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Postharvest application of calcium chloride and 1-methylcyclopropene for quality conservation on organic ripe fig

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Key words: Brown Turkey fig, *Ficus carica*, fruit conservation, shelf life.

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Abstract: The postharvest phase is an important step in the fruit production chain. Fig is an especially perishable fruit, which has encouraged researchers to study the effects of various substances on the postharvest life of this commodity. The objective of the present work was to evaluate the effects of calcium chloride ($CaCl_2$) and 1-methylcyclopropene (1-MCP) on the postharvest quality of the 'Roxo-de-Valinhos' fig cultivar. This study aimed to verify the effects of applying a 4% solution of $CaCl_2$ and a 1% solution of 1-MCP to figs and evaluating at four different storage times (0, 2, 4, and 6 days). The results showed that a 4% solution of $CaCl_2$ promoted better firmness, and when $CaCl_2$ at 4% solution was applied in combination with 1-MCP at 10 µg l^{-1}, the maturation index increased. In contrast, the 1-MCP treatment alone did not improve the postharvest quality of 'Roxo-de-Valinhos' ripe fig. We conclude that application of 4% solution of $CaCl_2$ and 1-MCP at 10 µg l^{-1} promote firmness and increase maturation index of 'Roxo-deValinhos' figs.

1. Introduction

Fig (*Ficus carica* L.) is an important crop globally, particularly in Southeast Europe, the Middle East, North Africa, and the Americas, where the USA and Brazil are highlighted for fig production (Rosianski *et al*., 2016; Uzundumlu *et al*., 2018; Paolucci *et al*., 2020). In Brazil, 19.6 tonnes were harvested from 2.114 hectares in 2020 (IBGE, 2022), where fig trees are cultivated mainly in the southeast and south regions where ripe figs are harvested for consumption and unripe figs for industrialization (Tofanelli *et al*., 2018).

Although fig tree cultivation has the potential to escalate, some production bottlenecks have inhibited this expansion, such as lack of commercial cultivars, unfamiliarly about ripe fig as excellent fruit for food from consumers, relative high price of ripe figs in the market, inefficient fresh ripe fig distribution and difficult to conserve quality of ripe figs after

harvested. Thus, one of the principal challenges is the high perishability of ripe fig after it has been harvested (Mirshekari *et al*., 2020), especially in Brazil where the main cultivar used is 'Roxo-de-Valinhos', also known as 'Brown Turkey', which produces highly-perishable fruit (Ferraz *et al*., 2020). 'Roxo-de-Valinhos' figs have a very short postharvest life of 1 to 2 days when stored under environmental conditions of market shelf, or 4 to 10 days under refrigerated storage (Lakshmi *et al*., 2018).

Several studies have been conducted on the effects of postharvest treatments on figs to extend their storage duration. The majority of research has focused on substances such as calcium chloride ($CaCl_2$), 1-methylcyclopropene (1-MCP), fungicides, and sodium hypochlorite (Irfan, 2013; Song *et al*., 2019; Jusoh *et al*., 2020).

$CaCl_2$ has been applied to fruits both in preharvest and postharvest to promote quality conservation (LI *et al*., 2014). This nutrient has been considered the most consequential for fruit quality promotion through its contributions to decreased softening, browning, and senescence of fruits, thereby extending their postharvest life (Suriati *et al*., 2021). 1-MCP has an unsaturated cyclic olefin that acts as a competitive ethylene antagonist and blocks ethylene receptors, consequently inhibiting maturation processes such as ethylene production, respiration rate, browning, and softening, and extending the shelf life of fruits (Sozzi *et al*., 2005; Watkins, 2008; Freiman *et al*., 2012).

The response of $CaCl_2$ or 1-MCP application on fruits varies according to the application form, fruit species, cultivar, maturation, and ripening stage of the fruit (Sozzi *et al*., 2005; Watkins, 2006; Irfan, 2013). Currently, there are few reports applying $CaCl_2$ and 1-MCP to figs to evaluate their effects on fruit quality.

Therefore, the objective of this study was to evaluate the effects of the application of $CaCl_2$ and 1-MCP on quality of fresh ripe fig 'Roxo-de-Valinhos' after harvested to extend their shelf life.

2. Materials and Methods

Plant materials

The experiment was conducted at the Federal University of Paraná State. Figs were harvested from plants grown in the orchard located in the Canguiri Experimental Farm (Pinhais County, Paraná State, Brazil, 25°26' S and 49°16' W, 947 m above sea level), considered Cfb by the Köppen climate classification, with annual maximum and minimum temperatures of approximately 24°C and 11°C, respectively, and annual average rainfall of 1,500 mm.

Fig trees used were 5-year-old Roxo-de-Valinhos cultivars, also known as 'Brown Turkey'. The first harvest was done when plants were 2 years old. Plants were cultivated in an organic plantation system, subjected to heavy annual pruning, and spaced at 1×2.5 m. Fertilization was done using cattle manure at 60 L per plant annually, separated into three 60 days intervals beginning in August. Phytosanitary management was performed by spraying a 2% lime sulfur solution after pruning and a 0.2% bordeaux mixture (biweekly in rainy period and monthly in dry period) from the vegetative and production stages of the fig trees to two weeks prior to harvest, mainly to prevent rust (*Cerotelium fici*). To control pests, such as tree borer (*Azochis gripusalis*) and borer beetle (*Coleobogaster cyanitarsis*), a 1% solution of oil extracted from neem (*Azadirachta indica* A. Juss) was sprayed on plants when these pests were detected damaging the fig trees. Mechanical weeding in crop rows and mowing between rows were used to control weeds, integrated with black oat (*Avena strigosa* L.) planted in July in 2012 and 2013.

Fruit materials

Figs were hand-harvested on April 1, 2014. Overall, this period is the late season for harvest of ripe fig in Brazil. Fruits were harvested when they showed at least a 50% change to their skin color, from green to reddish brown at stage 6 (Freiman *et al*., 2012) by visual evaluation. This maturation stage is usually better accepted by Brazilian market. After harvesting, figs were immediately transported to the Postharvest Laboratory and located in the same experimental farm for experimental proceedings. Figs were divided into lots containing 8 figs each lot for storage during 0, 2, 4, and 6 days, which were then placed in a paper box commonly used in ripe fig markets.

Postharvest treatments

After figs were harvested, they were treated with calcium chloride ($CaCl_2$) (Oliveira Junior *et al*., 2018) and 1-methylcyclopropene (1-MCP) (Tofanelli *et al*., 2018). Half of the selected figs were dipped in the

solution of $CaCl_2$ at 4%, and the other half were dipped in distilled water and immersed for 15 min. After that, figs were taken from the treatments to dry their surface by natural air-drying under the laboratory bench for 30 min.

After drying, figs were placed in a specific commercial paper box that fit 8 figs each. The packed figs were placed into a plastic container (70 L) when they were ready to be treated with 1-MCP. SmartFresh^{Ò} powder (0.14% active ingredient) was used to prepare the 1-MCP solution. This powder was measured with a precision balance and dissolved in distilled water to create a concentration of 10 µg l^{-1} inside the container, where figs were kept after treatment for 24 h. Untreated figs (control) were compounded only with distilled water and stored under the same conditions. Thus, 1-MCP powder was introduced into a syringe and supplemented with water until a specific volume was reached. The 1-MCP solution and control were injected into the respective plastic containers, which were immediately blocked. Therefore, both untreated figs and those treated with $CaCl_2$ were also treated with 1-MCP or the control (distilled water), resulting in a combination of 2 levels of $CaCl_2$ (0 and 4%) *vs.* 2 concentrations of 1-MCP (0 and 10 µg l^{-1}).

After 1-MCP treatment, packed figs were removed from boxes and stored at 4±1°C and 90-95% RH for 6 days, when they were removed to procedure analyses.

Fruit quality parameters

Fig quality conservation was evaluated using firmness, total soluble solids (TSS) concentration, titratable acidity (TA), maturation index (MI), and weight loss.

The samples were withdrawn from the refrigerator and 2 figs per replicate were analyzed every two days. Fruit-peel firmness was determined on the equator of the fig using a manual penetrometer (PTR-100) with a 7.9-mm-diameter tip and expressed in terms of lb force.

