between T2 (50% PRD) and the two treatments T0 (100% PRD) and T1 (75% PRD) as shown in Table 3. The mean yield was slightly higher in the control treatment as compared to T1 (75% PRD) and the stressed treatment (T2). The yield ranged from 4.5kg per tree under the treatment T2 to 6.5kg/tree under the control treatment. These results are similar to the study of Memmi et al. (2016) showing that the mean yield of pistachio trees under regulated deficit irrigation was not reduced in T1 and T2 compared to the control treatment with water savings of 40 % in T1 and 45 % in T2. The nut fresh weight was affected by the water regime with the treatment T2 showing statistically significant ($P<0.05$) difference with the control and T1. Regarding the nut fresh weight (FW) the highest value (0.75g) was observed under the control treatment whereas the lowest value (0.50g) was shown under the T2 treatment. In almond trees, Egea et al. (2010) reported that with exception of the partial root drying (PRD_{70}) treatment, the nut weight was significantly reduced in remaining deficit irrigated treatments.

Table 2. Blooming and harvesting dates of the cultivar 'Mateur'.

Traits	Mateur T0	Mateur T1	Mateur T2
Initial blooming	24 March	24 March	24 March
Full blooming	06 April	06 April	06 April
End blooming	13 April	13 April	13 April
Harvest day	16 August	16 August	16 August
Development period (day)	145	145	145

T0 = 100% PRD; T1 = 75% PRD; T3 = 50% PRD

Table 3. Tree vigor and agronomical measurements of the cv. Mateur under three water regimes.

Treatment	Height	Canopy	TCSA	Yield	FW	Y.E
T0 (100% PRD)	2.9 ± 0.1^a	3.2 ± 0.5^a	65.9 ± 5.1^a	6.5 ± 1.2^a	0.75±0.2^a	0.09±0.01^a
T1 (75% PRD)	2.5 ± 0.1^a	3.1 ± 0.6^a	66.5 ± 1.4^a	5.8 ± 1.0^a	0.65 ±0.5^a	0.08 ± 0.02^a
T2 (50% PRD)	2.2 ± 0.1^a	3.0 ± 0.2^a	60.6 ± 0.9^a	4.5 ± 0.7^b	0.50 ± 0.3^b	0.07 ± 0.02^a

Values are mean ± Standard error. Abbreviations: Tree height (m); Canopy (m); TCSA= Trunk cross sectional area (cm^2); Average yield (kg/tree); Yield efficiency (kg /cm^2); FW=fresh weight (g).
Mean separation within columns by Scheffe test at ($p \leq 0.05$).
Different letters indicate statistically significant ($P<0.05$) differences between treatments.

Vegetative growth

The apical shoot lengths of trees were affected by the water restriction generated under the treatment T2 (50% PRD) as observed in Figure 2a. The measurements made in May, presented a shoots length of 26 cm in the treatment T0, 21 cm in the treatment T1, and 19 cm in the treatment T2. The control treatment (100% PRD) and the treatment T1 (75% PRD) showed similar pattern of shoot length and diameter whereas the treatment T2 showed statistically significant differences ($P<0.05$) in all measurements from May to August.

The shoot diameter (Fig. 2b) presented the same behavior as shoot length being similar between T0 and T1 treatments whereas the T2 presented statistically significant ($P<0.05$) lower values. Hence, this parameter varied from 2 mm in April to 5 mm in August for the 50% PRD treatment.

Our results are in accordance with the findings of Robyt and White (1987), reporting that shoot growth ranged from 11 to 25 cm being higher in the control treatment. Spann et al. (2007) reported that the length of shoot growth produced from terminal buds on mature trees was quite variable, with the shortest shoots being less than 10 cm long and the longest shoots approaching a meter in some years. Baccari et al. (2020) reported that the effects of water stress on growth may be considered the first line of defense, due to the inhibition of cell elongation by the interruption of water flow from the xylem to the surrounding cells and serves to reduce the amount to total water transpired by the plant under drought conditions. Abboud et al. (2019) reported that the lowest shoot length was observed under the treatment 50%PRD. Similar results were found by Grattan et al. (2006), showing a clear reduction in shoot growth of olive trees under the lowest irrigation treatments. The PRD irrigation regime (50% PRD) decreased the vegetative growth and was consistent with several reports on partial root zone drying experiments on olive tree (Abboud et al., 2019; Dbara et al., 2016). Hence, Dbara et al. (2016) reported that the PRD50 induced a slight reduction of shoot elongation compared to control, while those of the PRD100 treatment did not statistically differ from the control.

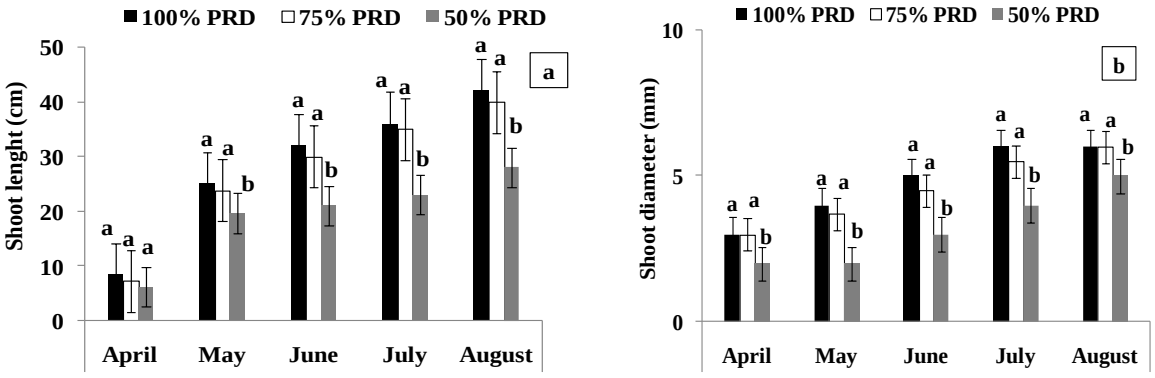

Fig 2. Shoot length (a) and diameter (b) of the pistachio cultivar Mateur. Different letters indicate significant differences among treatment.

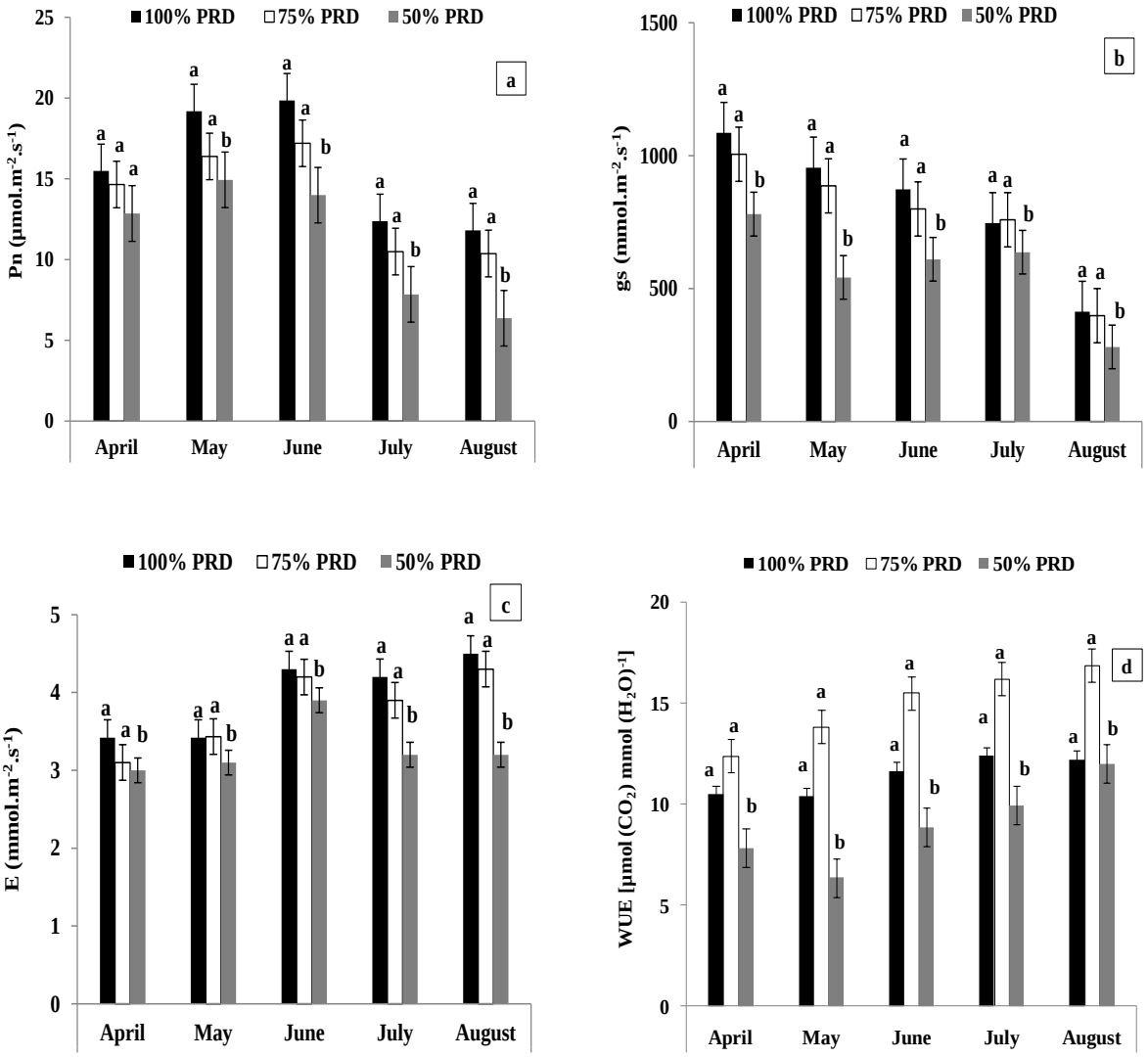

Fig. 3. Evolution of the photosynthetic assimilation (a), stomatal conductance (b), transpiration rate (c), and photosynthetic efficiency (d) of the pistachio cultivar Mateur. Different letters indicate significant differences among treatments.

Leaf gas exchange parameters

Photosynthetic assimilation

The photosynthetic assimilation (P_n) of the cultivar 'Mateur' under the PRD treatments was presented in Figure 3a. The Pn increased gradually during the rapid growth and the stone hardening stages of the pistachio nut (April, May and June). During the kernel growth stage (July and August), the Pn decreased gradually showing the high-water demand during this period of nut development. The treatment (T2) showed significantly ($P<0.05$) lower values of P_n.

Stomatal conductance

The stomatal conductance (g_S) of pistachio leaves (Fig. 3b) ranged from 320 to 760 mmol H_2O $m^{-2}s^{-1}$ in the 100% PRD treatment, showing statistically significant ($P<0.05$) differences among the three water regimes except for the values registered in April (Fig. 2b). Results showed that the water stressed treatment (T2) affected gs in all the nut development period whereas the treatment T1 (75% PRD) was less affected.

Transpiration (E)

The evolution of transpiration in the cv. Mateur is presented in Figure 3c. The evapotranspiration showed high values in August, being 4.5, 4.3 and 4.2 mmol m^{-2} s^{-1} for 100% PRD, 75 and 50% PRD treatments, respectively.

Water use efficiency (WUE)

The intrinsic water use efficiency (WUE) was significantly ($P<0.05$) affected by the irrigation regimes (Fig. 3d). The high values of WUE were detected under the 75% PRD. The lowest value of WUE (6.36 µmol (CO_2) mol $(H_2O)^{-1}$ was observed under the 50% PRD treatment during the month of May.

It has also been reported that the decrease in leaf photosynthesis in summer could be due to the temperature damage of the photosystems and the increase in the rate of photorespiration (Angelopoulos et al., 1996). Reddy et al. (2004) reported that the reduction of P_n in drought stressed plants could be attributed to the stomatal limitation and may also be explained by inhibited leaf photochemistry as well as metabolic impairment. According to Gijón et al. (2011) water stress applied during stages II (shell hardening) and III (kernel growth) were the most adversely circumstances for the plant physiological response. Leaf gas exchange is among the first processes that are affected by water deficit, through reducing the photosynthetic productivity, osmotic adjustment, and the capacity of plants to cope with drought (Ranjbar et al., 2021). Guerrero et al. (2006) reported that the RDI affected g_S later than Ψstem, and the greatest reduction in g_S (60% of control) was at the end of the regulated deficit irrigation period. Interestingly, the close relationship between Pn and gs reflects the role of gs in regulating the supply of CO_2 to the site of carboxylation and suggests that the decline in net photosynthesis over the season is largely a consequence of stomatal limitation (Abboud et al., 2019). Ranjbar et al. (2021) reported that the water use efficiency defined as the ratio of dry accumulation matter to water consumption during a season or the ratio of Pn to gs, is a significant indicator in assessing drought resistance.

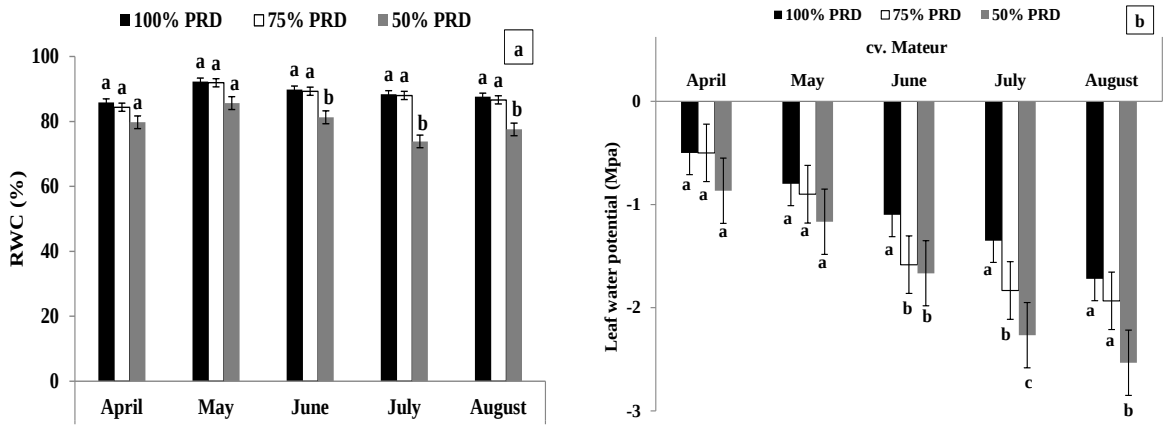

Fig. 4. Relative water content (RWC) (a), and leaf water potential (b) in leaves of the pistachio cultivar Mateur. Different letters indicate significant differences among treatments.

Water status

Relative water content

The Relative water content **(**RWC) of pistachio leaves under the three PRD treatments is presented in Figure 4a. The control treatment (T0) in our experiment maintained high RWC during all the growing season. In the current study, the PRD irrigation significantly ($P<0.05$) affected the tree water status. Under the treatment (T2), the RWC was significantly ($P<0.05$) reduced, especially during the months of June, July and August.

Leaf water potential

The leaf water potential of the cv. Mateur was evaluated during the growing season (Fig. 4b). At the beginning of the experiment, the leaf water potential values (about –1 MPa) no varied among the three treatments. During the stone hardening (June), the leaf water potential declined in the stressed treatments (75% PRD and 50% PRD). The greatest treatment differences in leaf water potential values occurred during July. At the end of the kernel growth stage, the control treatment (100% PRD) and the treatment (75% PRD) presented similar values.

Water is one of the main limiting factors of pistachio production in Tunisia. Under water deficiency, drought affects different physiological, biochemical, and molecular traits of the pistachio trees, whereas irrigation enhances the yield and improves the tree's nut quality (Haghighi et al., 2021). Several studies carried out in *Prunus* species reported a decrease in leaf water status and photosynthetic parameters (Jiménez et al., 2013; Escobar-Gutiérrez et al., 1998). Baccari et al. (2020) reported that the plant growth decrease is mainly due to the loss of turgor pressure through the decrease of trees water status attaining leaf water potential values between −6 and −4 MPa, thus indicating severe water deficit. These results are in accordance with the study of Abboud et al. (2021) in three olive cultivars conducted under partial root drying. Behboudian et al. (1986) reported that pistachio trees are able to pursue their photosynthetic activity even when leaf registers extraordinary low water potential (Ψleaf) values due to the unusual capability for leaf thermoregulation. Indeed, these authors showed that although photosynthesis declined with decreasing leaf water potential, plants continued to photosynthesize until a leaf water potential of as low as −5 MPa was reached which is a typical response of xerophytic plants. In the same line, Germana (1997) highlighted the great ability of pistachio to swiftly compensate water losses without displaying visible water stress symptoms. Additionally, Memmi et al. (2016b) considered that the rootstock *P.*

atlantica is adequate for deficit irrigated plantations due to its reputation as permissive to water stress under rain fed system.

Biochemical analysis

Results showed that the proline content (Fig. 5a) in pistachio leaves was significantly higher ($P < 0.05$) under the 50% PRD treatment as compared to 100 % PRD and 75% PRD treatments. Proline content reached its maximum value in the month of August under 50 % PRD (2.10 µmol g^{-1}FW). The proline accumulation was generally higher from June to the end of the trial. These findings are in accordance with the study of Abboud et al. (2021) showing that the proline accumulation was significantly ($P<0.05$) higher under the stressed water regime (50% PRD). This result matches previous works on olives (Abboud et al., 2021; Ben Ahmed et al., 2009) and citrus (Zandalinas et al., 2016), in which tolerant cultivars accumulate higher amounts of proline.

The total soluble sugars content was influenced by the irrigation treatments (Fig. 5b). The applied water regimes caused a significant increase ($P<0.05$) in sugar content for the treatment 50% PRD during the growing season. Furthermore, the maximum value of soluble sugar was 275.60 µg g^{-1} FW, belonged to the 50 % PRD treatment during the month of August. These results are in line with the study of Abboud et al. (2021) showing a total sugar accumulation in leaves of the three olive cultivars. Proline has been considered to act as an osmolyte, and ROS scavenger, its accumulation has been described as a tolerance mechanism used by plants to face drought stress (Jiménez et al., 2013). Dutra et al. (2017) reported that in addition to its conventional role as an osmolyte, proline protects membrane integrity and prevents enzyme/ protein denaturation by functioning as a potent ROS scavenger. Regarding the soluble sugar evolution, Escobar-Gutiérrez et al. (1998) showed that sorbitol rather than sucrose is preferentially photosynthesized at the low photosynthetic rates of drought-stressed peach leaves. Owing the putative role of soluble sugar and proline as antioxidants (Jimenez et al., 2013), they could improve deleterious effects of drought-induced oxidative stress by protecting membranes and enzymes.

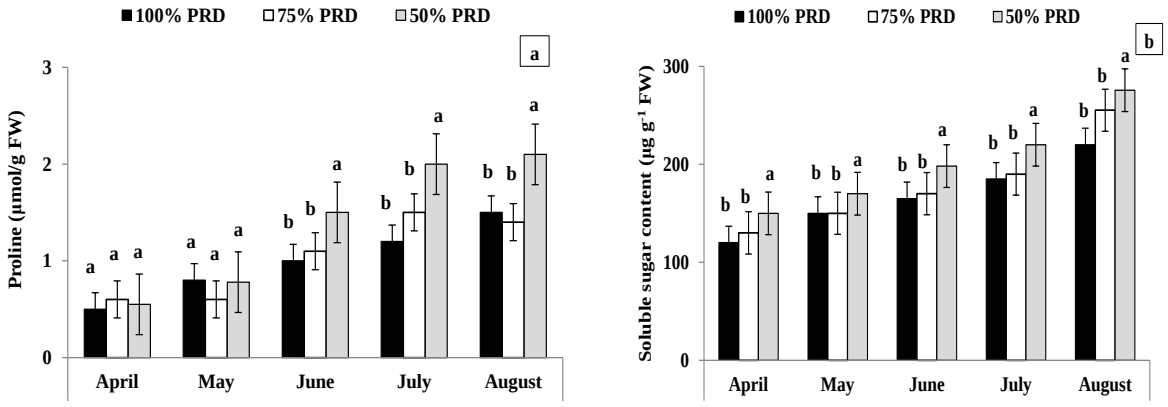

Fig. 5. Proline (a) and soluble sugar content (b) in the leaves of the pistachio cultivar Mateur. Different letters indicate significant differences among treatments.

CONCLUSION

The cv. Mateur presented quite similar response concerning the phenological traits and the tree vigor under the three PRD (100% PRD, 75% PRD, and 50% PRD) water regimes. A differential response to PRD irrigation was observed in the vegetative growth, water status, and the physiological parameters. However, the 75% PRD and 100% PRD showed similar behavior whereas the 50% PRD treatment led to a reduction in vegetative growth and leaf gas exchange. The reduction of irrigation volumes by 25% over the control (100% PRD) could be an efficient irrigation strategy to be implemented in high density pistachio orchards in arid and semi-arid conditions.

Conflict of interest

Authors declare no conflict of interest.

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Alleviating adverse effects of salt stress in pot marigold (*Calendula officinalis* L.) by foliar spray of silicon and nano-silicon under greenhouse and field conditions

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ABSTRACT

Purpose: In order to assay the impact of silicon (Si) and nano-Si on morphological and physiological traits of pot marigold (*Calendula officinalis* L.) under salt stress conditions, an experiment was conducted under greenhouse and field conditions. **Research Method**: The experiment was based on a completely randomized design including two levels of saline water (1.1 (control) and 6.1 dS m-1) and three levels of foliar spray (0, 2.5 mM Si and nano-Si) with 4 replications. **Findings**: Salinity stress decreased the vegetative and flowering parameters of pot marigold in the both conditions. Supplemental Si and nano-Si increased the dry weight of flowers under salt stress in the greenhouse (47 and 71%) and field (86 and 94%) conditions, respectively. Foliar application of nano-Si enhanced the flower total phenols of salt-stressed plants by 76% (greenhouse) and 50% (field), respectively. Under saline conditions, the use of nano-Si increased the flower antioxidant activity in the field by 17% in comparison to the control. Supplemental Si and nano-Si could reduce the negative impacts of salinity through increasing enzymatic and non-enzymatic antioxidants, accumulating soluble sugars, improving water relations, and enhancing chlorophyll content. **Research limitations:** No limitations were found. **Originality/value:** Based on the results of present study, the use of Si and nano-Si improved the growth and physiological characteristics of pot marigold under saline conditions.

INTRODUCTION

Pot marigold is an annual herbaceous plant belonging to the family Asteraceae and is used as an ornamental-medicinal plant (Khalid & da Silva, 2012). The flowers of this plant have a wide range of secondary metabolites, including flavonoids, carotenoids, glycosides, steroids, terpenoids, phenolic acids, mucilages, and saponins (Danila et al., 2011; Garcia-Risco et al., 2017). Pot marigold flower has diuretic, blood purifying and healing properties, and it can be used as a tonic, anticonvulsant, and anti-vomiting agent (Yoshikawa et al., 2001).

Salinity stress is one of the most critical environmental stress causes of a considerable loss in crop yield and growth. It decreases the amount of freshwater and land that can be used for agriculture (Zargar et al., 2019). About 20% of irrigated land is affected by salt, accounting for one-third of food-producing land (Gregory et al., 2018). The destructive effect of salt stress on plant growth is due to osmotic inhibition, ionic toxicity, and disruption of nutritional balance (Munns & Tester, 2008). Under salt stress, plants increase the osmotic pressure of the cells and facilitate the entry of water into plants through the accumulation of compatible solutes, which is known as the osmotic regulation process (Ghafiyehsanj et al., 2013). Compatible solutes generally include amino acids, ions, proline, soluble proteins, polyamines, and carbohydrates (Vyrides & Stuckey, 2017). Salinity, like other abiotic stresses, leads to increased reactive oxygen species (ROS) that can damage the membranes, nucleic acids, and lipids (Foyer, 2018).

Silicon (Si) is the second most abundant element in soil (Epstein, 2009). It plays a vital role in improving the growth and yield of crops, especially under abiotic and biotic stress (Frew et al., 2018; Wu et al., 2019). Improvement of salt tolerance has been reported following the application of Si in zinnia (Kamenidou et al., 2009), in *Borago officinalis* L. (Torabi et al., 2015), in rose (Soundararajan et al., 2018), mung bean (Ahmad et al., 2019), and *Bellis perennis* L. (Oraee & Tehranifar, 2023). The mechanisms by which Si increases salinity tolerance in plants include reduced sodium mobility, increased potassium uptake, enhanced activity of enzymatic and non-enzymatic antioxidant systems, improved photosynthesis, accumulation of osmolytes in the cells, and facilitated water relations (Ma, 2004; Khan et al., 2018; Ahmad et al., 2019).

Recently, the application of nanoparticles in different aspects of plant science has been considered by many researchers (Haghighi & Pessarakli, 2013). Nanoparticles display different properties than their bulk material due to the high surface area over volume ratio (Monica & Cremonini, 2009). Nano fertilizer is an essential approach in agriculture to improve the yield and quality of crops through increased nutrient use efficiency and reduction in fertilizer waste (Sharifiasl et al., 2019). It has been reported that the application of nano-Si had positive effects on the growth of cherry tomato (Haghighi & Pessarakli, 2013) and rice (Abdel-Haliem et al., 2017) under salinity stress. In this regard, Suriyaprabha et al. (2012) demonstrated that nano-Si, compared to Si had more positive impacts on the morphological and physiological properties of maize. Avestan et al. (2021) reported that supplemental nano-Si reduced the negative effects of salt stress on physiological changes in strawberry plants. Ismail et al. (2022) reported that the application of Si and nano-Si increased leaf relative water content (RWC) and improved antioxidant defense systems in *Pisum sativum* plants under salt stress. In addition, the beneficial effects of nano-Si application on the growth and fruit yield of tomato plants under salt stress conditions have been reported (Sayed et al., 2022). The nanoparticles have higher solubility and reactivity due to a larger surface area than bulk materials (Monica & Cremonini, 2009). The beneficial effects of Si on attenuating the negative impacts of salinity stress in various crops have been reported (Tuna et al., 2008; Khan et al., 2019). Nevertheless, based on the literature review, no research has been reported

the effect of salinity stress and Si and nano-Si on the morpho-physiological attributes of calendula. In this study, two greenhouse and field experiments were done to evaluate the impacts of Si and nano-Si on the morphological and physiological characteristics of pot marigold under salt stress.

MATERIALS AND METHODS

Greenhouse experiment

The experiment was based on a completely randomized design including two levels of saline water 1.1 (control) and 6.1 dS m^{-1}) and three levels of foliar spray (0, 2.5 mM Si and nano-Si) with 4 replications in early November 2018. The sources used for Si and nano-Si were silicon dioxide (SiO_2) and silicon dioxide nanopowder, respectively (Merck Co, Germany). The particle size of nano-Si was 20-30 nm with a purity of 98%. Two seeds of calendula (*Calendula officinalis* L. cv. Orange star) seeds were sown in plastic pots with a diameter of 16 cm filled with sandy loam soil (Table 1). Forty days after sowing; saline water and Si treatments were applied to the plants. The application method of Si and nano-Si was foliar spraying, two times with 10 days' intervals. Sodium chloride was also applied to the plants through irrigation water (300 ml per pot) twice a week. The plants were treated with saline water for 80 days and then the desired traits were measured.

The experiment was carried out based on a randomized complete block design with three replications in a research field located in Torbat-e-Jam city (35° 14′ 38″ N, 60° 37′ 21″ E), Khorasan Razavi province, Iran in April 2019. The physicochemical analysis of the used soil and the meteorological data during the test period are given in Tables 1 and 2, respectively. Salinity and Si treatments were similar to the greenhouse conditions. The calendula seeds were planted in plots with dimensions of 2 × 2 m with a distance of 30 cm between the rows and 20 cm on the rows. Forty days after sowing (at 6-8 leaf stage), saline water and Si treatments were applied to the plants. The volume of the solution for each plot was 40 liters per irrigation. The application method of Si and nano-Si was foliar spraying, two times with 10 days' intervals. The plants were treated with saline water for 80 days and then the desired traits were measured.

Measurements

The measured traits were leaf number, plant height, flower diameter, flower number, days from seed to flowering, and dry weight of shoots and flowers.

The leaf RWC was measured by the method of Pieczynski et al. (2013). The electrolyte leakage (EL) was measured by the method of Lutts et al. (1996). Chlorophyll content was measured by the method of Arnon (1949).

Total phenols were measured using Folin–Ciocalteu reagent, described by Blainski et al. (2013). Total flavonoids were measured by the method of Yoo et al. (2008). Antioxidant activity was measured by the method of Koleva et al. (2002) using 2, 2-diphenyl-1-picrylhydrazyl (DPPH). Total soluble sugars were measured by the method of Irigoyen et al. (1992) using anthrone reagent.

Data analysis

Statistical analysis of data was done using JMP software (version 13, 2016). Mean comparison of data was evaluated using the Least Significant Difference (LSD) test at 5% probability level.

Table 1. The physical and chemical characteristics of the soils used in the greenhouse and field experiments.

Experiment	Soil texture	pH	EC (ds/m)	Organic matter (%)	Field capacity (%)	Permanent wilting point (%)	N (%)
Greenhouse	Sandy loam	7.9	1.3	0.07	13.0	6.5	0.02
Field	Clay loam	7.8	4.5	0.5	16.5	8.5	0.02

Table 1. (Continued**).**

Experiment	K (meq l^{-1})	Ca (meq l^{-1})	Na (meq l^{-1})	Cl (meq l^{-1})	Mg (meq l^{-1})	Sodium adsorption ratio (SAR)
Greenhouse	8.2	8.6	23.1	25.3	1.5	10.3
Field	17.6	6.2	32.4	25.9	4.2	14.2

Table 2. Minimum and maximum monthly average temperatures and rainfall of the study location during the field experimental period in year 2019.

Month	Average monthly maximum temperature (°C)	Average monthly minimum temperature (°C)	Rainfall (mm)
April	22.0	8.3	57.8
May	23.8	11.8	4
June	30.1	16.4	0
July	35.6	22.4	0
August	36.7	22.7	0

RESULTS

Salt stress reduced the growth factors in both conditions (Table 3). Nano-Si supplementation enhanced the plant height under salt stress by 36% (greenhouse) and 24% (field). The highest leaf number of the salt-stressed plants was obtained from nano-Si treated plants by 41% (greenhouse) and 22% (field) increase related to the control. In the greenhouse, supplemental nano-Si increased shoot dry weight of the salt-stressed plants more than 2-fold. Also, under field and salt stress conditions, application of Si and nano-Si enhanced dry weight of shoot by 13 and 24%, respectively (Table 3).