Fruit juice was extracted from the stored figs using a centrifuge in order to assess TSS and TA. TSS was measured with a handheld refractometer using a drop of juice and expressed as a percentage while TA was expressed as % citric acid and measured using 100 ml of the solution (10 ml of juice + 90 ml distilled water) that was immediately titrated with 0.1 N NaOH using 3 drops of phenolphthalein as an indicator. MI was determined by dividing TSS by TA (TSS/TA ratio).

Weight loss of stored figs was obtained using the relative weight variation (%) of 2 figs per replicate during a storage period of 6 days. The figs were weighed at harvest time and at every storage period of 2 days, and weight loss was determined. Fig weights were assessed using an electronic balance.

Experimental design

Three replicates of samples with 8 figs per replicate for each treatment were used for physical and chemical quality analyses; 2 figs were analyzed immediately after 1-MCP proceedings and the other 6 figs were stored. Four storage periods (0, 2, 4, and 6 days) were used to evaluate postharvest quality conservation. Theses samples of figs were randomized selected avoiding to select damage figs by pests and mechanical injury.

The experimental design was completely randomized. Treatments included the application or absence of 1-MCP (10 µg l^{-1}) combined with the application or absence of $CaCl_2$ (4%) and with no storage (0) and three storage times (2, 4, and 6 days). Thus, the experiment was designed using a 2^3 factorial arrangement (2´2´4): (2) $CaCl_2$ at 2 levels (0 and 4%), (2) 1-MCP at 2 levels (0 and 10 µg l^{-1}), and (4) storage period at four levels (0, 2, 4, and 6 days).

Statistical procedures

Analyses were carried out with analysis of variance (ANOVA) using the Sisvar Statistical Program (version 5.3) (Ferreira, 2011). Treatments that showed a significant effect were subjected to multiple comparisons of the mean using the Tukey test at a 5% probability level.

3. Results

Results from ANOVA showed that $CaCl_2$ promoted significant effect on firmness and soluble solids of the 'Roxo-de-vlinhos' ripe figs, that storage periods had significant influence on soluble solids, titratable acidity, weight loss, and maturation index, whereas 1-MCP only affected significantly the maturation index (Table 1). The exclusive interaction that showed a significant effect was $CaCl_2$ x 1-MCP on the fig ratio. As expected, storage influenced the quality parameters evaluated in the ripe figs, except in terms of firmness. There was no significant effect of $CaCl_2$ and 1-MCP on all parameters evaluated in this present work after storage of 6 days (Table S1).

Table 1 - Analysis of variance (ANOVA) for parameters evaluated on storage of ripe figs treated with calcium chloride ($CaCl_2$) and 1-methylcyclopropene (1-MCP)

Source of Variation	df	FI (Lb)		SS (°Brix)		TA (%)		WL (%)		MI (Ratio)	
		MS	*F*	MS	*F*	MS	*F*	MS	*F*	MS	*F*
CC	1	25.37	17.06 **	6.31	9.84 **	0.02	0.09 NS	102.11	0.36 NS	404.26	1.70 NS
MCP	1	2.34	1.57 NS	0.08	0.13 NS	0.64	3.15 NS	28.29	0.11 NS	1029.53	4.33 *
SP	3	3.94	2.65 NS	4.57	7.14 **	1.04	5.09 **	97.99	104.25 **	765.64	3.22 *
CC×MCP	1	0.07	0.05 NS	0.61	0.95 NS	0.81	3.97 NS	2.53	1.69 NS	987.36	4.15 *
CC×SP	3	0.48	0.33 NS	1.01	1.57 NS	0.18	0.86 NS	0.04	0.14 NS	9.85	0.01 NS
MCP×SP	3	1.39	0.93 NS	0.86	1.35 NS	0.09	0.44 NS	0.09	0.05 NS	342.21	1.44 NS
CC×MCP×SP	3	3.90	2.62 NS	0.58	0.91 NS	0.46	2.27 NS	0.33	0.39 NS	84.59	0.36 NS
CV (%)		46.2		9.6		19.9		13.2		22.2	

CC= Calcium Chloride; MCP= Methylcyclopropene; SP= Storage Periods; CV= Coefficient of Variation; FI= Firmness; SS= Soluble Solids; TA = Titratable Acidity; WL= Weight Loss; MI= Maturation Index; MS= Mean Square; F= F test;
** Significant at P≤0.01; * Significant at P<0.05. NS = not significant.

Except for effect of the interaction between $CaCl_2$ x 1-MCP on the maturation index of figs, we could also observe from ANOVA that there was no significant effect of interactions between the factors on parameters evaluated in this present work (Table 1).

When ripe figs were post-harvest treated with $CaCl_2$, they demonstrated more firmness than untreated figs (3.37 lb and 1.91 lb, respectively) (Table 2). $CaCl_2$ postharvest treatment also influenced TSS on ripe figs, where untreated fruits showed a higher value (8.70%) compared to the treated figs (7.98%).

Figs stored for 4 (8.74%) and 6 days (8.81%) showed higher TSS than non-stored fruits (7.47%) (Table 3). The titratable acidity was higher after 6 days of storage (2.58%), when compared with no storage (1.98%) and 2 days of storage (2.07%) (Table 3). The weight loss of ripe figs was significantly

Table 2 - Effects of calcium chloride ($CaCl_2$) treatments on quality parameters of ripe figs stored over different periods

Fruit parameter	$CaCl_2$	
	Firmness (Lb)	Soluble Solids (%)
Untreated	1.91 ± 1.34* b	8.70 ± 1.00 a
Treated	3.37 ± 1.29 a	7.98 ± 0.91 b
Coefficient of Variation (%)	46.2	9.6

* Standard deviation. Values followed by the different lowercase letter in a column are significantly different from each other (P<0.05).

Table 3 - Effects of storage periods on quality parameters of ripe figs

Storage (days)	Fruit parameters			
	Soluble solids (%)	Titratable acidity (%)	Weight loss (%)	Maturation index
0	7.47 ± 0.99* b	1.98 ± 0.35 b	0 ± 0.00 d	69.22 ± 12.56 ab
2	8.35 ± 0.68 ab	2.58 ± 0.73 b	7.67 ± 0.86 c	62.22 ± 16.49 b
4	8.74 ± 0.82 a	2.07 ± 0.47 ab	13.67 ± 2.84 b	80.47 ± 21.62 a
6	8.81 ± 1.02 a	2.46 ± 0.22 a	18.75 ± 3.96 a	65.23 ± 11.54 ab
Coefficient of Variation (%)	9.6	19.9	27.3	22.2

* Standard deviation. Values followed by the same lowercase letter in a column are not significantly different from each other (P<0.05).

enhanced as storage duration increased, as expected. The greatest weight loss was 18.75% after 6 days of cold storage.

The maturation index (TSS/TA ratio) was the parameter that showed a significant interaction effect (Table 1). When ripe figs were treated with $CaCl_2$ after with 1-MCP, they showed a higher ratio (75.55) than those treated only with $CaCl_2$ (57.22) (Table 4).

4. Discussion and Conclusions

$CaCl_2$ has been widely used for food conservation, mainly because it can maintain firmness during the shelf life of vegetables, especially in fruits immersed in calcium chloride solution after harvest (Li *et al*., 2014). Calcium plays an important role in maintaining the structure of the cell wall, since its cation binds to pectins producing calcium pectates that structure the cell wall, thereby inhibiting enzymes such as polygalacturonase and polymethylesterase.

Irfan *et al*. (2013) recorded the lowest sugar content when figs were treated with $CaCl_2$ at 4%, as reported before when we showed that figs treated with $CaCl_2$ obtained lower sugar content against untreated figs. These authors concluded that this likely occurred because calcium caused a delay in fruit ripening through the inhibition of certain events such as starch hydrolysis and formation of sugars, a response to the high amounts of calcium deposition in the fruit cell wall of the treated figs. These authors also mentioned that components such as pectic substances, calmodulin, and hemicellulose polysaccharides are the most likely binding sites for calcium, preserving the texture and stability of the treated fruit and promoting higher fruit resistance by maintaining high calcium concentration in the cytosol, as well as less free sugars released during storage.