The flowering attributes were affected by salt stress in both conditions. Under salinity stress, the treatment of calendula plants with nano-Si had the lowest days to flowering in both conditions. Under salt stress, nano-Si supplementation increased the flower diameter by 19% (greenhouse) and 49% (field) in comparison to control plants, respectively. Supplemental nano-Si enhanced the flower number by 43% (greenhouse) and 2.2 times (field) in comparison to control plants, respectively (Table 3). Salinity stress decreased the flower dry weight. However, supplemental Si and nano-Si enhanced dry weight of flowers in the greenhouse (47 and 71%) and field (86 and 94%) conditions in comparison to control plants, respectively (Fig. 1).

Salt stress decreased the leaf RWC in both conditions. However, foliar spray of Si and nano-Si enhanced this parameter. Under field conditions, Si and nano-Si supplementation increased the RWC by 5 and 10%, respectively (Table 4). The EL of leaf was increased by salt in both conditions. However, use of Si and nano-Si reduced the EL of salt-stressed plants (Table 4). Salt stress reduced the total chlorophylls. However, nano-Si supplementation increased total chlorophylls (Table 4). In the greenhouse, the use of nano-Si enhanced the total soluble sugars in the leaf of salt-stressed plants by 37%. Under field and salinity stress conditions, nano-Si supplementation increased root total soluble sugars by 19% (Table 4).

Table 3. Effects of silicon (Si) and nano-Si on plant height, leaf number, dry weight of shoot, days to flowering, flower diameter, and flower number of salt-stressed pot marigold (*Calendula officinalis* L.) under greenhouse and field conditions.

Salinity (dS m^{-1})	Foliar spray (mM)	Plant height (cm)	Leaf number/plant	Dry weight of shoot (g)	Days to flowering	Flower diameter (mm)	Flower number/plant
	Greenhouse						
1.1 (control)	0	14.05±0.33 c	18.75±0.47 d	0.51±0.02 d	107.00±2.85 a	33.04±0.20 bc	4.50±0.28 b
	Si 2.5	16.87±0.20 b	29.00±0.91 b	1.34±0.03 b	100.25±1.49 b	34.51±0.67 ab	5.93±0.06 a
	Nano-Si 2.5	18.31±0.33 a	39.75±1.23 a	1.49±0.02 a	96.25±2.05 bc	35.76±0.25 a	6.60±0.21 a
6.1	0	10.00±0.54 e	16.75±0.85 d	0.27±0.03 f	98.75±1.10 b	26.50±0.34 e	2.25±0.25 d
	Si 2.5	12.12±0.23 d	21.50±0.64 c	0.40±0.01 e	89.75±1.84 cd	29.14±0.41 d	2.80±0.33 cd
	Nano-Si 2.5	13.65±0.64 c	23.37±0.37 c	0.64±0.03 c	84.25±2.25 d	31.80±0.59 c	3.23±0.27 c
	Field						
1.1 (control)	0	29.50±0.38 b	152.03±1.22 b	2.26±0.05 b	124.33±1.20 a	24.26±0.56 b	9.50±0.52 c
	Si 2.5	30.83±0.44 b	158.58±2.66 b	2.68±0.06 a	123.33±0.88 a	28.13±0.31 a	18.93±0.59 b
	Nano-Si 2.5	35.91±0.30 a	197.13±3.13 a	2.83±0.08 a	112.33±1.15 bc	28.28±0.65 a	21.58±0.87 a
6.1	0	19.80±0.85 e	98.66±3.14 e	1.42±0.09 d	113.00±1.52 b	17.17±0.29 d	8.91±0.91 c
	Si 2.5	21.75±0.66 d	111.00±1.01 d	1.62±0.10 cd	105.00±3.24 c	20.63±0.49 c	19.35±0.37 b
	Nano-Si 2.5	24.69±0.18 c	121.08±0.90 c	1.77±0.05 c	96.13±3.40 d	25.60±0.21 b	19.58±0.88 ab

Means in the columns followed by the same letter are not significantly different according to LSD test at $P < 0.05$.
The numbers following ± sign are the standard errors.

Table 4. Effects of silicon (Si) and nano-Si on relative water content (RWC), electrolyte leakage (EL), total chlorophyll content, and total soluble sugars of the root and shoot tissues of salt-stressed pot marigold (*Calendula officinalis* L.) under greenhouse and field conditions.

Salinity (dS m^{-1})	Foliar spray (mM)	RWC (%)	EL (%)	Total chlorophyll (mg g FW^{-1})	Root soluble sugars (mg g DW^{-1})	Shoot soluble sugars (mg g DW^{-1})
		Greenhouse				
1.1 (Control)	0	85.46 ± 0.35 c	14.36 ± 0.94 d	0.48 ± 0.011 c	1.26 ± 0.10 e	2.02 ± 0.28 e
	Si 2.5	90.75 ± 0.84 b	10.05 ± 0.71 e	0.56 ± 0.008 b	3.92 ± 0.11 c	2.76 ± 0.37 d
	Nano-Si 2.5	92.83 ± 0.68 a	14.46 ± 0.83 d	0.77 ± 0.006 a	4.85 ± 0.12 b	5.50 ± 0.07 b
6.1	0	72.66 ± 0.53 e	34.58 ± 0.56 a	0.28 ± 0.015 e	3.04 ± 0.23 d	4.76 ± 0.12 c
	Si 2.5	75.80 ± 0.64 d	27.33 ± 0.22 c	0.29 ± 0.007 de	4.48 ± 0.07 b	4.79 ± 0.07 c
	Nano-Si 2.5	76.23 ± 0.44 d	29.65 ± 0.40 b	0.31 ± 0.016 d	5.33 ± 0.17 a	6.57 ± 0.25 a
		Field				
1.1 (Control)	0	80.77 ± 1.44 bc	20.27 ± 1.79 c	0.84 ± 0.003 cd	1.18 ± 0.16 c	2.16 ± 0.07 b
	Si 2.5	86.04 ± 1.47 ab	12.60 ± 0.45 d	0.93 ± 0.036 b	1.08 ± 0.03 c	2.26 ± 0.29 b
	Nano-Si 2.5	88.43 ± 1.23 a	13.14 ± 0.53 d	1.10 ± 0.008 a	1.33 ± 0.13 c	2.91 ± 0.30 b
6.1	0	70.05 ± 1.25 d	39.10 ± 0.55 a	0.77 ± 0.022 de	4.35 ± 0.09 b	5.85 ± 0.25 a
	Si 2.5	75.65 ± 2.09 cd	30.83 ± 1.06 b	0.76 ± 0.037 e	4.58 ± 0.13 b	5.85 ± 0.09 a
	Nano-Si 2.5	80.39 ± 2.02 bc	32.49 ± 1.09 b	0.89 ± 0.015 bc	5.22 ± 0.06 a	6.42 ± 0.22 a

Means in the columns followed by the same letter are not significantly different according to LSD test at $P < 0.05$. The numbers following ± sign are the standard errors.

Total phenols and flavonoids, and antioxidant activity of the flowers and leaves were affected by salt, Si, and nano-Si (Table 5). The use of nano-Si enhanced the flower total phenols by 76% (greenhouse) and 50% (field). The application of Si and nano-Si increased the leaf total flavonoids under salinity stress in the greenhouse (29 and 36%) and field (19 and 28%), respectively. Under field and salinity stress conditions, application of nano-Si enhanced the flower antioxidant activity by 17%. Under saline conditions, the use of nano-Si enhanced the antioxidant activity of the leaves by 4% (greenhouse) and 21% (field), respectively (Table 5).

Table 5. Effects of silicon (Si) and nano-Si on total phenols, total flavonoids, and the antioxidant activity of flowers and leaves of salt-stressed pot marigold (*Calendula officinalis* L.) under greenhouse and field conditions.

Salinity (dS m^{-1})	Foliar spray (mM)	Total phenols of flowers (mg g DW^{-1})	Total Flavonoids of flowers (mg g DW^{-1})	Antioxidant activity of flowers (%)	Total phenols of leaves (mg g DW^{-1})	Total Flavonoids of leaves (mg g DW^{-1})	Antioxidant activity of leaves (%)
			Greenhouse				
	0	5.22±0.15 e	0.21±0.01 d	50.01±0.21 e	9.71±0.18 e	1.00±0.03 d	50.57±0.78 d
1.1	Si 2.5	10.75±0.59 c	0.58±0.06 c	51.56±0.65 d	10.73±0.47 de	1.48±0.01 b	62.19±0.16 c
(Control)	Nano-Si 2.5	11.02±0.32 c	0.79±0.02 ab	54.35±0.38 c	15.68±0.36 c	1.50±0.05 b	64.88±0.52 ab
	0	9.16±0.34 d	0.47±0.04 c	61.94±0.39 b	11.24±0.83 d	1.19±0.01 c	61.60±1.15 c
6.1	Si 2.5	13.00±0.10 b	0.70±0.03 b	62.55±0.47 ab	21.01±0.54 b	1.54±0.04 ab	62.84±0.44 bc
	Nano-Si 2.5	16.16±0.45 a	0.86±0.02 a	63.72±0.25 a	24.30±0.39 a	1.62±0.05 a	65.82±1.10 a
			Field				
	0	7.49±1.06 c	1.27±0.02 e	57.50±1.32 c	10.40±0.39 e	1.33±0.12 d	52.21±2.47 d
1.1	Si 2.5	11.07±0.22 b	1.73±0.01 d	68.60±0.16 b	22.49±0.67 c	1.90±0.10 c	54.88±0.76 d
(Control)	Nano-Si 2.5	12.27±0.91 b	1.82±0.03 d	75.01±2.62 a	23.50±1.37 bc	2.28±0.08 b	71.01±1.58 b
	0	12.25±0.33 b	2.01±0.09 c	63.46±2.07 b	14.53±0.26 d	2.52±0.08 b	63.00±0.37 c
6.1	Si 2.5	17.36±0.59 a	2.51±0.02 b	68.70±0.16 b	26.67±1.00 ab	3.02±0.09 a	70.62±0.74 b
	Nano-Si 2.5	18.41±0.35 a	3.01±0.06 a	80.05±2.36 a	28.33±0.97 a	3.25±0.04 a	84.24±0.83 a

Means in the columns followed by the same letter are not significantly different according to LSD test at $P < 0.05$.
The numbers following ± sign are the standard errors.

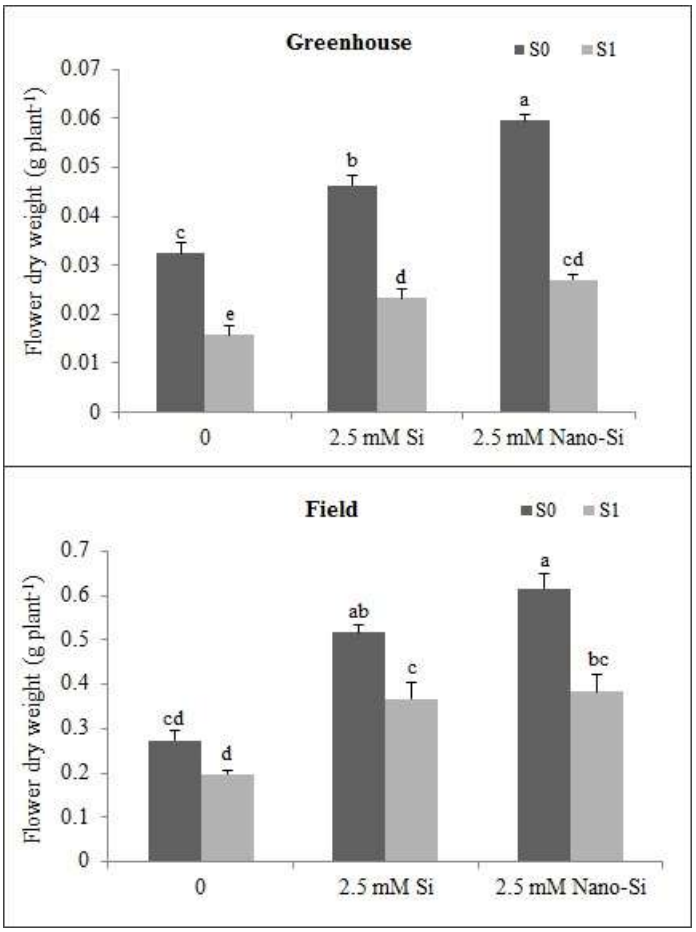

Fig. 1. Effects of silicon (Si) and nano-Si on flower dry weight of salt-stressed pot marigold (*Calendula officinalis* L.) under greenhouse and field conditions. Values followed by the same letter are not significantly different according to the LSD test at $P < 0.05$. Vertical bars indicate ± SE. S0 and S1: 1.1 (control) and 6.1 dS m^{-1}, respectively.

DISCUSSION

In this study, salinity reduced the vegetative parameters of calendula. However, foliar spray with Si and nano-Si was useful in alleviating the negative impacts of salt stress. Decreasing effects of salt stress on the vegetative parameters have been reported in various crops (Jaffel-Hamza et al., 2013; Soundararajan et al., 2018; Kamran et al., 2019). The increase in growth with the use of Si has been reported in zinnia (Kamenidou et al., 2009), in cherry tomato (Haghighi & Pessarakli, 2013), in pot marigold (Bayat et al., 2013), and rice (Mahdieh et al., 2015). Silicon probably improves plant growth under salinity stress conditions by increasing photosynthesis machinery and reducing respiration (Zhu et al., 2015; Ahmad et al., 2019). Moreover, the growth-promoting ability by applying Si may be due to the reduction in root Na uptake and, or its root-to-shoot transport in salt-stressed plants (Kafi et al., 2011; Khan et al., 2019). In this respect, Garg and Bhandari (2016) found that Si supplementation reduced the Na contents in the roots and the leaves of chickpeas (*Cicer arietinum* L.). Also, it has been shown that Si nanoparticles, by forming a layer on the root cell wall, can increase plant stress tolerance and improve crop yield (DeRosa et al., 2010). In this study, although the salinity of soil in the field experiment was higher than that in the greenhouse experiment, all the growth parameters measured in the field were higher than those recorded in the pot experiment. Similarly, Hamdi et al. (2019) demonstrated that although the salinity of irrigation water in the field experiment was higher than that in the greenhouse, the yield parameters of durum wheat grown in the field were higher than those in the greenhouse. A possible reason for higher growth and yield in the field conditions compared to the greenhouse can be attributed to greater soil depth, better root growth and expansion, higher light intensity, and greater access to water and nutrients (Arena et al., 2017; He et al., 2017).

Salt stress negatively affected the flowering of pot marigold. However, foliar spray of Si and nano-Si improved these parameters. Kamenidou et al. (2010) reported that the application of Si increased the flower quality of gerbera. Bayat et al. (2013) also reported that application of Si improved the flower quality of salt-stressed calendula. The positive impact of Si on flowering may be due to its role in enhancing gibberellin levels, which was detected in wheat shoots (Hanafy Ahmed et al., 2008). Gibberellin stimulates flowering in many plant species through participation in floral induction and development (Jang et al., 2018).

In our experiment, salt stress reduced the RWC of pot marigold leaves. Increased sodium concentration in plant tissues is a possible reason for the decrease in RWC of the leaves (Cicek & Cakirlar, 2002). The reduction in leaf RWC indicates a reduction in turgor pressure, which reduces water required for morphological and physiological processes such as cell elongation, stomatal opening, and photosynthesis (Acosta-Motos et al., 2017). The use of Si and nano-Si increased the leaf RWC under salt stress. Silicon facilitates water absorption and transport under osmotic stress conditions and increases the leaf RWC (Khan et al., 2019). In cucumber, Zhu et al. (2015) reported that Si supplementation increased root water uptake and RWC by stimulating root growth and root hydraulic conductance. Matoh et al. (1986) also demonstrated that Si supplementation maintained RWC in rice plants through the decrease in the transpiration rate of leaves.

In this study, salinity increased the EL of leaf in accordance with the results of Bayat et al. (2012) in pot marigold. Increased EL indicates reduced membrane stability, which is probably the result of oxidative stress under saline conditions (Foyer, 2018). Bayat et al. (2013) found that Si supplementation reduced the EL of calendula under salt stress.

In this study, salinity stress reduced the total chlorophyll content. Salt stress increases the ROS production in chloroplasts and reduces chlorophyll content (Haghighi & Pessarakli, 2013). Various studies have shown the positive effect of Si on chlorophyll biosynthesis under

saline conditions. For example, Falouti et al. (2022) demonstrated that the use of Si reduced the negative effects of salinity stress on chlorophyll content and total biomass in barley. Increased total chlorophylls following the application of Si may be attributed to its role in preventing chlorophyll chain degradation (Bayat et al., 2013). In addition, the treatment with Si nanoparticles increased the activity of antioxidant enzymes in potato under saline conditions, thereby maintaining chlorophyll stability (Mahmoud et al., 2020).

Based on the present results, salinity enhanced the total soluble sugars. The soluble sugars accumulate under environmental stress conditions and protect plants through osmosis regulation via continuous water influx, maintaining membrane and protein stability, and scavenging the ROS (Kumar et al., 2007; Turkan, 2011). Several studies have shown that Si supplementation can increase the salt resistance of different plants by regulating the synthesis of compatible solutes (Yin et al., 2013; Zhu et al., 2016). The accumulation of soluble sugars following the use of Si helps the plant to maintain metabolic activity by retaining water in their tissues under salinity stress conditions (Zhu et al., 2019).

In this experiment, salinity increased the total phenols, total flavonoids, and antioxidant activity.

Salt stress causes an increase in the ROS, which damages the membranes, proteins, and organelles (Foyer, 2018). Plants use enzymatic and non-enzymatic antioxidant systems to detoxify the ROS (Bayat & Moghadam, 2019). In current study, Si and nano-Si supplementation improved the antioxidant system of pot marigold under salinity. Similar results were reported on cucumber (Khoshgoftarmanesh et al., 2014), sunflower (Conceição et al., 2019), and *Bellis perennis* L. (Oraee & Tehranifar, 2023). Similarly, Ahmad et al. (2019) found that Si supplementation increased the activity of antioxidant enzymes in salt-stressed mung bean plants.

The treatment of plants with nano-Si was more effective in alleviating the adverse effects of salt stress than Si in the studied traits. These results are in agreement with previous studies on maize (Suriyaprabha et al., 2012) and rice (Abdel-Haliem et al., 2017). Silicon nanoparticles are better adsorbed and transported by plants due to their smaller size (Abdel-Haliem et al., 2017). Nano-Si has a larger surface area than its bulk material and therefore has higher solubility and reactivity (Monica & Cremonini, 2009).

CONCLUSION

The use of Si and nano-Si alleviates harmful impacts of salt stress on the morphological and physiological traits of calendula by increasing the activity of antioxidant systems, improving water relation, maintaining cell membrane integrity, and enhancing chlorophyll content. However, the positive effects of nano-Si were higher than that of Si in reducing the negative impacts of salinity in the greenhouse and field conditions, which can be used as an alternative source of Si fertilizer to help the sustainable farming of pot marigold.

Conflict of interest statement

The authors at this moment hereby declare that there is no conflict of interest.

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Effect of pre - and post-harvest factors on 'Benny' Valencia fruit rind phenolics on mitigation of chilling and non-chilling disorders during cold storage

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ABSTRACT

Purpose: This study investigated the effect of harvest time, postharvest dehydration + waxing and storage temperature on rind-free and conjugated phenolics and their ability to alleviate chilling injury and pitting of 'Benny' Valencia oranges during cold storage. **Research method:** Fruit were harvested at early, mid- and late season, and thereafter, divided into control, dehydrated, waxed + dehydrated portions. After treatment, fruit were stored at -0.6 and 4.5°C for 28 days, thereafter, 7 days at ambient temperature (25°C). **Findings:** In general, peel pitting index (PPI) was significantly higher for late season fruit, while, CI was higher for early season fruit, especially at -0.6°C storage. Furthermore, dehydration stress without waxing resulted in significantly higher PPI and CI at -0.6°C when compared with 4.5°C storage. With respect to both free and soluble conjugated phenolics, the control fruit showed higher levels of rind phenolics, especially at late harvest across all the storage temperatures. Therefore, untreated fruit appeared to tolerate cold stress by up-regulating endogenous systems of total rind phenolics. Postharvest dehydration repressed endogenous phenolics synthesis. In conclusion, susceptibility to pitting disorder increases with harvest time, dehydration stress, while fruit harvested early were highly susceptible to CI. **Research limitations:** The main limitation of this study is the lack of specific phenolics. **Originality/Value**: The study found that dehydration plus waxing has a significant effect on chilling and non-chilling citrus 'Benny' Valencia fruit. Furthermore, these treatments induced an increase in rind total phenolics to mitigate rind physiological disorders during extended cold storage.

INTRODUCTION

In South Africa, citrus is the third largest horticultural industry after deciduous fruit and vegetable industries (Dodd et al., 2010; CGA, 2015). Globally, the industry is the second highest exporter of citrus fruit after Spain, exporting approximately 1,7 million tons annually (CGA, 2015). Exported citrus fruit from South Africa to USA, Korea, Thailand and China must be cold sterilised at -0.6°C for 22 days in order to comply with the phytosanitary requirements (Dodd et al., 2010; Hordijk, 2013). In general, cold sterilisation has been proved by Hordijk (2013) to be the best practice that kills insect larvae of major pest in citrus, including false codling moth (*Cryptophlebia leucotreta*) and Mediterranean fruit fly (*Ceratitis capitata*), However, it has previously been found that cold sterilisation may result in rind physiological disorder such as; chilling injury (CI) and rind pitting disorder (RPD) which manifest during shipment; and therefore, affecting fruit quality (Dou, 2004; Dou, 2005; Magwaza, 2008).

According to Magwaza et al. (2013), symptoms of non-chilling (pitting) and chilling rind disorders are non-distinguishable. Chilling injury manifest in the citrus rind initially as small sunken lesion that later enlarge and become dark-brown sport, thus reducing fruit marketability (Dou, 2004; Dou, 2005). While non-chilling rind disorders manifest as the collapse of the sub-epidermal rind cells with no discoloration taking place at early stages, at later stages, the clusters of oil gland are affected which release intercellular content that change colourless lesions to brown colour (Alquezar et al., 2010). Previous studies have attempted to study and understand CI and RPD mostly in citrus fruit types such as lemon fruit (Mathaba, 2012), mandarin fruit (Medeira et al., 1999; Joubert, 2016), navel oranges (Alférez, & Zacarías, 2000; Agustí et al., 2001) and 'Marsh' grapefruit (Alférez & Burns, 2004; Olarewaju et al., 2020). Recently, 'Benny' Valencia were found to be highly susceptible to peel pitting in South Africa (Ehlers, 2016; Cronje et al., 2017; Mothapo et al., 2022), of which the cause is unknown. However, as part of solution, various factors have been previously related with the occurrence of rind physiological disorders on other cultivars with no clear solutions.

Previously researchers investigated various factors that relate to development of rind physiological disorders on other citrus cultivars. In Spain and Florida, researchers investigated post-harvest RPD on grapefruit (*Citrus paradisi* Macfadyen) and found that the fluctuation in relative humidity at harvest and during storage caused rind physiological disorder (Alférez & Burns, 2004; Alférez et al., 2010; Alquezar et al., 2010) and similar findings were observed in 'Brigitta' norther highbush blueberry (*Vaccinium corymbosum*) (Moggia et al., 2023). According to Alférez et al. (2003), exposure to low relative humidity followed by rehydration at high relative humidity induces difference in cellular water potential between the rind tissues (flavedo and albedo), thereby, leading to albedo cells collapse. In contrast, Alférez and Zacharías (2014) reported that changes in rind morphological and their potential relation to the alteration in water status as induced by fluctuations of relative humidity during post-harvest handling is dependent on fruit maturity at harvest. The effect of maturity has yielded various conclusions in different citrus cultivars (Gonzalez-Aguilar et al., 2000).

Several researchers studied the effect of fruit maturity on physiological disorder in citrus and in accordance to their results early and late harvest times usually leads to more post-harvest RPD (Dou, 2005; Khumalo, 2006; Magwaza et al., 2013) and 'Brigatta' blueberries harvested early, peak, and late (Moggia et al., 2023). Furthermore, fruit maturity has been found to also influence phytochemical substances (alkaloids, cyanogenic glycosides, flavonoids, terpenoids and phenolic compounds) with antioxidant capacity. In citrus,

phenolics are the most effective phytochemical substances and bioactive compounds with antioxidant properties, playing a crucial role to maintain fruit quality (Mditshwa, 2012). Under stress conditions, phenolics act as scavenging compounds against various reactive oxygen species (ROS), especially during cold storage (Sala, 1998). Oxidative stress occurs when production of ROS exceeds the capacity of the cell to scavenge and resulting in the occurrence of physiological disorders (Huang et al., 2007; Sala, 1998).

In pomegranate fruit, Fawole and Opara (2013) found that as maturation continues, several antioxidants (scavengers) are synthesized and such compounds determines the fruit quality attributes under cold storage. In the study of Wang and Lin (2000), higher total phenolics concentrations and total antioxidant activity were reported on late harvested blackberry, raspberry, and strawberry fruit. Conversely, there is sufficient evidence suggesting that waxing play a significant role in regulation of total phenolic metabolism (Meighani et al., 2015). However, the information is unclear. Thus, the aim of this study was to investigate the combined effect of harvest time, postharvest dehydration plus waxing and storage temperature on rind phenolics in order to alleviate manifestation of chilling injury and pitting of 'Benny Valencia sweet oranges after cold sterilization.

MATERIALS AND METHODS

Experimental sites and procedures

Fruit were randomly harvested at early (3 weeks before commercial harvest - June 2021), mid- (commercial harvest on the - July 2021) and late (three weeks after commercial harvest - August 2021) from Mahela Boerdery commercial farm at Letsitele in Limpopo province, South Africa (23° 88′ 36″ S, 30° 82′ 34″ E). After harvest, fruit were transported to the Agricultural Research Council - Institute for Tropical and Subtropical Crops (ARC-ITSC) postharvest laboratory in Nelspruit (25° 45′ 18″ S, 30° 96′ 97″ E) and drenched with water containing Sporekill® (Dimethyldidecyl ammonium chloride, Hygrotech Pty LTD, South Africa) (120 mg L^{-1}), thereafter air dried. After air-drying, fruit were packed inside 18 crates (0.14 m^{-2}), each containing 25 fruit. At each harvest, ten fruit sample were collected before storage and used for immediate external and internal quality, while the additional ten fruit per each treatment were stored for analysis after storage.

Post-harvest treatments

The experiment comprised of three treatment factors: harvest time (early, mid- and late), three post-harvest treatments [Wax only (A), wax plus dehydration (B) and dehydration only (C)] and two storage temperatures (-0.6±0.1 and 4.5±0.1°C). From the 18 crates, one crate fruit (n = 25) were randomly allocated to each of the three treatments, which were replicated three times. Fruit allocated for treatment A (n = 25) served as control and were waxed using Citrishine® (Citrishine Pty Ltd, Johannesburg, South Africa), thereafter transferred directly to cold storage temperature (0.6±0.1 and 4.5±0.1°C) with 90±1% relative humidity (RH). Fruits for treatment B (n = 25) were also waxed before storage. However, prior to storage, 3 days' dehydration stress was applied to fruit allocated for treatment B by dehydrating fruit at constant condition of 25°C with 45±1% RH for 3 days, in order to create an environment conducive to induce high vapour pressure deficit (0.7-1.1 kPa) (Alférez et al., 2003). Fruit allocated for treatment C (n =25) received similar dehydration stress as treatment B; however, did not receive wax application. Thereafter, fruit were exposed to 3 days of dehydration stress (Treatments B and C) thereafter transferred to different cold storage temperature (-0.6±0.1 and 4.5±0.1°C), for up to 28 days. After 28 days of cold storage, fruit were kept at room temperature for 7 days to allow citrus rind pitting and chilling injury to manifest. Storage

temperature and relative humidity were monitored by means of temperature/relative humidity logger (Tinytag View 2 TV-4500 Gemini Data Loggers (UK) Ltd. After 7 days' shelf-life, fruit were evaluated for rind physiological disorder (peel pitting and chilling injury), electrolyte leakage, weight loss, firmness loss, rind colour and internal fruit quality.

Estimation of Peel pitting/ chilling injury rind physiological disorders

Fruit were evaluated visually before cold storage and after 7 days' shelf-life. In each fruit, number and cluster of sunken glands were counted. Visual appearance (peel pitting) was assessed based on a 4-point hedonic scale: [0 (no post-harvest pitting: 0%), 1 (low post-harvest pitting: 25%), 2 (low to moderate post-harvest pitting: 50%), 3 (moderate to high pitting: 75%) and 4 (severe post-harvest pitting: 100%)]. Peel pitting incidence was calculated using the formula previously reported by Lafuente and Sala (2002) as follow (1):

$$\text{Peel Pitting Index (PPI)} = \frac{\sum(\text{Peel damage scale (0–4)} \times \text{number of fruit within each class})}{\text{Total number of fruit}} \quad (1)$$

Weight loss

Fruit weight was measured before cold storage (W_0) and after 7 days' shelf-life (W_1) using weighing scale (Scaltec, SBA, Heilingenstadt, Germany). Weight loss was calculated using the following formula (2):

$$\text{Weight loss (\%)} = \left[\frac{W_0 - W_1}{W_0}\right] \times 100\% \quad (2)$$

Electrolyte leakage

Membrane leakage was determined using fruit disks following the method of Bajji et al., (2002) and calculated using the following formula (3):

$$\text{Total electrolyte leakage (\%)} = (EC_1/EC_2) \times 100 \quad (3)$$

Firmness loss

Fruit firmness loss was measured before cold storage (F_1) and after 7 days of shelf-life (F_2) using Sinclair desktop machine (Model: 51DFTB, International LTD, Jarrold, Bowthorpa, Norwich, NR5, 9.D, England) and calculated using the following formula (4):

$$\text{Firmness loss (\%)} = \left[\frac{F_1 - F_2}{F_1}\right] \times 100\% \quad (4)$$

Determination of total phenolic concentration

Total phenolic concentration (TPC) was measured using the Folin–Ciocalteu (Folin–C) method as described by Abeysinghe et al. (2007) with some modification. Pulverized lemon peel (0.5 g) was accurately weighed in a test tube. For both free and conjugated phenolics, samples were extracted with 5 ml of 50% DMSO: 50% of 1.2 M HCl in 80% methanol/water, vortexed for 1 minute and centrifuged at 10 000 rpm for 10 min to remove the solid fraction. The resultant supernatant was used for determination of free phenolics. Therefore, for extraction of conjugated phenolics, samples were heated at 90°C for 3 hours, with vortexing every 30 minutes and after allowed to cool down to room temperature, following which they were diluted to 10 ml with methanol and centrifuged at 10 000 rpm for 10 minutes to remove the solid fraction. Thereafter, Folin-Ciocalteu reagent (0.5 ml) was added to the solution and allowed to react for 3 minutes. The reaction was neutralized with 1 ml of sodium carbonate (2%) solution. The mixture was then vortexed, and absorbance read at 760 nm using a UV–visible spectrophotometer (Thermo Scientific Technologies, Madison, Wisconsin). Gallic acid

was used as standard (0.02−0.10 mg mL^{-1}) and data were expressed as milligram gallic acid equivalent per 100 mL DW (mg GAE 100 mL^{-1} DW).