Storage having no influence on firmness likely relates to the figs being harvested at a relatively advanced ripening stage, when they showed at least a 50% change in skin color, from green to reddish brown at stage 6. In Brazil, the late season to harvest ripe figs for table takes place from late March to early April, when they usually get better prices in the market. Thereby, it would be interesting whether growers had a technique that was able to extend shelf life of ripe figs in order to add value to their product. Thus, at this harvest time, figs already displayed preharvest firmness reduction; therefore, the collected data of firmness from harvest to last storage period did not show a significant difference. According to Freiman *et al*. (2012), postharvest treatment on figs at the maturation stage can be ineffective for their quality conservation or may have a slight effect on softening, promoting its retardation, especially when figs are collected at an advanced stage. These authors mentioned that marketable ripe figs are defined as commercially mature when they have changing skin coloration from 20 to 70% of their surface.

Tofanelli *et al*. (2018) also showed TSS increasing during storage in 'Roxo-de-Valinhos' figs collected at stage 5 (Freiman *et al*., 2012) and treated with 1-MCP. According to those authors, this TSS increase likely occurs either due to the hydrolysis of several polysaccharides, such as pectins, starch, and other oligosaccharides in the cell wall, which become part of the cellular juice when they are solubilized in the aqueous phase, or due to starch accumulation during fruit maturation, which is degraded into sugars by the action of enzymes such as α-amylase, β-amylase, and starch phosphorylase, consequently increasing the TSS concentration.

Although the acidity of figs tends to decrease during the storage period (Irfan *et al*., 2013; Song *et al*., 2019), it has been observed that in the initial storage time, acidity tends to increase for a short period in the first few days. Gözlekçi *et al*. (2008) evaluated the effect of 1-MCP on the quality of figs stored during three different periods (5, 10, and 15 days) and

Table 4 - Effects of calcium chloride ($CaCl_2$) and 1-methylcyclopropene (1-MCP) treatments on the ratio (%) of ripe figs

$CaCl_2$	1-MCP	
	Untreated	Treated
Untreated	72.09 ± 11.65* aA	72.28 ± 18.67 aA
Treated	57.22 ± 11.10 bB	75.55 ± 20.28 aA
Coefficient of Variation (%)	22.2	

* Standard deviation. Values followed by the same uppercase letter in a column and lowercase letter in a row are not significantly different from each other (P<0.05).

observed that fruit acidity slightly increased until the first storage period. In addition, Álvarez-Herrera *et al*. (2016) studied the effect of 1-MCP on the postharvest conservation of pitahaya (*Selenicereus megalanthus*) during seven storage periods (4, 8, 12, 16, 20, 24, and 28 days), and discovered an increase in acidity in the treated pitahayas until the second storage period (8 days). In the present work, the last evaluated storage period was 6 days; therefore, it was expected that the acidity of ripe figs would increase during the entire experimental period.

Fruit transpiration and several physiological disorders of figs, such as skin side cracking and ostiole-end cracking/splitting occur during storage, and these could promote increasing levels of TA and TSS, as well as the maintenance of several organic acids during the storage period (Byeon and Lee, 2020).

In our study, 1-MCP treatments only affected on maturation index (ratio). Cantín *et al*. (2020) also did not obtain improvement on shelf life of 'Cuello Dama Negro' dark-skin commercial figs treated with 1-MCP at 1 μL l^{-1} and according to these authors the advance maturity stage of the figs at the moment of harvest could be promoted a high increase in ethylene production even under 1-MCP treatment. They also highlighted that, although the best moment to harvest fresh-market figs is variable according to each cultivar or variety, it is usually done as early as possible. In our work, studied figs were harvested at maturation stage 6 (Fig. 1B), thus an early harvest (Fig. 1C) could be promoted different results, what we would release here as a challenge for future studies, as well as evaluate how these figs harvested on early maturation stage would be taken up by the market. However, figs may be a climacteric or non-climacteric fruit due to their maturation shows rapid changes of compositional features that are typical of climacteric fruit, whereas figs are not capable to keep ripening after harvested (D'Aquino *et al*., 2015). Thus, that is a good question when would be the better time to harvest ripe figs for post treatment in order to enlarge their shelf life as well as promote their quality conservation.

Fig fruits are highly perishable products in which physiological events such as transpiration, respiration, and degradation occur quickly (Gözlekçi *et al*., 2008; Ozkaya *et al*., 2014; Byeon and Lee, 2020). It is interesting to consider the unique morphological structure of figs, as they have a thin skin over the entire fruit with a distal end orifice called the ostiole where water loss through evaporation occurs by exudation of a syrupy liquid (D'Aquino *et al*., 2003; Freiman *et al*., 2012).

$CaCl_2$ combined with 1-MCP applied on postharvest ripe figs likely promoted sugar content accumulation and was not favorable to acidity oscillation of fruits during storage.

Some structural effects on figs have been observed when they are treated with calcium, such as mechanical strength improvement and increased resistance of fruit tissue due to absorbed calcium in the apoplast primarily complexing with the cell wall, where the plasma membrane works as a cementing material. Calcium is also involved in the maintenance of the cell wall structure by interacting with pectic acid and forming calcium pectate (Irfan *et al*., 2013). Thus, in the present study, $CaCl_2$ application likely promoted these events in figs, resulting in sugar content accumulation and acidity stabilization, consequently enhancing the maturation index (ratio).

In addition, when 1-MCP was applied after calcium on the ripe figs, the MI ratios increased, potentially due to ethylene inhibition, decreased fruit respiration, and glucose and fructose stabilization during cold storage (Ozkaya *et al*., 2014; Song *et al*., 2019).

Overall, the results showed that a 4% $CaCl_2$ solution was capable of promoting higher firmness and sugar content stabilization, whereas when $CaCl_2$ was applied in combination with 1-MCP at 10 μg l^{-1}, the maturation index increased. These results reinforce

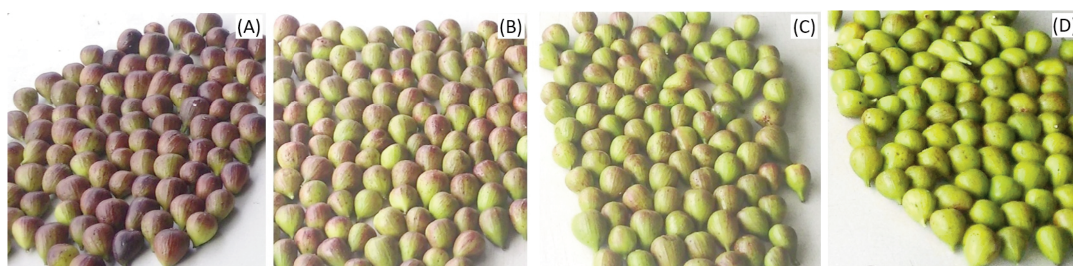

Fig. 1 - Figs of Roxo-de-Valinhos harvested at different stages of ripening. A - Advanced maturation; B - Maturation at 50% change skin color; C - Maturation at 25% change skin color, D - Maturation at 10% change skin color.

that calcium really may maintain more stable the structure of the cell wall, seeing that when it was combined with 1-MCP there was no effect on firmness. An explanation in this case, may be because humidity into the container increased softly due to 1-MCP solution applied indoor, what could be sufficient to promote moisture saturation from $CaCl_2$ reducing its absorbing capacity.

The present work encourages the development of further investigation in order to verify the effects of both $CaCl_2$ and 1-MCP applied at numerous different concentrations on quality conservation of 'Roxo-de-Valinhos' ripe figs harvested at earlier maturation stage and during a longer storage period than studied here.

In conclusion, this study showed the effect of calcium chloride and 1-methylcyclopropene on conserve quality of 'Roxo-de-Valinhos' ripe figs to market for table. The calcium chloride solution applied at 4% improves firmness and promotes soluble solid stabilization of ripe figs stored for 6 days in a refrigerator, whereas 1-MCP treatment at a dose of 10 µg l^{-1} does not contribute for maintaining postharvest quality of ripe fig, nor its shelf life. However, treatment with 4% $CaCl_2$ combined with 1-MCP 10 µg l^{-1} increases the maturation index (ratio) of fresh ripe figs. Finally, a better understanding of harvest seasons, maturation stages and postharvest treatments on ripe figs is essential for crop fig, grower and market.