Statistical analysis

Analysis of variance was performed using using GenStat 16th edition (VSN international, UK). Means were separated using Least Significant Difference Test (LSD) at probability level of ($P < 0.05$). Relationship between weight loss vs peel pitting index and chilling injury index vs electrolyte leakage was performed using Pearson correlation method.

RESULTS AND DISCUSSION

Peel pitting

In this study, peel pitting symptoms appeared as cluster of sunken areas ('pits') on the flavedo that eventually affected oil glands and were higher in fruit stored at -0.6°C when compared with fruit stored at 4.5°C, irrespective of harvest time (Fig. 1). This could be as result of rind ultrastructural changes and breakdown of the external cellular strata due to chilling temperature (Vercher et al., 1994). Moreover, peel pitting index (PPI) increased with maturity and was significantly higher at late harvest, especially for unwaxed fruit exposed to dehydration stress after removal from storage at -0.6 and 4.5°C. These results were similar to those previously reported by Alférez and Zacarías (2014), whereby, peel pitting incidence were significantly higher for late season and dehydrated 'Navelin' navel fruit stored at 20°C for 28 days. Meanwhile, waxed fruit either with dehydration or non-dehydration treatment showed less susceptibility to the incidence of peel pitting when compared with unwaxed fruit, irrespective of harvest time and storage temperature. Generally, fruit exposed to dehydration stress at low RH experience higher moisture loss, reducing flavedo and later albedo cells water potential (Agustí et al., 2001; Alférez et al., 2003; Alférez et al., 2005). In the findings of Alquezar et al., (2010), higher rind pitting was observed on 'Nave' orange fruit stored at 45% RH when compared with 95% RH at 20°C for 5 weeks. According to Alférez et al. (2003), Alférez et al. (2004), and Ehlers (2016), when fruit is transferred to the water-saturated atmosphere (95% RH) where vapour pressure deficit (VPD) was low, water moves from the surroundings to the epidermal cells, resulting into rehydration of cells. Flavedo and outer albedo cells rehydrate fast when compared with subtending albedo cells, creating variation in water potential between the cells (Alférez et al., 2003; Alférez & Burns, 2004), which in turn depends on fruit maturity (Storey & Treeby, 1994). This variation creates a suction force between cells; and subsequently, cause collapse of internal flavedo and external albedo cell layers, resulting into peel pitting development (Agustí et al., 2001; Alquezer et al., 2010). Alférez and Zacarías (2014), reported that late harvested 'Navelate' navel fruit albedo cells were shapeless and compacted after ehydration, thereby failed to recover after it was transferred to higher RH. This could be the reason for the occurrence of peel pitting on unwaxed late harvested fruit exposed to dehydration stress prior storage. So far, findings have shown contradictory wax effects on pitting, whereby, non-waxed citrus fruit also showed higher pitting due to prior dehydration (Lado et al., 2019; Strano et al., 2022).

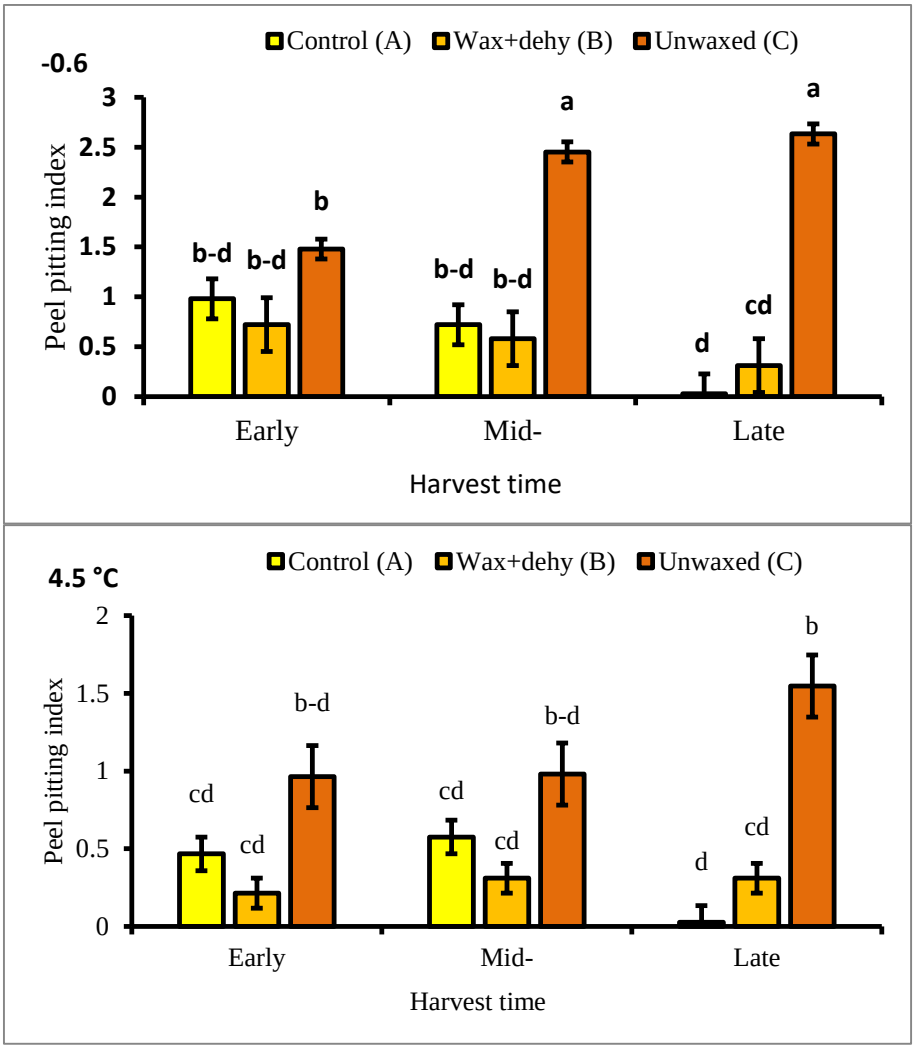

Fig. 1. Effect of harvest time and post-harvest treatment in peel pitting index (PPI) (0-4) of 'Benny' Valencia fruit cold stored at -0.6 and 4.5°C. Means with same letter are not significantly different at $P < 0.05$ (±SE, n = 25).

Chilling injury

Several studies have shown that chilling susceptibility of citrus fruit could be affected by harvest time (Lafuente et al., 1997), waxing and dehydration of fruits and extended cold storage (Mothapo et al., 2018). In the present study, the combination of harvest time, post-harvest treatment (waxing plus dehydration) and cold storage temperature had a significant effect ($P < 0.05$) on chilling injury (CI) of 'Benny' Valencia fruit (Fig. 2). CI symptoms manifested as skin browning lesion after withdrawal from cold storage, particularly on fruit stored at -0.6°C. However, fruit stored at 4.5°C did show no chilling symptoms, irrespective of wax plus dehydration, although, slight symptoms manifested in unwaxed dehydrated fruit at early harvest. This could be an indication that cold storage temperature caused an increase in reactive oxygen species (ROS) activities while reducing antioxidant activity (Sala, 1998; Lado et al., 2019; Liang et al., 2020). Unwaxed dehydrated fruit harvested early and stored at -0.6°C showed higher chilling injury index (CII) when compared with mid- and late harvested fruit of the same treatments. Furthermore, wax treatment effectively reduced chilling injury on fruit stored at -0.6°C, as waxed fruit had lower chilling injury symptoms when compared to untreated fruit at all harvest times. These results agreed with Wild (1993), who observed

lower chilling symptoms on waxed 'Washington' navel oranges when compared with unwaxed fruit stored at 1°C for 8 weeks with additional 1 week at 20°C. In pineapple (*Anana comosus* (L.), wax application was shown to improve the chilling injury in two cultivars (Star-Fresh and 2952 and Sta-Fresh 7055) for 21 days cold storage at 7°C and 90% relative humidity (RH) (Hu et al., 2011). The effectiveness of wax in reducing the fruit susceptibility to chilling injury may be as a result of anti-transpiration effect caused by wax (Wang, 1993; Dou, 2004; Germano et al., 2019). Therefore, this study confirmed the result previously reported by Hu et al. (2011), that wax treatment could be a potentially method to alleviate chilling injury.

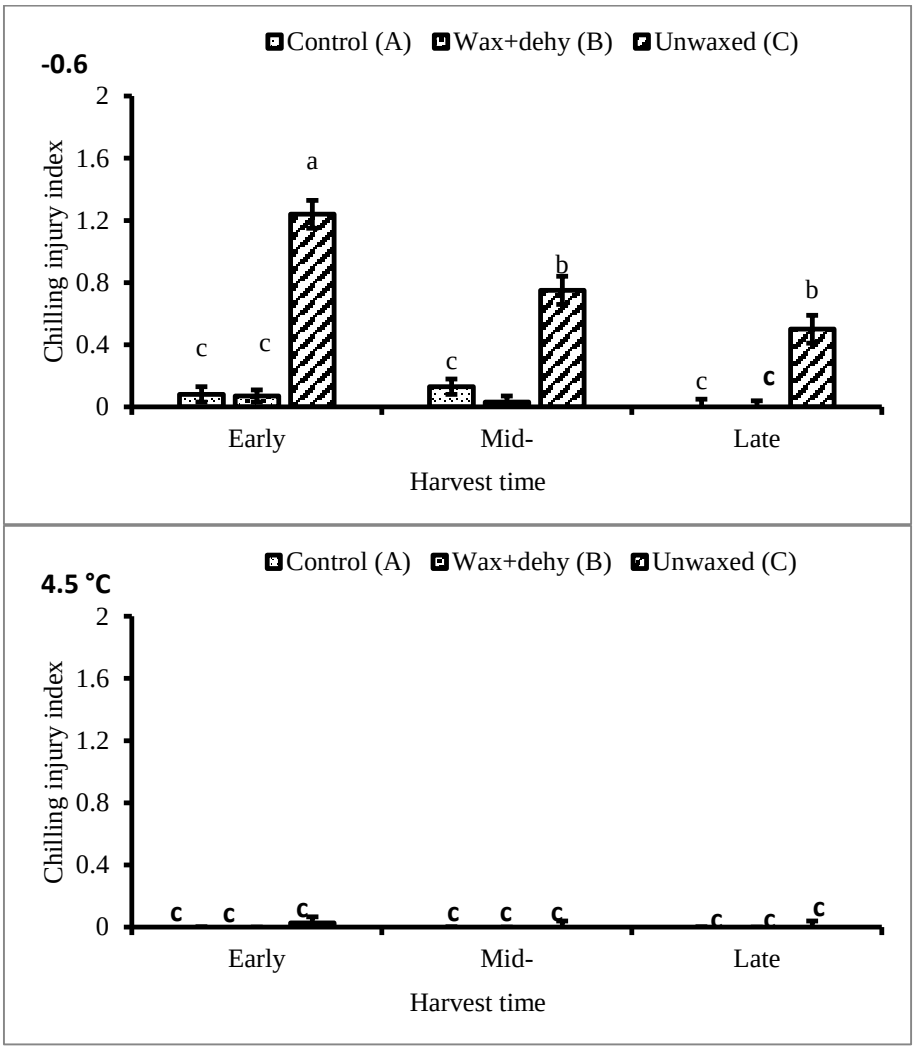

Fig. 2. Effect of harvest time and post-harvest treatment on chilling injury index (CII) (0-4) of 'Benny' Valencia fruit cold stored at -0.6 and 4.5°C. Means with same letter are not significantly different at $P < 0.05$ (±SE, n = 25).

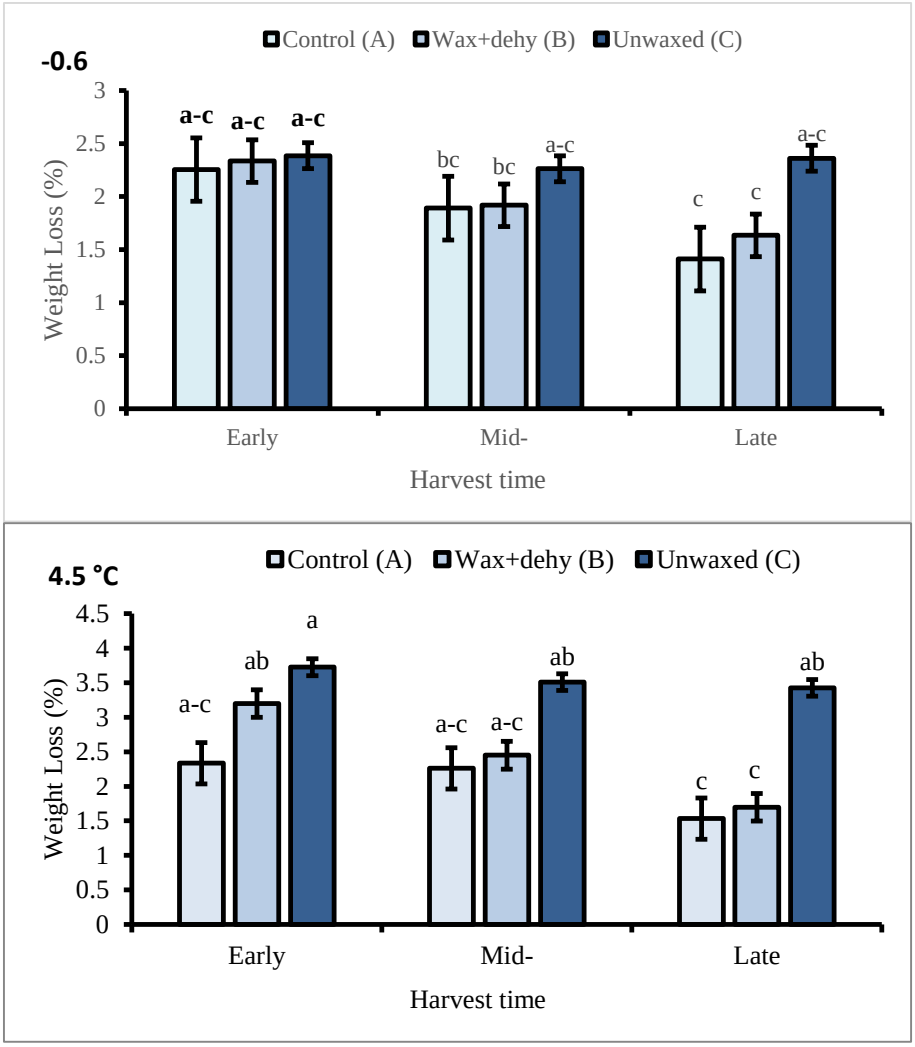

Fig. 3. Effect of harvest time and post-harvest treatment in weight loss of 'Benny' Valencia fruit stored at -0.6 and 4.5°C. Means with same letter are not significantly different at P < 0.05 (±SE, n = 25).

Weight loss

There was significant interaction effect (P<0.05) between harvest time, post-harvest treatment and cold storage temperature on fruit weight loss. The weight loss in fruit stored at 4.5°C was higher when compared with those stored at -0.6°C across all post-harvest treatment and harvest time (Fig. 3). These results agreed with those reported by Undurraga et al. (2014), who observed lower weight loss in 'Eureka' lemon fruit stored at lower temperature (3°C) compared to fruit stored at higher temperature (7 and 11°C). This could be due to high respiration under high evaporative demand (Yaman & Bayoindurh, 2002). Furthermore, higher weight loss was observed in early harvested fruit in comparison to mid- and late harvested fruit stored at both temperatures for all post-harvest treatment. Mid- and late unwaxed harvested fruit exposed to dehydration stress and stored at -0.6°C showed higher percentage of weight loss, while there was no variation between control and waxed plus dehydrated fruit. These findings were agreeing with those reported by Pailly et al. (2004), whereby, higher weight loss rate for early harvested 'Star Ruby' grapefruit stored at 6°C when compared with late harvested fruit. Moreover, passion fruit (*Passiflora edulis*) weight loss was significantly reduced in response to bees and carnauba wax when compared with waxed fruit (Sang & Hai, 2020), and shellac and decco wax reduced 'Ngowe' mango fruit weight loss during storage at 12°C during 28 days' storage (Maina et al., 2019). It is

presumed by some that higher weight loss on early harvested fruit relates to poor development of wax layering on the fruit (Moon et al., 2003; Sala, 1998). Generally, the rate of weight loss in wax treated fruit progressively decreased with the delay of harvest time; and consequently, increased the postharvest behavioural differences between waxed fruit and unwaxed fruit. In general, wax act as a semi permeable barrier against oxygen, carbon dioxide and moisture loss on the fruit, thus, reducing the rate of weight loss (Baldwin et al., 1999; Sang & Hai, 2020).

Firmness loss

There was a significant interaction effect ($P<0.05$) between harvest time, post-harvest treatment and cold storage temperature on fruit firmness loss. The results revealed that storage at -0.6°C reduced fruit firmness loss when compared with 4.5°C across all post-harvest treatment and harvest time (Fig. 4). Moreover, late harvested fruit showed higher firmness loss, while lower firmness loss was observed in early harvested fruit stored at -0.6 and 4.5°C for unwaxed and waxed dehydrated fruit. It has been found that an increase in firmness loss at late harvest occur due to high enzymatic activity by polygalacturonase (PG) breaking down pectin substances in the soluble fraction (Seymour et al., 1996). The breakdown of pectin substances in the middle lamellae and cell wall occur as maturity continues and has been reported to cause loss of cell wall integrity and firmness (Tzoutzoukou & Bouranis, 1997). Interestingly, the present study, showed waxed fruit had lower firmness loss when compared with unwaxed dehydrated fruit stored at both storage temperatures. Our results agreed with previous findings, whereby, wax significantly reduced 'Ngowe' mango fruit (Hu et al., 2011) and 'Sta-Fresh 2952 and 'Sta-Fresh 7055' pineapple fruit during cold storage (Maina et al., 2019). In these fruit, firmness loss was retained by waxing treatment, which retarded enzymatic pectin degradation (Zhou et al., 2011).

Electrolyte leakage (EL)

In this study, there was a significant interaction effect ($P< 0.05$) between harvest time, post-harvest treatment and cold storage temperature on fruit electrolyte leakage. Fruit stored at -0.6°C had higher electrolyte leakage when compared with those stored at 4.5°C at all harvest times, regardless of post-harvest treatment (Fig. 5). Generally, low storage temperature is a major factor that increases fruit membrane permeability and the subsequent rate of ion leakage (Raison & Orr, 1990; Saltveit, 2000). Cold storage temperature induces lipid phase transition from a more flexible liquid crystalline structure to a solid gel (Hordijk, 2013). According to Raison and Orr (1990), when gel phase coexists, the lipids do not pack well, causing cracks and result into high solutes leakage. In accordance to these findings, the present study found that storing fruit at -0.6°C resulted in higher electrolyte leakage after removal from storage.

A decreasing trend of electrolyte leakage was observed as harvest time progressed after removal from both cold storage temperatures. Late harvested fruit showed the lowest electrolyte leakage when compared with early and mid-harvested fruit stored at -0.6 and 4.5°C for all post-harvest treatments. Fruit exposed to dehydration stress without wax showed higher electrolyte leakage when compared with control and waxed plus dehydrated fruit, irrespective of harvest time (Fig. 5). These results were similar to those previously reported by Negi and Roy (2000), whereby, electrolyte leakage was higher for dehydrated 'Nantes' carrots stored at 7.5°C than non-dehydrated fruit due to higher water loss. In addition to our result, we found that waxing treatment repressed electrolyte leakage across all harvest time and storage temperature. These results were supported to those found by Zhou et al. (2011), whereby, electrolyte leakage during storage could effectively be inhibited by wax treatment due to higher peroxidase (POD) activities induced by wax on 'Huanghua' pears fruit stored at

4°C. In addition, relative leakage was correlated with membrane damage (malondialdehyde (MDA) content) and found to be significant lower in waxed pineapple fruit during cold storage at 7°C plus 90% relative humidity (RH) for 21 days (Hu et al., 2011). Peroxidase activity has the ability to scavenge peroxides (superoxide and peroxides) that contribute in damaging cell membrane and increase membrane permeability (Yuan et al., 2010).

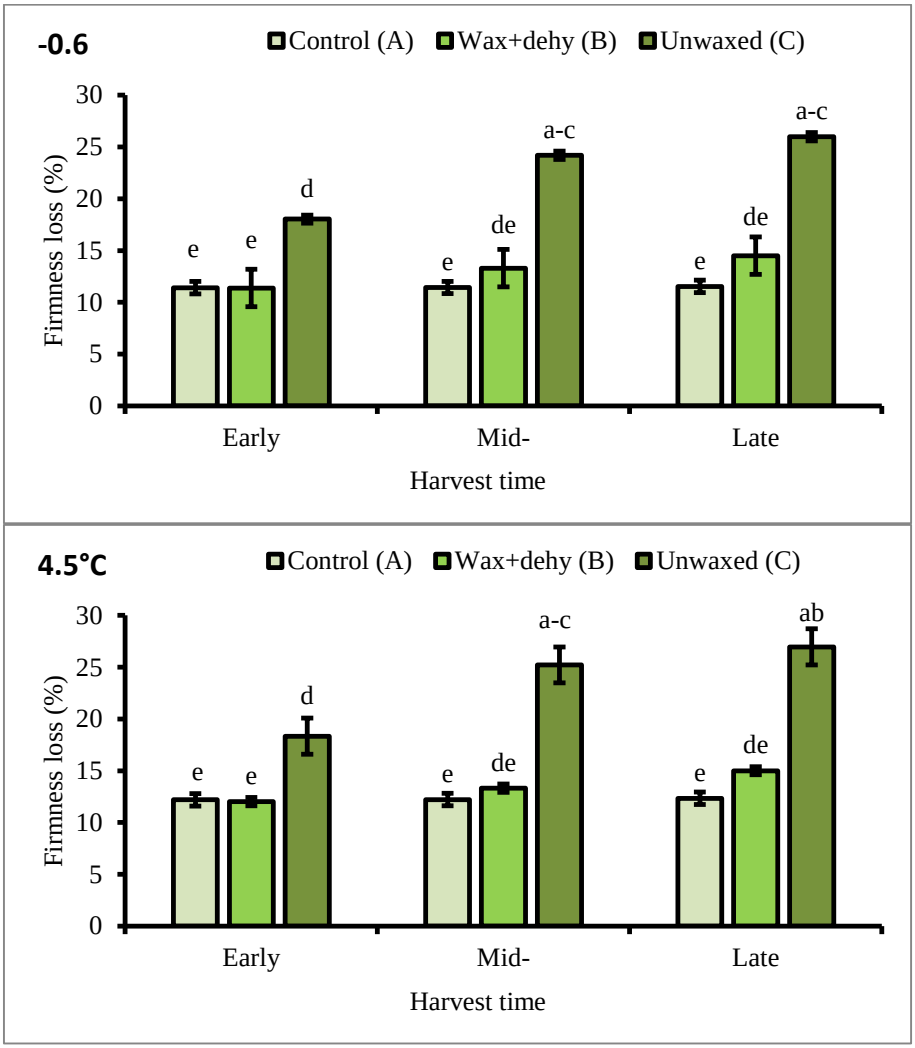

Fig. 4. Effect of harvest time and post-harvest treatment in firmness loss of 'Benny' Valencia fruit stored at -0.6 and 4.5°C. Means with same letter are not significantly different at $P < 0.05$ (±SE, n = 25).

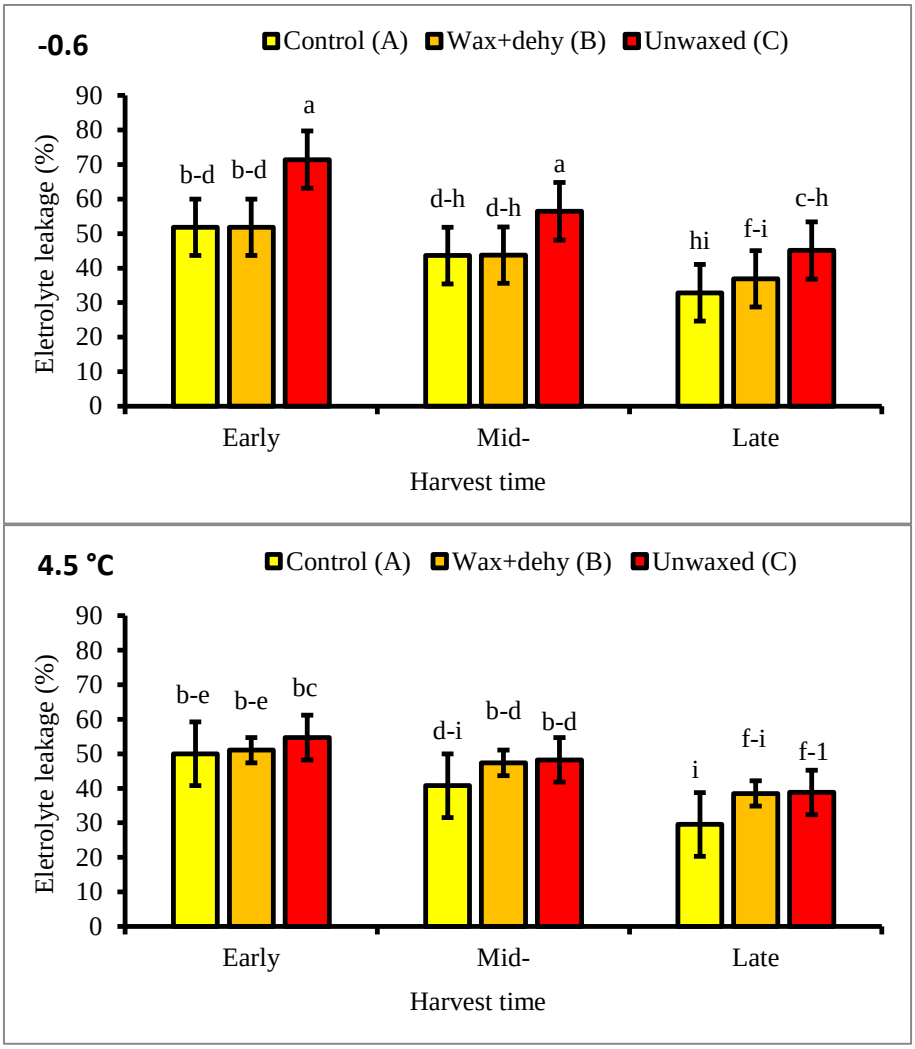

Fig. 5. Effect of harvest time and post-harvest treatment in electrolyte leakage (%) of 'Benny' Valencia fruit stored at -0.6 and 4.5°C. Means with same letter are not significantly different at P < 0.05 (±SE, n = 5).

Total phenolic concentration

Free phenolic

The interaction of harvest time, wax plus dehydration and storage temperature had no significant effect on total phenolic concentration of 'Benny' Valencia fruit (P>0.05) (Table 1). However, the free phenolic concentration increased at late harvested fruit, both stored at -0.6 and 4.5°C, especially in control fruit. These results agreed with Davis and Albrigo (1994), who indicated that over matured fruits have high percentage of amino acids, vitamin C, minerals and small quantities of lipids, proteins, and secondary metabolites. Fruit stored at 4.5°C showed lower free phenolic content when compare with fruit stored at -0.6°C, across all harvest time and post-harvest treatment. This result was similar to those previously reported by Chaudhary et al. (2016) who found high total phenolic in 'Rio Red' grapefruit stored at 5°C compared with fruit stored at 11°C due to high CI observed on fruit stored at 5°C. Across all harvest time and storage temperature, unwaxed fruit exposed to 3 days dehydration stress, showed lower total phenolic concentration when compared with control and waxed dehydrated fruit. Similar results were reported in the study of Li et al. (2017), whereby, higher levels of total phenolics were found in 'Sweet Charlie' strawberry fruit waxed with polysaccharide and stored at 3°C when compared with unwaxed fruit, thereby significantly

inhibited fruit decay and respiration after 12 days of storage. In 'Muscat' grapes, dehydration slightly increased total phenolics and flavonoids, thereby increasing oxidative stress tolerance (Corona et al., 2020). In addition, Petriccione et al. (2015) suggested that waxing could be used to prolong postharvest fruit life through reducing moisture, respiration, gas exchange, and oxidative reaction rates by regulating antioxidant activity.

Soluble conjugated phenolic

There was no significant interaction effect (P>0.05) between harvest time, post-harvest treatment and cold storage temperature on soluble conjugated phenolic (Table 1). Conjugated phenolic content was higher in fruit harvested late compared with early and mid- harvested fruit (Table 1). Storage temperature had no effect on the level of conjugated phenolics. For all harvest time and storage temperatures, fruit that received wax application showed higher conjugated phenolics. The study of Tavarini et al. (2008) associated moisture loss with total antioxidant activity. In generally, the study emphasized that moisture loss led to antioxidant degradation. Therefore, high soluble conjugated phenolic observed on this study could have resulted due to rind moisture content.

Correlation

Weight Loss vs. Peel pitting

Additionally, this study further suggested that weight loss after removal from storage temperature can serve as a non-destructive predictor of peel pitting, since it was significantly ($P < 0.05$) correlated with peel pitting incidence during mid- ($r^2 = 0.98$) and late ($r^2 = 0.70$) harvest (Fig. 2). These findings were supported by previous work by Cronjé et al. (2017), whereby, weight loss and peel pitting of 'Navelate Navel' and 'Tangor Ortanique' mandarin fruit correlated during storage for 3 weeks at 30 or 90% relative humidity.

Table 1. Effect of wax plus dehydration on conjugated and free phenolics of 'Benny' Valencia fruit harvested at three different times and stored at -0.6 and 4.5°C.