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Postharvest application of calcium chloride and 1-methylcyclopropene for quality conservation on organic ripe fig

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The authors declare no competing interests.

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Key words: Brown Turkey fig, *Ficus carica*, fruit conservation, shelf life.

Abstract: The postharvest phase is an important step in the fruit production chain. Fig is an especially perishable fruit, which has encouraged researchers to study the effects of various substances on the postharvest life of this commodity. The objective of the present work was to evaluate the effects of calcium chloride ($CaCl_2$) and 1-methylcyclopropene (1-MCP) on the postharvest quality of the 'Roxo-de-Valinhos' fig cultivar. This study aimed to verify the effects of applying a 4% solution of $CaCl_2$ and a 1% solution of 1-MCP to figs and evaluating at four different storage times (0, 2, 4, and 6 days). The results showed that a 4% solution of $CaCl_2$ promoted better firmness, and when $CaCl_2$ at 4% solution was applied in combination with 1-MCP at 10 µg l^{-1}, the maturation index increased. In contrast, the 1-MCP treatment alone did not improve the postharvest quality of 'Roxo-de-Valinhos' ripe fig. We conclude that application of 4% solution of $CaCl_2$ and 1-MCP at 10 µg l^{-1} promote firmness and increase maturation index of 'Roxo-deValinhos' figs.

Table S1 - Sample identifications (Sample ID), local names, their meanings of the 86 86 local mango cultivar from southern of Iran

Fruit parameters	Treatment				CV
	CC		MCP		
	Untreated	Treated	Untreated	Treated	
Soluble solids (%)	9.13 * ± 1.32 a	8.48 ± 0.54 a	9.15 ± 1.08 a	3.14 ± 0.93 a	9.6
Titratable acidity (%)	2.43 ± 0.23 a	2.48 ± 0.22 a	2.43 ± 0.20 a	2.48 ± 0.23 a	19.9
Weight loss (%)	19.35 ± 5.14 a	18.14 ± 2.66 a	19.10 ± 5.21 a	18.40 ± 2.66 a	27.3
Firmness (Lb)	2.31 ± 0.54 a	3.53 ± 2.67 * a	2.70 ± 0.88 a	3.14 ± 1.35 a	46.2
Maturation index	68.43 ± 14.29 a	62.03 ± 8.00 a	65.72 ± 12.51 a	64.75 ± 11.66 a	22.2

* Standard deviation. Values followed by the same uppercase letter in a column and lowercase letter in a row are not significantly different from each other ($P<0.05$).

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Diallel analysis of selected yield-contributing traits in Okra [*Abelmoschus esculentus* (L.) Moench]

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Key words: Diallel analysis, gene action, general combining ability, hybrid, specific combining ability.

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All relevant data are within the paper and its Supporting Information files.

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Abstract: Information on gene action controlling quantitative traits is important for effective selection. A five-parent diallel cross, which generated 10 F_1 hybrids of okra (*Abelmoscus esculentus*) were evaluated during the early and late planting seasons of 2019 in Ibadan, Nigeria. Data obtained were subjected to diallel analysis and genotype by yield-trait (GYT) biplot analysis to estimate combining ability effects and identify stable hybrids for measured traits respectively. Genotype mean squares were significant (p≤0.01) for all most measured traits. Furthermore, General Combining Ability (GCA) and Specific Combining Ability (SCA) mean squares were significant (p≤0.05/0.01) for most measured traits, indicating the influence of additive and non-additive gene actions in expression of these traits. Preponderance of non-additive gene effects shows the high influence of the environment on most of the considered traits in this study. Iwo Nla had the most desirable GCA estimates of -0.98 and 1.14, for days to 50% flowering (DTF), number of fruits per plant (NoF) respectively while IK11 had the most desirable GCA values for mature-fruit width (0.21) and 1000-seed weight (5.71). SCA estimates were most desirable for NH47-4 × LD88, NH47-4 × Iwo Nla, with values of -4.21 and 4.32 for DTF and NoF respectively. Hybrids NH47-4 × Iwo Nla and IK11 × Clemson associated with higher NoF x trait might be useful for improvement of number of fruits per plant in this population.

1. Introduction

Okra (*Abelmoschus esculentus* L. Moench) is an important food crop in Africa and belongs to the Malvaceae family (Kochhar, 1986). It is a widely-cultivated vegetable across West Africa and is well adapted to tropical environments. The vegetable is grown in almost all the agro-ecological regions across Nigeria because of its importance to the economic development of rural dwellers, and can be found in most markets across the country (Siemonsma and Kouame, 2004; Christo and Onuh, 2005; Mohammed and Miko, 2009).

Fresh okra pods serve as soup thickeners because of its unique mucilage properties and are known to be good sources of vitamins and minerals (Schippers, 2000). The edible fruits contain 86% water, 2.2% protein, 10% carbohydrate, 0.2% fat and vitamins A, B, and C (Berry *et al*., 1988). Despite the important role played by okra in meeting the daily nutritional and economic needs of the society in the region, minimum efforts and resources have been directed towards its genetic improvement to meet the desired preferences of farmers and consumers, such as earliness and increased number of fruits with smooth pod per plant. Okra research in developing countries has for long been concentrated on germplasm characterization and development of pure line varieties, with little or no efforts directed towards development of hybrid varieties despite its huge potential and market. Okra hybrids are gradually becoming the favorites of many farmers regardless of associated high cost of seeds since hybrid varieties are usually characterized by higher yield and uniformity, coupled with tolerance to diseases and pests. Heterosis which is widely referred to as the superiority of F_1 hybrid over the mean performance of its better inbred parents, has been reported to enhance yield and component traits in okra by about 86% (Elmaksoud *et al.*, 1986, Ahmed and Adam, 2014). Hybrid variety development is a quick path to combining economic and desirable horticultural traits in vegetable crops such as okra. The floral pattern of okra which enables easy emasculation and pollination coupled with the ability to produce a large number of seeds from a single pollination, has made commercial exploitation of heterosis a profitable venture in okra hybrid seed production (Reddy, 2010).

Heterosis breeding is a means to improve yield and its component traits in okra (Jindal *et al*., 2009). In many developing countries of West Africa, minimal work has been reported on the use of two or more okra genotypes in heterotic studies for estimating hybrid vigor relative to fruit yield and its component traits. Positive heterosis favors genetic improvement of yield and yield contributing traits whereas negative heterosis is encouraged when breeding for traits such as earliness (Biswas *et al.,* 2005). The objective of this study was to (i) determine gene action and identify superior parent combination(s) for measured traits and (ii) identify stable hybrids for desired traits of okra with a view to providing information for future breeding programs.

2. Materials and Methods

Study area

Two experiments were conducted at the National Horticultural Research Institute (NIHORT), Ibadan, Oyo state, Nigeria during the early planting season (April to July) and late planting season (from August to December) of 2019. NIHORT is located in the humid forest savanna transition zone (210 masl, 7°30'N, 3°54'E), with a bimodal annual rainfall pattern spanning across 120-128 rainy days, amounting to 1200-1400mm. Pan Evaporation is between 1550-1600mm. Wet season extends from March through October and dry season from November to February, with annual maximum temperature ranging between 27-34°C and annual minimum temperature ranging between 20-23°C (Ogungbenro and Morakinyo, 2014).

Plant material and field establishment

Genetic materials evaluated in this study comprised of 10 newly-developed F_1 hybrids obtained from diallel mating and their 5 parents namely: NH47-4, IK11, Iwo Nla, LD 88, and Clemson. The parents and their hybrids were grown in Randomized Complete Block Design with two replicates during the early and late planting seasons of 2019. Three seeds per hill were sown directly in 3-cm holes and later thinned to one plant per hill after seedling establishment. Plants were spaced at 60 cm x 50 cm between and within rows respectively on a 2-m bed constituting 10 stands per plot. Regular plant protection and other agronomic activities were carried out as when due to ensure full expression of desired traits and safeguard crops from pests. Manual weeding was done at three weeks after planting while a compound fertilizer, NPK 15:15:15, was applied at three-week interval after sowing to enhance vegetative growth at the recommended rate of 60 kg/ha (Adigun *et al*., 2018). Insect pest control was done by spraying Cyperfits (synthetic pyrethrum) at the rate of 80 g ai/ha.