Storage Temperature	Harvest time	Treatment	Conjugated (mg GAE 100 ml^{-1} DW)	Free
-0.6°C	Early	Control (A)	409^{abc}	392.5^{abc}
		Waxed-dehydrated (B)	431^{abc}	332.7^{abc}
		Unwaxed-dehydrated (C)	309.7^{abc}	309.7^{abc}
	Mid-	Control (A)	443.1^{abc}	423^{abc}
		Waxed-dehydrated (B)	395.7^{abc}	376.4^{abc}
		Unwaxed-dehydration (C)	263.8^{c}	215.5^{c}
	Late	Control (A)	508.6^{abc}	471.6^{a}
		Waxed-dehydrated (B)	477.7^{abc}	355.7^{abc}
		Unwaxed-dehydration (C)	447.6 ^{abc}	394.8^{abc}
4.5°C	Early	Control (A)	390.2^{abc}	413.2^{bc}
		Waxed-dehydrated (B)	348.8^{abc}	254.6^{bc}
		Unwaxed-dehydrated (C)	337.3^{abc}	351.1^{abc}
	Mid-	Control (A)	362.6^{abc}	334.8^{abc}
		Waxed-dehydrated (B)	390.2^{abc}	420.1^{ab}
		Unwaxed-dehydration (C)	275.3^{c}	289.3^{abc}
	Late	Control (A)	456.5^{abc}	437.8^{ab}
		Waxed-dehydrated (B)	446.5^{abc}	420.1^{ab}
		Unwaxed-dehydration (C)	397.1 ^{abc}	344.7^{abc}
	P value		0.350	0.550

Chilling Injury vs. Electrolyte Leakage

The positive correlation between electrolyte leakage and chilling injury was observed on fruit stored at -0.6°C and only strong and significant for early and mid-harvested fruit (Table 2). Electrolyte leakage on fruit stored at 4.5°C was observed; however, fruit did not show any chilling symptoms. In this case, it was noticeable that electrolyte leakage could not be used as predictor for chilling injury in citrus as previously reported by Cohen et al. (1994).

Table 2. Correlation between fruit peel pitting index (PPI) vs weight loss (%) and chilling injury index (CII) vs electrolyte leakage (EL).

Harvest time	Variables	-0.6°C		4.5°C	
		Pearson (r)	t distribution	Pearson (r)	t distribution
Early harvest	PPI vs WL	0.39	0.4	0.68	1.1
	CII vs EL	0.99	7.1	N/A	N/A
Mid-harvest	PPI vs WL	0.98	4.9	0.71	1.3
	CII vs EL	0.98	4.9	N/A	N/A
Late harvest	PPI vs WL	0.95	3.2	0.99	7.1
	CII vs EL	0.88	1.7	N/A	N/A

For 3 degree of freedom and one tailed test, t critical value = 2.93

CONCLUSION

This study demonstrated that peel pitting and chilling injury occurrence together with the overall quality of 'Benny' Valencia fruit are influenced by harvest time, dehydration stress, waxing and cold storage temperature. Exposing fruit to dehydration stress at low relative humidity (RH) (45%) as previously documented to other cultivars is indeed a key factor leading to the occurrence of rind physiological disorders during storage. Therefore, maintaining of a rind constant water status prior to and during postharvest handling by waxing and/or other factors may have a crucial role in retaining peel quality and reduce the occurrence of rind disorders in 'Benny' Valencia fruit. However, for the successful reduction of these disorders during commercial shipment of 'Benny' Valencia fruit, the focus should not be on a single factor but rather a strategy that encompasses multiple factors known to decrease the impact of rind disorders. With respect to both free and soluble conjugated phenolics, the control fruit showed higher rind phenolics, especially at late harvest, across all the storage temperatures. Therefore, late harvested, waxed (control) fruit seem to be naturally responding to cold stress by up-regulating endogenous systems of total rind phenolics. Contrarily, postharvest treatments (dehydration) suppressed endogenous phenolics synthesis, therefore, poor defence against chilling and non-chilling disorders of 'Benny' Valencia fruit.

Conflict of interest

The authors declare that there is no conflict of interest.

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Effects of combined Red and Blue light spectra as supplemental light on yield and fruit quality of sweet pepper

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ABSTRACT

Purpose: The use of supplementary light in regions with low natural sunlight is necessary to fulfill the increasing consumer requests for fresh vegetables. This study aimed to investigate the effect of different combinations of red and blue LEDs on yield and quality of greenhouse-grown sweet pepper (*Capsicum annuum* L.) fruits during the growth period. **Research method**: The experiments were conducted in Rasht, Iran as split plots in the form of a completely randomized design in three repetitions (four plants per plot) on two cultivars of sweet pepper (Padra and Shadlin). With the appearance of the first flower buds, plants were exposed to different light treatments including: three combinations of red (R) and blue (B) LEDs (T1:R8B1, T2:R7B2, and T3:R6B3), with a same intensity of 200 $\mu molm^{-2}s^{-1}$ as supplement light to the natural light, together with natural light as control treatment (CT). Sweet pepper fruits were harvested weekly over 27 weeks and fruit yield and quality were assessed. **Findings**: Supplemental light using LEDs significantly increased yield and fruit quality parameters (except titratable acidity and maturity index) compared to the control. Marketable yield was differed among the light treatments and plants exposed to T3 showed the highest marketable yield (14.58 kg/m^2). The effect of supplemental light on total yield was more detectable when the average daily light integral was the lowest (for example, the difference between T3 and the control treatment in January was 1.27 kg/m^2, while this difference was 0.68 kg/m^2 in June). No significant difference was observed between cultivars and T3 was the best treatment in most parameters. **Research limitations:** No limitations were found. **Originality/Value**: In the northern regions of Iran, even in the months that do not seem to have light limitations, the use of supplementary light is recommended to increase the yield of sweet peppers in the greenhouse.

INTRODUCTION

Sweet pepper is a widely cultivated greenhouse crop, which is considered as one of the most consumed vegetables worldwide for fresh consumption or ready-to-eat foods, due to its taste, physicochemical compounds and various antioxidants (Guo et al., 2016; Jokinen et al., 2012; Kim & Son, 2022; Naznin et al., 2019). During the winter months in northern temperate climate, light is a limiting factor for yield and fruit quality of greenhouse vegetables (Lanoue et al., 2022). In the northern regions of Iran, the average daily light integral (DLI) is below 10 $molm^{-2}day^{-1}$duringthe autumn and winter seasons (obtained from the Rasht agricultural meteorological station). At this low DLI, flower abortion occurs in sweet peppers, which leads to a decrease in fruit production (Lanoue et al., 2022). As a countermeasure, artificial supplemental light sources are used to promote photosynthesis and yield and to improve fruit quality of year-round fresh greenhouse crop production, especially in the days with low intensity of natural light (Jokinen et al., 2012).

Traditionally, the commercial greenhouses increase DLI using high-pressure sodium (HPS) lamps as a supplemental lighting source above crop canopy. However, HPS lamps have fixed spectral compositions and may have adverse effects on greenhouse crops due to the conversion of a large portion of the input energy into heat (Klamkowski et al., 2014). Among different types of supplemental lights, light emitting diodes (LEDs) offer many benefits, such as reduced electricity consumption, safety and longevity (Dąbrowski et al., 2015). In addition, the low surface temperature and possibility of manipulation of light spectrum introduced the LEDs as a suitable alternative to HPS lamps (Pattison et al., 2018). The low surface temperature make LEDs feasible to use in close proximity to plant tissue, and the possibility of manipulating the spectral composition of LEDs can lead to biochemical, physiological, and photomorphogenic changes and subsequently improve yield (Guo et al., 2016).

Plants have certain responses to different light wavebands. Red (R) and blue (B) have been highly recommended by the scientific and greenhouse production communities because they are in the chlorophyll absorption region and bring higher photosynthetic and quantum efficiency (Hao et al., 2017; Lin & Jolliffe, 1996). It has been found that blue light plays an important role in pigment accumulation, stomatal opening, photomorphogenesis, leaf expansion and plant growth, and red light controls the function of the reproductive system, chloroplast, as well as petiole and stem growth (Li, et al., 2012). Moreover, the highest photosynthetic photon efficiency (PPE) among LEDs is related to these two wavebands (Hernández & Kubota, 2016). Usually a combination of R and B are used in controlled environment agriculture (CEA) for growth and production of different crops (Esmaeili et al., 2022; Javadi Asayesh et al., 2021). According to the mentioned contents, the suitable light spectra of LEDs are of great importance for the horticulture industry. Although R and B light spectra are proposed as the main spectra absorbed by chlorophyll a and b pigments, however there are scarce of information regarding their effects as the supplemental light for the main fruity greenhouse crops needing supplemental light for keeping economic yielding during seasons with light intensity limitations. In the regions with high precipitation such as Guilan province in North of Iran, at least in half of the year, average of DLI in late autumn and early winter times is lower than 15 $molm^{-2}day^{-1}$ and the amount of light transmitting into the greenhouse is less than 10 $molm^{-2}day^{-1}$. So, the objective of this study was to investigate the effect of different combinations of R and B LED supplemental lighting on the yield and fruit quality of two greenhouse sweet pepper cultivars during two consecutive growing periods in Rasht, Iran, finally, introducing the best light combination for greenhouse production of sweet pepper.

MATERIALS AND METHODS

Location

The experiments were conducted in the research greenhouse of the Faculty of Agricultural Sciences of Guilan University, Rasht, Iran (longitude 37° N, latitude 49° E, and 7 m above sea level (Fig. 1) during 2019-2021. The information about the number of sunny hours and photosynthetically active radiation in the 40-year statistical period (1970-2010) was obtained from the Rasht Agricultural Meteorological Station (Table 1).

Plant materials and growth conditions

This research was carried out as split plots in the form of a completely randomized design in 3 repetitions (fourplants per plot) on two cultivars of greenhouse sweet pepper (*Capsicum annum* L.) including red (Padra) and yellow (Shadlin). Seeds (purchased from Meridiem seeds. Co, Iran) were planted in 45-cell trays (4 × 4 × 8 cm) containing a mixture of 50% perlite and 50% cocopeat at the depth of 0.5 cm. At two-cotyledon stage, healthy, strong, identical and same-sized seedlings were transferred to 1 L plastic pots. With the appearance of the first flower buds, the seedlings were transferred to the main pots with a diameter of 23 cm and a depth of 21.5 cm (7 L). At this stage, the plants were exposed to four light treatments (in total 96 plants). Drip irrigation was done with a modified nutrient solution (Papadopoulos, 1994) based on the plant's water needs on an average of once a day (Fig. 2, Table 2). Stem, flower and fruit pruning were done regularly from the beginning of growth until harvest time.

Fig 1. Location map of the research greenhouse of the Faculty of Agricultural Sciences, Guilan University, Rasht, Iran.

Table 1. Number of sunny hours and photosynthetically active radiation in Guilan (1970-2010). †

Season	Average of total sunny hours (h)	Average of daily sunny hours (h)	Average of daily light integral ($molm^{-2}d^{-1}$)
Spring	489	5.26	20.82
Summer	598	6.43	28.93
Autumn	295	3.28	9.45
Winter	268	2.98	7.52
Yearly	1650	4.52	15.01

† (Rasht Agricultural Meteorological Station)

Table 2. Nutrient solutions used during the growth period of sweet pepper plants.

Stock	Fertilizer	The month after germination				
		First	Second	Third	Fourth	The beginning of the harvest - the end of the period
Stock A	Calcium nitrate	150 g	160 g	170 g	170 g	170 g
	Potassium nitrate	44 g	44 g	44	44 g	44 g
Stock B	Potassium nitrate	34 g	44 g	44	44 g	44 g
	Magnesium Sulphate	40 g	45 g	50	56 g	62 g
	Potassium monophosphate	60 g	60 g	50	50 g	50 g
Stock C	Manganese sulfate	1 g	1 g	1	1 g	1 g
	Zinc sulfate	0.5 g	0.5 g	0.5	0.5 g	0.5 g
	Copper sulfate	0.2 g	0.2 g	0.2	0.2 g	0.2 g
	Sodium molybdate	0.1 g	0.1 g	0.1	0.1 g	0.1 g
Stock D	Borax	2.4 g	2.4 g	2.4 g	2.4 g	2.4 g
Stock E	Sequestrene Fe	10 g	10 g	10 g	10 g	10 g

Fig 2. Sweet pepper plants used in the present study in the fruiting stage.

Table 3. Lighting treatments based on red (R) and blue (B) spectra and the contribution of each light spectrum in the overall light composition. †

Treatment	Description
T1	R:B (8:1)
T2	R:B (7:2)
T3	R:B (6:3)
CT	Control treatment (without supplemental light)

† Peak wavelength λp was 660 nm for red LED and 460nm for blue LED.

Supplemental light application

LED modules (36 W, with an exposure area of 50 × 100 cm) were purchased from Iran Growlight Company, Tehran, Iran. The lamps were installed with a distance of 20 cm above the plants canopy (the lamps were movable and were moved based on the plant's height to maintain the distance). The light intensity of 200 µmol $m^{-2}s^{-1}$, was applied for all light treatments (Hikosaka et al., 2013; Naznin et al., 2019; Nederhoff & Marcelis, 2010). 24 wall washer lamps of one-meter length were used, and 36 LEDs were installed in each lamp. The LEDs were divided into 4 groups of 9. Out of 9 LEDs, 1, 2 and 3 of them were blue and the rest were red in treatments 1, 2 and 3, respectively (Table 3). In order to avoid overlapping of lamps and light diffusion among the treatments, each lamp was installed in the center of each plot. A photoperiod of 14 hours (5 am to 7 pm) was applied to the treatments, and according to the literature (Guo et al., 2016; Maureira et al., 2022) when the intensity of solar radiation was above 400 µmol$m^{-2}s^{-1}$ (10 am - 3 pm on completely sunny days), supplemental lights were turned off. Photosynthetic photon flux density on the plant surface was measured with a photometer (SKP 200, Skye Instruments Ltd).

Data collection

Fruit yield

Fruits at maturity stage (85 days after transferring the seedlings to the main pots) were harvested weekly for 189 days and measurements were taken every week. Fruits weight was measured with an electronic scale and the total yield, marketable yield (fruits weighing more than 100 grams and without blossom-end-rot) and the number of marketable fruits was calculated. The length and diameter of fruits were measured using Vernier calipers.

Fruit quality

Fruits were cut into two halves and flesh thickness was measured at two different points of each half. Soluble solid content was determined from filtered pepper fruit extract using a refractometer (CETI-BELGUM). Titratable acidity (TA) was recorded by titration of 10 mL filtered fruit extract with 0.1N NaOH to pH 8.1 and the quantity (mL) of NaOH was converted into citric acidity (Ghasemnezhad, et al., 2011). Maturity index (MI) was obtained using the following equation (1) (Martínez-Zamora, et al., 2021):

$$MI = \frac{TSS}{TA} \qquad (1)$$

Where, TSS is total soluble solids and TA is titratable acidity. The measurement of vitamin C in filtered pepper fruit extract was done by titration against 2,6-dichlorophenol-indophenol solution (Zayed, 2012). The samples were placed in a 65 °C dryer for 72 h, and then the dry matter (DM) was obtained using the equation (2) (Lanoue, et al., 2022):

$$DM = \frac{\text{Dry weight}}{\text{Fresh weight}} \times 100 \qquad (2)$$

Statistical analysis

Comparison of normality tests under skewness and kurtosis coefficients in the range of -2 to 2 was performed using Statistical Product and Service Solutions for Windows, (SPSS, version 16.0) (Ghasemi & Zahediasl, 2012). Analysis of variance was run on yield indices and fruit quality indices using Statistical Analysis Software (SAS, version 9.1) (Littell, 1989) to investigate whether theses indices have a significant relationship with light, pepper cultivars, and also with interactive effects of light × cultivar. Tukey's multiple comparison test ($P<0.01$ and $P<0.05$) was used to check the difference between means.

RESULTS

Fruit yield

The results of variance analysis indicated that total yield, marketable yield, number of fruit, average fruit weight, fruit length and fruit diameter were significantly ($P < 0.01$) influenced by light treatments, but the individual effect of cultivar as well as the interactive effects of light × cultivar were not statistically significant (Table 4). Differences between the means at the 1% level showed that the total yield, marketable yield, number of fruits, average fruit weight, fruit length and fruit diameter of sweet peppers under all levels of supplemental light were significantly higher than their values in control treatment (Table 5).

As shown in Table 5, marketable yield was differed among the light treatments and increased with additional blue light levels, so that T3 (R6B3) had the highest marketable yield (14.58 kg/m^2) and statistically, there was a significant difference between T3 and two other treatments. This is while, study of the differences between the means of total yield, number of fruit, average fruit weight and fruit size ($P < 0.05$) showed that there was no statistical difference among T1, T2, and T3 plants.

Figure 3 compares the average of total yield under four light treatments in different fruit harvested month. With the increase of natural light, an increase in the yield of the control treatment is observed (0.45 kg/m^2 in January vs 2.86 kg/m^2 in June), which makes the difference between the control treatment and the light treatments to be less in months with higher light intensity. For example, the difference between T3 and the control treatment in January was 1.27 kg/m^2, while this difference was 0.68 kg/m^2 in June; however, this difference was still significant.

Table 4. Variance analysis for yield parameters of sweet pepper plants grown under different qualities of supplemental light.

Source	Df	Mean Square (MS)†					
		Total yield	Marketable yield	Number of Fruit	Average fruit weight	Fruit length	Fruit diameter
Light	3	69.71**	70.88**	2145**	0.001**	314**	102**
Light Error	8	0.137	0.04	4	0.001	8.59	11.13
Cultivar	1	0.02 ^{ns}	0.01 ^{ns}	0.17 ^{ns}	0.001^{ns}	20.17 ^{ns}	57.1 ^{ns}
Light × Cultivar	3	0.02 ^{ns}	0.02 ^{ns}	1.62 ^{ns}	0.001 ^{ns}	24.62 ^{ns}	8.49 ^{ns}
Residual Error		0.27	0.05	8.75	0.001	5.75	3.62
Coefficient of variation	-	4.09	1.77	3.71	3.37	2.79	2.55

† ns, **: Non significant and significant at 1% probability level, respectively.

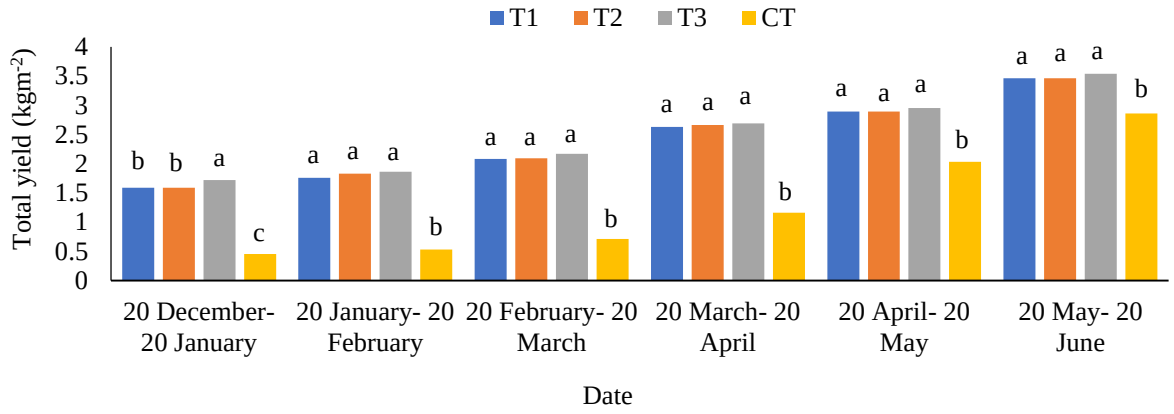

Fig 3. Total yield of sweet pepper under four light treatments during different dates of study.

Table 5. Effect of light treatment on yield parameters of sweet pepper plants.

Light treatment	Yield indices†					
	Total yield (kgm^{-2})	Marketable yield (kgm^{-2})	Number of fruit	Average fruit weight (kgm^{-2})	Fruit length (mm)	Fruit diameter (mm)
T1	14.28^{b}	14.06^{b}	88.17^{a}	0.161^{a}	90.32^{a}	75.83^{ab}
T2	14.35^{ab}	14.18^{b}	89.33^{a}	0.160^{a}	88.00^{a}	84.83^{ab}
T3	14.99^{a}	14.58^{a}	90.33^{a}	0.166^{a}	89.67^{a}	78.83^{a}
CK	7.75^{c}	7.41^{c}	51.50^{b}	0.151^{b}	75.00^{b}	69.00^{b}

† Data are shown as treatment average of three replicates.

Mean values followed by different letters in the same column indicate significant differences by the Tukey's test at $p \leq 0.01$.

Table 6. Variance analysis for fruit quality parameters of sweet pepper fruits under different qualities of supplemental light**.**

Source	Df	Mean Square (MS) †					
		Flesh thickness	Dry matter	Vitamin C	Total Soluble Solids	Titratable acidity	Maturity index
Light	3	2.15**	0.001**	69.15**	0.66**	0.01^{ns}	0.03*
Light Error	8	0.015	0.001	3.00	0.047	0.01	0.01
Cultivar	1	0.91^{ns}	0.001^{ns}	1.05^{ns}	0.02^{ns}	0.01^{ns}	0.01^{ns}
Light × Cultivar	3	0.02^{ns}	0.001^{ns}	6.94^{ns}	0.01^{ns}	0.2^{ns}	0.01^{ns}
Residual Error		0.06	0.001	8.33	0.06	0.3	0.01
Coefficient of variation	-	3.78	3.03	3.78	3.52	5.12	4.62

† ns, *, **: Non significant and significant at 5% and 1% probability level, respectively.

Table 7. Effect of supplemental light with different spectra of red and blue light on fruit quality parameters of sweet pepper plants.

Light treatment	Yield indices†					
	Flesh thickness (mm)	Dry matter (%)	Vitamin C **(**mg/100gFW**)**	Total Soluble Solids (%)	Titratable acidity(%)	Maturity index
T1	6.58^{a}	13.7^{a}	78.83^{a}	7.17^{a}	3.08^{a}	2.32^{a}
T2	6.72^{a}	13.4^{ab}	77.67^{a}	7.15^{a}	3.10^{a}	2.31^{a}
T3	6.83^{a}	13.2^{ab}	78.17^{a}	7.28^{a}	3.12^{a}	2.33^{a}
CK	5.53^{b}	12.2^{b}	71.50^{b}	6.55^{b}	3.07^{a}	2.16^{a}

† Data are shown as treatment average of three replicates; mean values followed by different letters in the same column indicate significant differences by the Tukey's test at $p \leq 0.01$.

Fruit quality

As can be seen in Table 6, light treatment significantly affected the fruit quality parameters except for the titratable acidity and maturity index. However, no significant differences in fruit quality parameters were found between red and yellow fruits. Also, the interactive effects of light × cultivar were not statistically significant. Tukey's multiple comparison test at the 1% level showed that with a higher ratio of light B, flesh thickness and total soluble solids of fruits increased, while vitamin C decreased (78.17 mg in T3 vs. 78.83 mg in T1). However, these differences were not statistically significant (Table 7). Dry matter showed a significant difference among T1 and CK plants, so that T1 (R8B1) had the highest dry matter (13.7%).

DISCUSSION

Fruit yield

Light limitation or uneven light distribution impose restriction on photosynthesis system, which can cause a decrease in plant yield and fruit quality (Yamori et al., 2016). As shown in Table 5, differences between the means of total yield at all levels of supplemental light were significantly greater than control treatment. This is consistent with other reports which showed that LED supplemental lighting improves yield in different crops (Jokinen et al., 2012; Takahashi et al., 2020). It has been found that applying LED lighting on the leaves, elevates carbon dioxide fixation, decrease flower and fruit abortion, improves fruit growth (González-Real et al., 2009), and accelerate fruit maturation, consequently leading to yield improvement (Jokinen et al., 2012).

During low light intensity seasons, cloudy days, or in high latitudes when the average DLI is lower than a threshold level required for induction of flowering or fruit growth, the use of supplemental light is of vital importance to keep yield in many crop species (Fig. 3). Jokinen et al. (2012) showed that mean fruit weight increased due to LED supplemental light as the natural DLI decreased, which led to an increase in total yield (Jokinen et al., 2012). In addition, the results of the present study showed that with the increase of natural light, LED supplemental lighting still has a significant effect on increasing yield. Therefore, using LED supplemental lighting is a promising approach to increase fruit yield in areas that are not necessarily light-limited (at least on the below part of the plant canopy). This finding is in agreement with the report of Joshi et al. (2019).

Malformed fruits, fruits with blossom-end-rot, and small fruits were classified as fruits with low marketable yield. A decrease in blossom-end-rot and as a result an increase in marketable yield was observed in the presence of LED supplemental lighting in the present study (Table 5). This is exactly the opposite of the result reported in the presence of HPS light (Stadler, 2011). The increase in occurrences of blossom-end-rot in the presence of HPS lamp has been considered to be related to the high thermal radiation of HPS compared to LED (Prinzenberg et al., 2021).

There was significant difference among three supplemental light spectra for production of marketable yield. The results showed that a high proportion of B light increases the total yield; thus, the highest marketable yield (14.58 kg/m^2) was observed in T3 plants (R6B3), which is in agreement with the findings of Javadi Asayesh on Guzmania and Vriesea (Javadi Asayesh et al., 2021), and Aalifar on Carnation (Aalifar et al., 2020a; Aalifar et al., 2020b).

As shown in Table 5, differences between the means of number of fruit at all levels of supplemental light were significantly greater than the control treatment. The fruit load of sweet pepper is high and it has been found that at low DLI (less than 10 molm^{-2}day^{-1}), due to insufficient photosynthesis, sweet peppers tend to flower drop, which reduces fruit production (Lanoue et al., 2022; Takahashi et al., 2020). Maximum photosynthetic efficiency can be achieved by increasing the incident light level through using supplemental light. It has been

found that the spectral energy distribution of R and B lights corresponds to chlorophyll pigment absorption, which causes high photosynthetic activity. Therefore, increase in number of fruit in plants grown under red and blue lights may be as the result of enhanced carbohydrate production due to improvement in photosynthetic capacity, followed by a reduction in fruit drop (Javadi Asayesh et al., 2021). Previous studies have also reported an increase in the number of sweet pepper fruits under LED light treatments (Jokinen et al., 2012; Naznin et al., 2019). Of course, it has been found that red light alone increases starch content by inhibiting the transfer of photosynthates out of the leaves, which may have a negative effect on fruit production (Sæbø et al., 1995). In the present study, increasing the number of fruit under supplementary light led to an increase in total yield, which is in agreement with the results of other studies (Guo et al., 2016; Takahashi et al., 2020).

Fruits with an standard and unique size are more marketable than small - and diversified fruits, which is a criterion for price determination of the product (Lanoue et al., 2022). The results obtained from the fruit length data in the present study showed that the differences between the means of fruit length at all levels of supplementary artificial light were significantly higher than the control treatment. Previous studies also showed that fruit yield parameters such as fruit size and number were highest in plants grown under Rand BLEDs (Gómez & Mitchell, 2016; Pepin et al., 2013). However, in another research that used supplemental intra-canopy LED illumination for sweet pepper, the fruit yield increased in the spring season only by affecting the number of fruit, without affecting the fruit size or weight (Joshi et al., 2019).

Fruit quality

Fresh sweet pepper is rich in biologically active substances, including chlorophyll, carotenoids and vitamin C, which can effectively scavenge active oxygen free radicals in the human body and reduce the risk of Brain and cardiovascular diseases as well as cancer (Blekkenhorst et al., 2018; de Sá Mendes & de Andrade Gonçalves, 2020; Olatunji & Afolayan, 2018). In the present study, all levels of supplementary artificial light significantly increased amount of vitamin C in the fruit compared to the control treatment. Increase in the proportion of blue light in overall spectrum, induced accumulation of vitamin C in fruits, although there was not a statistically significant difference among T1, T2, and T3 plants. Increase of vitamin C content by B light has been reported in tomato and strawberry (Javanmardi & Emami, 2013; Kim et al., 2011). It has been found that there is a significant relationship between the vitamin C content and soluble sugars in leaves of lamb's lettuce, so it seems that blue light plays a role in regulating vitamin C synthesis not only through its effect on blue light receptors, but also through increasing the rate of photosynthesis and the formation of sugars (Wojciechowska et al., 2015). However, Liu et al., who investigated the effects of different LED spectra lightings on the post-harvest nutritional quality of chili peppers, stated that blue light has a negative effect on vitamin C content in several cultivars (Liu et al., 2022a).

Soluble solids and titratable acidity are essential physicochemical factors that can determine the taste of sweet pepper fruits (Ghasemnezhad et al., 2011). In the present study, all LED light treatments significantly increased soluble solids and titratable acidity compared to the control treatment. These results are in consistent with the findings of Kim et al. (2022) who showed that blue and red light increased the content of soluble solids in peppers compared to soluble solid content of fruits obtained from plants grown under natural light (Kim & Son, 2022).

All supplementary light treatments significantly increased the maturity index compared to the control treatment (Tukey's test at $p \leq 0.05$). Maturity index is a reliable index to determine

the pepper fruits harvesting time (Navarro et al., 2002). To offer high-quality sweet peppers to the market, the fruits should be harvested at their optimal maturity stage to meet the needs of consumers. At the optimal maturity stage, the fruits must show a variety-specific color, shape, size, acidity and total soluble solids content, but the maturity of the fruit should not be excessive (Ignat et al., 2013).

Higher fruit dry matter content means more nutrients per unit of fresh fruit (Lanoue et al., 2022). Reports have shown that increasing red light can increase the dry matter content of fruit (Liu et al., 2022a; Liu et al., 2022b). In the current study, by increase in the proportion of R light in overall spectrum, the percentage of dry matter increased. This result is in agreement with previous study in pepper (Lan et al., 2022), which showed the increase in red light increased the dry matter content of the fruit compared to its content in the plants grown under natural light, while increase in blue light did not influenced the fruit dry matter content.

CONCLUSION

In conclusion, using combination of red and blue light spectra as supplemental lighting increased total yield of greenhouse-grown red and yellow sweet pepper fruits due to increase in number of fruits and fruit size, compared the fruits produced under natural light conditions. The blue light addition from 10% to 30% to the growing light spectrum improved fruit yield parameters. Therefore, the results showed that T3 (R6B3) was the best treatment. Furthermore, our results showed that LED supplemental lighting by increasing flesh thickness, fruit dry matter, vitamin C, and total soluble solids is a promising approach to improve fruit quality.

Conflict of interest

The author has no conflict of interest to report.

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