Data collection

Data on agronomic attributes were recorded on five randomly selected competitive plants in each plot according to okra descriptors by Charrier (1984) and IPGRI (1991) for days to 50% flowering (DTF), plant height at maturity (PH) (cm), matured fruit width and length (cm), average number of fruits (fruits per plant) obtained by the ratio between the

total number of fruits and the number of plants in the plot, internode length (cm), number of ridges per pod and 1000-seed weight (g).

Data analysis

Each season was considered an environment. Pooled data for the two environments were subjected to analysis of variance (ANOVA). The MIXED MODEL procedure of Statistical Analysis System (SAS) (SAS Institute, 2002) was used, with replication within environment treated as random factor and genotype (crosses) as fixed factor. The statistical model used for the combined analysis is:

$$Yijg = \mu + Ei + Rj(i) + Gg + EGig + \varepsilon ijg$$

where Yijg is the measurement for the gth Genotype grown in Replicate j within Environment i; μ is the grand Mean; Ei is the main effect of Environment i; Rj(i) is the effect of Replicate nested within Environment effect; Gg is the effect of the Genotype; EGig is the interaction effect between Genotype and Environment, and εijg is the error term.

Mid- and better-parent heterosis were calculated according to the procedure of Singh (1973) described by Amiteye *et al*. (2019).

General (GCA) and specific combining ability (SCA) estimates were generated for each of the traits according to the procedure of Griffing (1956), employed by Medagam *et al*. (2012), for diallel analysis model B (Mixed), method 2 using AGD-R software version 3.0 (Rodríguez *et al*., 2015).

The statistical model for the diallel analysis is as follows:

$$Yijk = \mu + Ee + gi + gj + sij + gEeg + sEes + \varepsilon ijk$$

where Yijk is the observed measurement for the ijth cross grown in the kth environment; μ is the grand mean; Ee is the main effect of Environment; gi and gj are the GCA effects; sij is the SCA effect; gEeg is the interaction effect between GCA and Environment; sEes is the interaction effect between SCA and Environment, and εijk is the error term.

The GCA and SCA effects were tested for significance using t-test at 5 and 1% levels of probability as suggested by Mather and Jinks (1982), Kearsey and Pooni (1996). Standard error (SE) estimates of GCA and SCA were obtained from PB Tools software version 1.4.0 (PB Tools, 2014). The formulae for the SEs were described by Ahmed and Adam (2014) as follows:

SE (gi) = $[(n-1)\sigma^2e/n(n+2)]^{½}$ and SE (gi-gj) = $[(2\sigma^2e/n+2)]^{½}$ for GCA effects and SE (sij) = $[n(n-1)\sigma^2e/(n+1)(n+2)]^{½}$ and SE (sii-sjj) = $[2(n-2)\sigma^2e/n+2)]^{½}$ for SCA effects where S.E. (gi) = S.E. for GCA effects of parents, S.E. (gi-gj) = S.E. of difference between GCA effects of the ith and jth parents, S.E. (sij) = S.E. for SCA effects of the diallel hybrids, S.E. (sii-sjj) = S.E. of the difference between the SCA effects of the ith and jth hybrids, σ^2e is the error mean square value in the diallel analysis, and n is the number of parents.

The proportions of the additive and non-additive genetic variances were computed as the percentages of GCA and SCA sums of squares (SS) respectively of the cross SS across environments (Fasahat *et al*. 2016). Means of observed data were used to obtain pair-wise correlation (Pearson's) coefficients, to determine the level of association among measured traits. The modified genotype × trait (GYT) biplot approach of Yan and Fregeau-Reid (2018) was used to profile the 10 diallel F_1 hybrids for measured traits. The GYT incorporates yield into other traits, rather than as a standalone trait. Here, GYT is the interaction of genotype and the combination of other traits with NoF. In this study, the procedure for obtaining the NoF-trait combination estimates was based on the direction of association of other traits with NoF. Thus, the estimates were obtained by multiplication for all measured traits except DTF, FWT, and INL which were negatively correlated with NoF, and were obtained by division. Biplotswere obtained using the GGEBiplotGUI package in R.

3. Results

Analysis of variance for pooled data over the two environments (seasons) revealed significant ($P\leq0.01$) genotype (G) mean squares for all measured traits while environment (E) mean square was significant ($P\leq0.01$) for DTF and NOF. The mean square of the G×E interaction was found to be significant ($P\leq0.05$) for NOR. Number of days to flowering (DTF) ranged from 46.5 days for NH47-4 × Clemson to 57.75 days for LD88 while NOS ranged from 30.50 for NH47-4 × LD88 to 117.15 for LD88 × Iwo Nla. Iwo Nla × Clemson produced the longest peduncles with a mean length of 3.50 cm while LD88 had the shortest peduncle lengths averaging 1.50 cm (Table 1).

Diallel analysis of hybrids revealed significant ($P\leq0.05$ or 0.01) cross mean squares for all measured traits necessitating the partitioning of the mean squares into GCA and SCA components. Mean squares of GCA and SCA were significant ($P<0.05$ or

Table 1 - Analysis of variance of 15 genotypes of okra evaluated for selected traits across research environments

Genotype		DTF (days)	PH (cm)	NoF	LNT (cm)	FWT (cm)	PL (cm)	INL (cm)	NoS	THS (g)	NoR
Clemson		48.25 ef	62.25 de	5.00 cde	12.92 a	2.39 d	3.00 b	6.50 de	68.75 de	50.00 d	7.38 efg
IK11		49.25 def	104.00 abc	6.00 b-e	7.75 cd	3.4 abc	1.63 ef	8.63 cd	79.50 bcd	70.00 a	6.75 g
IwoNla		47.75 f	74.00 b-e	5.75 b-e	10.22 bc	2.91 dc	2.00 ed	6.75 de	64.75 e	60.00 c	5.25 h
LD88		57.75 a	122.75 a	4.50 de	6.70 d	3.34 abc	1.50 f	12.50 ab	82.25 bc	45.00 e	9.00 ab
NH47-4		52.50 bc	63.75 de	5.00 cde	7.95 cd	3.51 abc	2.50 c	6.75 de	65.50 e	70.00 a	8.00 def
NH47-4 × IK11		48.25 ef	73.50 b-e	8.75 b	8.77 cd	3.43 abc	2.38 cd	5.50 e	68.25 de	60.00 c	5.25 h
NH47-4 × LD88		52.13 bcd	106.25 ab	7.75 bc	12.16 ab	2.84 cd	3.00 b	10.50 bc	66.00 e	70.00 a	8.13 cde
NH47-4 × Clemson		51.25 b-e	73.00 b-e	8.25 b	9.71 bc	3.25 bc	3.00 b	6.00 e	72.50 cde	65.00 b	8.00 def
NH47-4 × Iwo Nla		49.38 def	42.25 e	4.25 e	8.44 cd	4.08 a	1.78 ef	7.13 de	36.00 f	60.00 c	5.75 h
IK11 × LD88		53.75 b	76.50 b-e	7.50 bc	13.93 a	3.16 dbc	3.50 a	5.75 e	61.00 e	60.00 c	8.88 abc
IK11 × Clemson		50.50 c-f	107.25 ab	7.75 bc	9.12 cd	3.82 ab	2.50 c	6.75 de	117.25 a	60.00 c	8.25 bcd
IK11 × Iwo Nla		47.50 f	70.25 cde	7.25 bcd	11.92 ab	2.91 dc	2.50 c	6.50 de	84.75 b	70.00 a	9.25 a
LD88 × Clemson		50.88 b-e	96.50 a-d	7.25 bcd	8.90 cd	3.93 ab	1.75 ef	13.25 a	72.25 cde	70.00 a	7.25 fg
LD88 × Iwo Nla		51.00 b-e	74.75 b-e	12.25 a	13.61 a	2.72 dc	2.25 cd	9.50 c	68.50 de	60.00 c	5.00 h
Clemson × Iwo Nla		48.25 ef	75.50 b-e	4.00 e	6.74 d	2.93 dc	2.00 de	13.00 a	30.50 f	42.50 e	7.75 def
Source	DF	DTF	PH	NOF	LNT	FWT	PL	INL	NOS	THS	NOR
Rep (Environment)	2	**	NS	**	NS	NS	NS	NS	NS	NS	NS
Environment (E)	1	**	NS	**	NS	NS	NS	NS	NS	NS	NS
Genotype (G)	14	**	**	**	**	**	**	**	**	**	**
G×E	14	NS	NS	NS	NS	NS	NS	NS	NS	NS	*
Error	28	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
R-Squared		0.86	0.71	0.82	0.84	0.72	0.92	0.88	0.94	0.95	0.95

*,** significant at p≤0.05 and p≤0.01 respectively; NS= not significant; Rep= Replicate; DTF= days to 50% flowering; PH= plant height at maturity; NOF= number of fruits per plot; LNT= fruit length; FWT= fruit circumference; PL= Pedicel length; INL= internodal length; NOS= number of seeds per pod; THS= 1000-seed weight; NOR= number of ridges per pod.

0·01) for all traits measured except GCA for FRTWDT while cross × environment and SCA × environment interaction mean squares were significant only for NOR. With the exception of 1000-seed weight, SCA effect consistently accounted for higher proportion of total the variation among measured traits compared to GCA effects. The SCA accounted for 93.35% and 41.56% of the total variation for number of seeds per pod and 1000-seed weight respectively (Table 2).

Clemson and Iwo Nla had significant (*P*<0.05 or 0.01) and negative General Combining Ability (GCA) effects for DTF while LD88 showed significant (*P*<0.01) positive GCA effect for PH. Iwo Nla had significant (*P*<0.01) positive GCA effect for NoF. Significant negative and positive GCA effects for FRTLNT and FRTWDT was recorded for IK11 and LD88 respectively while NH47-4, IK11 and LD88 showed significant (*P*<0.05 or 0.01) positive GCA effects for 1000-seed weight (Table 3). Days to flowering had significant negative Specific Combining Ability (SCA) for NH47-4 × LD88, NH47-4 × Clemson and IK11 × LD88 while NH47-4 × IK11, IK11 × KD88, and LD88 × Clemson had high significant positive SCA effects for PH. Similarly, NH47-4 × Iwo Nla and LD88 × Clemson had significant (*P*<0.05 or 0.01) and positive SCA for NOF and FRTLNT while significant positive SCA effects were observed for FRTWDT for NH47-4 × IK11, IK11 × LD88, LD88 × Iwo Nla and Clemson × Iwo Nla. Furthermore, NH47-4 × IK11, NH47-4 × Clemson, IK11× Clemson, LD88× Clemson and LD88 × Iwo Nla exhibited high significant positive SCA for NoS and 1000-seed weight (Table 3). The proportion of additive genetic variance ranged from 0.07 for NoS to 0.58 for 1000-seed weight while the non-additive genetic variance estimates ranged from 0.42 to 0.93 for THS and NoS respectively (Table 4). Generally, the non-additive variance was higher than the additive

Table 2 - Mean squares from diallel analysis of variance of okra genotypes evaluated in two environments

Source of variation	DF	DTF (days)	PH (cm)	NoF	LNT (cm)	FWT (cm)	PL (cm)	INL (cm)	NoS	THS (g)	NoR
Rep (Env)	2	22.92 **	1023.90 NS	40.08 **	4.13 NS	0.04 NS	0.08 NS	6.93 NS	41.22 NS	0.83 NS	0.19 NS
Environment (E)	1	39.20 NS	32.27 NS	40.02 NS	1.80 NS	0.02 NS	0.00 NS	0.00 NS	0.15 NS	0.00 NS	0.04 NS
Crosses	14	30.07 **	1817.21 **	18.89 **	23.45 **	0.90*	1.39 **	30.09 **	1588.48 **	327.38 **	8.10 **
GCA	4	38.99 NS	1187.64 **	18.52 *	38.17 *	1.32 NS	2.09 **	37.33 **	369.98 *	669.64 **	11.27 **
SCA	10	26.50**	2069.04 **	19.04 **	17.56 **	0.73 *	1.11 **	27.19 **	2075.88 **	190.48 **	6.83 **
Crosses × E	14	6.11 NS	58.48 NS	1.87 NS	2.70 NS	0.27 NS	0.01 NS	0.78 NS	47.69 NS	0.00 NS	0.69 *
GCA×E	4	8.56 NS	30.10 NS	1.87 NS	4.42 NS	0.48 NS	0.01 NS	2.10 NS	40.85 NS	0.00 NS	0.52 NS
SCA×E	10	5.12 NS	69.83 NS	1.88 NS	2.01 NS	0.19 NS	0.01 NS	0.25 NS	50.42 NS	0.00 NS	0.76 *
Residuals	28	3.3	416.11	3.23	2.55	0.23	0.06	2.1	49.43	7.98	0.25
GCA Proportion (%)		37.05	18.67	28.01	46.51	41.97	42.96	35.45	6.65	58.44	39.76
SCA Proportion (%)		62.95	81.33	71.99	53.49	58.03	57.04	64.55	93.35	41.56	60.24

*,** significant at p≤0.05 and p≤0.01, respectively; NS= not significant; Rep= Replicate; DTF= days to 50% flowering; PH= plant height at maturity; NOF= number of fruits per plot; LNT= fruit length; FWT= fruit circumference; PL= Pedicel length; INL= internodal length; NOS= number of seeds per pod; THS= 1000-seed weight; NOR= number of ridges per pod.

Table 3 - Estimates of general (GCA) and specific combining ability (SCA) effects of okra parents and hybrids respectively for measured traits

Parent/Hybrid	DTF (days)	PH (cm)	NoF	LNT (cm)	FWT (cm)	PL (cm)	INL (cm)	NoS	THS (g)	NoR
NH47-4	-0.1	-6.36	0.04	-0.35	0.01	-0.09 *	0.82	-4.01 *	2.50 *	0.19
IK11	-0.18	5.29	-0.14	-0.69 *	0.21	-0.19 *	0.59	-1.34	5.71 **	-0.19
LD88	2.00 **	8.29	-1.14 **	-1.13 **	0.17	-0.19 *	1.09 *	-0.84	-7.50 **	0.67 **
Clemson	-0.75 *	-5.43	0.11	1.87 **	-0.33 *	0.47 **	-1.25 *	0.34	-0.71	0.33 *
Iwo Nla	-0.98	-1.79	1.14 *	0.3	-0.06	0	-1.25 *	5.84 *	NE	-0.99 **
S.E. (gi)	0.31	3.45	0.3	0.27	0.08	0.04	0.25	1.18	0.48	0.08
S.E. (gi - gj)	0.49	5.45	0.48	0.43	0.13	0.07	0.39	1.88	0.75	0.13
NH47-4 × IK11	0.6	16.07 *	0.61	0.02	0.47 *	-0.32 **	3.51 **	8.42 **	0.95	-0.07
NH47-4 × LD88	-4.21 **	-7.93	-1.64 *	-1.70 **	-0.49*	-0.07	2.76 **	-33.83 **	-13.33 **	-0.43 *
NH47-4 × Clemson	-2.21 **	0.54	0.36	0.48	-0.01	-0.23 *	-1.40 **	19.24 **	7.38 **	1.41 **
NH47-4 × Iwo Nla	1.52 *	1.39	4.32 **	3.74 **	-0.47 *	-0.02	1.60 **	-2.51	-3.33 **	-1.52 **
IK11 × LD88	-3.01 *	-52.82 **	-1.21	0.33	0.45 *	-0.20 *	-2.89 **	-31.01 **	0.95	-2.05 **
IK11 × Clemson	2.49 **	24.89 **	1.04	1.06	-0.27	0.37 **	2.83 **	-2.19	4.17 **	0.66 **
IK11 × Iwo Nla	1.85 *	-12	0.5	0.17	-0.14	0.84 **	-1.67 **	-1.19	-1.55	1.86 **
LD88 × Clemson	1.93 **	-7.86	1.79 *	3.27 **	0.08	0.87 **	-2.42 **	-7.69 **	7.38 **	0.55 **
LD88 × Iwo Nla	-1.08	19.25 *	1	0.03	0.47 *	0.34 **	-1.42 **	43.06 **	6.67 **	1.25 **
Clemson × Iwo Nla	-0.58	-0.79	0.75	-3.32 **	0.58 **	-0.45 **	-0.33	-7.12 *	-0.12	-1.41 **
S.E. (Sij)	0.63	7.04	0.62	0.55	0.17	0.09	0.50 **	2.43	0.97	0.17
S.E. (Sii-Sij)	0.84	9.44	0.83	0.74	0.22	0.12	0.67	3.25	1.31	0.23

*,** significant at p≤0.05 and p≤0.01 respectively; S.E.= standard error; gi= GCA effects of parents; gi-gj= difference between GCA effects of the ith and jth parents; Sij= SCA effects of the diallel hybrids; Sii-Sjj= difference between the SCA effects of the ith and jth hybrids; NE= not estimated; DTF= days to 50% flowering; PH= plant height at maturity; NOF= number of fruits per plot; LNT= fruit length; FWT= fruit circumference; PL= Pedicel length; INL= internodal length; NOS= number of seeds per pod; THS= 1000-seed weight; NOR= number of ridges per pod.

Table 4 - Proportion of additive and non-additive genetic variances for measured traits across the two environments

Trait	Additive variance	Non-additive variance
DTF (days)	37	63
PH (cm)	19	81
NoF	28	72
LNT (cm)	47	53
FWT (cm)	42	58
PL (cm)	43	57
InteL (cm)	35	65
NoS	7	93
ThSW (g)	58	42
NoR	40	60

DTF= days to 50% flowering; PH= plant height at maturity; NOF= number of fruits per plot; LNT= fruit length; FWT= fruit circumference; PL= Pedicel length; INL= internodal length; NOS= number of seeds per pod; THS= 1000-seed weight; NOR= number of ridges per pod.

variance for all measured traits except THS.

Estimates of mid-parent heterosis (MPH) and better-parent heterosis (BPH) of diallel crosses of okra are presented in Table 5. Significant positive and negative heterosis was observed for all traits. Desirable significant BPH and MPH for earliness was observed for NH47-4 × LD88 (-16.45% and -6.24%), NH47-4 × Clemson (-9.52% and -2.85%), IK11 × LD88 (-14.50 and -3.86%), and LD88× Iwo Nla (-12.55% and -2.13%). LD88 × Clemson also showed desirable high significant BPH of -6.93% for DTF. For NoF, all the hybrids showed desirable significant ($P<0{\cdot}05$ or 0.01) BPH and MPH with the exception of NH47-4 × IK11, NH47-4 × LD88, and IK11 × LD88 for BPH, and NH47-4 × LD88 and IK11 × LD88 for MPH. Intermodal length (Intel) recorded high negative significant BPH and MPH for IK11 × LD88, LD88 × Clemson, and LD88 × Iwo Nla while LD88 × Iwo Nla only had significant ($P<0.05$ or 0.01) negative BPH for the same trait.

Table 5 - Estimates (%) of heterobeltiosis of ten diallel crosses of okra for measured traits

Hybrid	DTF (days)		PH (cm)		NoF		FRTLNT (cm)		FRTWDT (cm)	
	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH
NH47-4 × IK11	-3.1	0	-7.21	7.53	20.83	15.91*	11.87*	6.66*	12.02*	6.91**
NH47-4 × LD88	-16.45**	-6.24**	-38.49**	-9.52*	-20	-7.89	-15.23*	-4	-16.45**	-7.19**
NH47-4 × Clemson	-9.52**	-2.85**	10.2	5.75	45.00**	22.50**	-7.68	7.14*	-17.18**	-0.72
NH47-4 × Iwo Nla	-2.86	0.87	1.01	4.26	113.04**	63.95**	33.24**	24.92**	-22.57**	-7.66**
IK11 × LD88	-14.50*	-3.86**	-65.58**	-31.37**	-29.17*	-9.52	8.85	8.37*	19.95**	10.50**
IK11 × Clemson	5.84*	3.46**	2.16	13.91**	29.17*	20.45**	-5.86	8.84*	-16.32**	-0.85
IK11 × Iwo Nla	4.06	2.84**	-29.81**	-8.99*	37.50**	20.21**	-4.99	4.02	-4.2	1.63
LD88 × Clemson	-6.93**	0.71	-37.68**	-8.65*	50.00**	28.95**	7.84	20.99**	-5.44	5.14*
LD88 × Iwo Nla	-12.55**	-2.13*	-12.63	4.51	34.78*	25.61**	-10.68	3.93	14.47*	11.19**
Clemson × Iwo Nla	0	0.26	-0.68	3.94	52.17**	31.40**	-32.13**	-12.10**	17.91**	14.75**
Standard error	2.31	0.91	7.22	3.92	11.86	6.19	5.35	3.2	4.93	2.3

Hybrid	PL (cm)		INL (cm)		NoS		ThSW (g)		NoR	
	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH
NH47-4 × IK11	-30.00**	-7.58	53.62**	36.18**	-9.12	-0.17	0	0	-9.38	-0.85
NH47-4 × LD88	-20.00*	0	4	17.53*	-62.92**	-29.36**	-39.29**	-13.04**	-13.89*	-4.41
NH47-4 × Clemson	-16.67*	-4.55	-3.7	-0.94	23.27*	13.13*	0	8.33**	15.63*	10.16**
NH47-4 × Iwo Nla	-10	0	40.74**	20.37**	4.58	2.59	-14.29**	-3.85	-37.50**	-12.26**
IK11 × LD88	9.23	6.8	-43.00**	-16.27*	-56.23**	-27.74**	-14.29**	2.17	-36.11**	-13.49**
IK11 × Clemson	0	14.86**	21.74	19.42**	-16.98	-5.48	0	8.33**	10.17	7.52*
IK11 × Iwo Nla	50.00**	32.76**	-30.43*	-10.98	-8.81	0.26	-7.14	0	18.52**	16.67**
LD88 × Clemson	16.67*	27.78**	-54.00**	-19.74**	-25.84*	-9.6	20.00**	13.16**	-1.39	4.2
LD88 × Iwo Nla	25.00**	21.43**	-46.00**	-14.94*	42.55**	29.76**	0	7.14**	-8.33	7.89*
Clemson × Iwo Nla	-20.83*	-2.5	-18.52	-8.49	-0.73	1.12	0	4.55*	-28.81**	-8.42*
Standard error	7.48	4.32	11.23	5.86	9.69	5.25	4.58	2.24	6.13	3.06

*,** significant at p≤0.05 and p≤0.01 respectively; BPH and MPH= Better- and Mid-parent heterosis, respectively; DTF= days to 50% flowering; PH= plant height at maturity; NOF= number of fruits per plot; LNT= fruit length; FWT= fruit circumference; PL= Pedicel length; INL= internodal length; NOS= number of seeds per pod; THS= 1000-seed weight; NOR= number of ridges per pod.

Significant positive percentage for BPH and MPH were also obtained for NH47-4 × Clemson (23.27 and 13.13%) and LD88 × Iwo Nla (42.55 and 29.76%) while NH47-4 × Clemson, IK11 × Clemson, LD88 × Clemson, LD88 × Iwo Nla and Clemson × Iwo Nla displayed significant positive (desirable) BHP and MPH for 1000-seed weight.

The tester vector view of the genotype × NoF-trait (GYT) biplot showing associations among the NoF-trait combinations is presented in figure 1. Since all NoF-trait combinations have NoF as a component, positive correlation was observed between all possible pairs. There was correspondence between the GYT biplot and Pearson correlation (Table 6) among the traits. Positive correlation was recorded between NoS and THS while INL associated negatively with NoS and THS (Table 6). The GYT biplot, shows the magnitude of angles among INL, NoS, and THS while figure 2 is the polygon view of the GYT biplot displayed in figure 1.

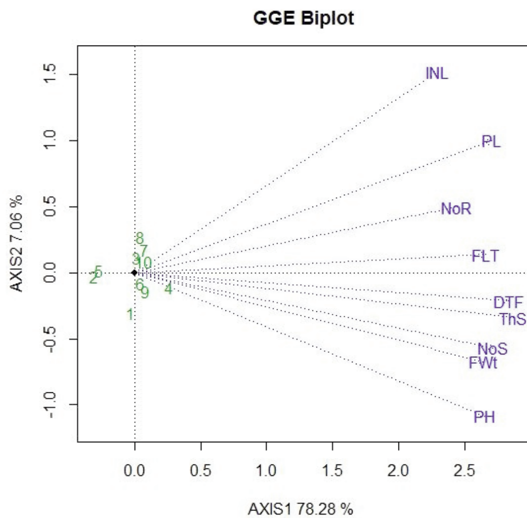

Fig. 1 - The tester vector view of the genotype by NoF x trait (GYT) biplot showing associations among the NoF x trait combinations.

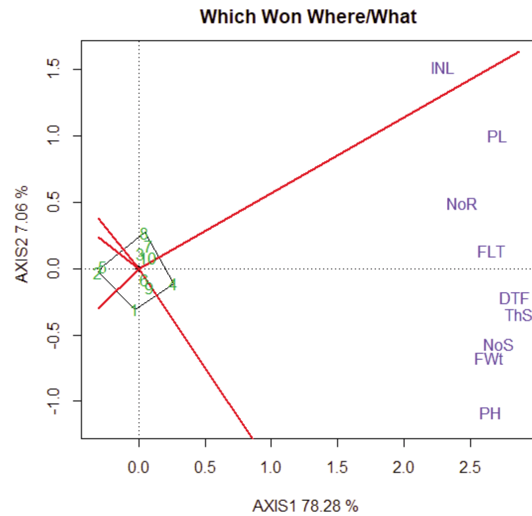

Fig. 2 - The polygon view of the genotype by NoE x trait (GYT) biplot to identify genotypes with outstanding trait profiles.

Hybrid was the vertex cultivar for the sector containing all the NoF-trait combinations except INL while LD88 × Clemson, NH47-4 × Clemson, IK11× Iwo Nla, and Clemson × Iwo Nla were associated with the polygon sector containing INL. The superiority ranks of the hybrids based on their NoF-trait combinations is shown in figure 3. Hybrid NH47-4 × Iwo Nla was the farthest above average from the origin, followed by LD88 × Iwo Nla and Clemson × Iwo Nla while NH47-4 × LD88 was the farthest below average performance across NoF-trait combination. The shortest vector lengths were observed for IK11 × LD88, NH47-4 ×

Table 6 - Pearson correlation coefficients among pairs of traits of okra hybrids

	PH	NOF	LNT	FWT	PL	INL	NOS	THS	NOR
DTF	0.52 *	-0.01	-0.09	0.18	0.02	0.32	0.18	-0.13	0.46
PH		0.09	-0.23	0.09	-0.23	0.52 *	0.58 *	-0.01	0.37
NOF			0.57*	-0.17	0.32	-0.17	0.35	0.37	-0.27
LNT				-0.58 *	0.71 **	-0.37	0.09	0.21	0
FWT					-0.42	0.05	0.11	0.27	-0.04
PL						-0.55*	0.08	0.16	0.34
INT							-0.2	-0.28	0.12
NOS								0.29	0.31
THS									-0.01

* and ** significant at p ≤ 0.05 and p ≤ 0.01 respectively; DTF, days to 50% flowering; PH, plant height at maturity; NOF, number of fruits per plot; LNT, fruit length; FWT, fruit circumference; PL, Pedicel length; INL, internodal length; NOS, number of seeds per pod; THS, 1000-seed weight; NOR, number of ridges per pod.

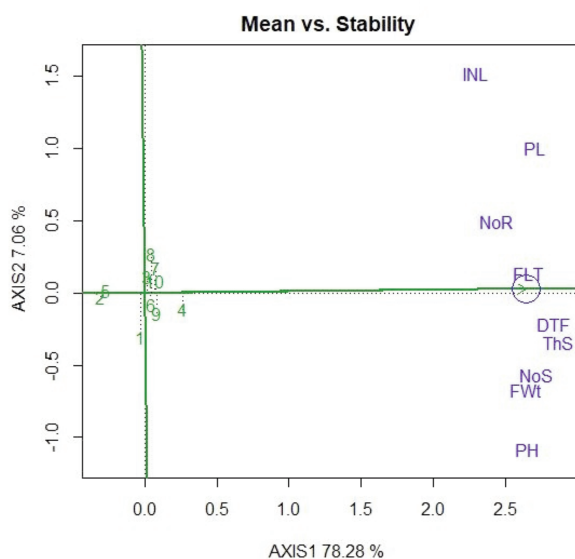

Fig. 3 - The average tester coordination view of the genotype by NoF x trait (GYT) biplot ranking the hybrids based on overall superiority and their strengths and weaknesses.

LD88 (below-average performers) and NH47-4 × Iwo Nla and IK11 × Clemson (above-average performers).

4. Discussion and Conclusions

The significant genotype mean squares observed for all traits is an indication of sufficient genetic variation among the genotypes for measured traits and this shows the feasibility of improving these traits through selection. Wammanda *et al*. (2010) observed similar level of genotypic variability for DTF, PH, NoF, INL, LNT and FWT in okra. The significant GCA and SCA mean squares for most measured traits indicates that both additive and non-additive gene actions were important in the inheritance of these traits. The proportion of total variation accounted for by SCA was larger than that of GCA for all the traits except 1000-seed weight thus indicating that 1000-seed weight is majorly controlled by additive gene action. Having most of the traits controlled by additive and non-additive gene effects implies that substantial breeding progress could be made using breeding methods such as backcrossing and heterosis breeding techniques which exploit the two modes of gene action. This supports the work of Reddy *et al*. (2012) on the preponderance of non-additive gene action for several traits of okra.

The significant negative GCA effects for DTF observed for Clemson and Iwo Nla implied that these genotypes could possess useful genes for improvement of earliness in okra while Iwo Nla with significant positive GCA effect for NoF might be a good donor for genes associated with increased number of fruits and seeds. In the same vein, Clemson and Iwo Nla, with significant negative GCA for INL could be useful in developing new okra breeding lines with short internode and enhanced branching thereby improving yield. This result agress Atanu and Sabesan (2009) who reported significant GCA effects for DTF, NoF, NoR, LNT and FWT through diallel crosses in okra.

The significant and desirable SCA effects (negative for DTF and INL) observed for most measured traits among the 10 hybrids suggests the presence of favourable gene combinations for most horticultural traits of interest in this study. Thus, NH47-4 × LD88, NH47-4 × Clemson, and IK11 × LD88 with significant and negative SCA effects for DTF contains favourable gene combinations for earliness while NH47-4 × IK11, NH47-4 × Clemson, and LD88 × Iwo Nla might be harbouring gene combination associated with seed-increasing effects. Majority of the hybrids except NH47-4 × LD88 and IK11 × Iwo Nla have gene combinations in favour of LNT, FWT and 1000-seed weight. The above mentioned sets of hybrids could be deployed for heterosis breeding in favour of associated horticultural traits such as earliness and increased number of seeds. Furthermore, they can be intercrossed and advanced to generate breeding populations with a large gene pool that might be useful in identification and selection of new promising segregants. Similar results have been reported for okra by Oyetunde and Ariyo (2015), Reddy *et al.* (2012) and Anyaoha *et al.* (2021).

Significant and negative MPH and BPH is desired for DTF and INL improvement of earliness and increased number of branches respectively. The significant and negative BPH and MPH observed for these traits in hybrids: NH47-4 × LD88, NH47-4 × Clemson, IK11 × LD88, IK11 × Iwo Nla, LD88 × Clemson and LD88 × Iwo Nla could be exploited through heterosis breeding. Khanorkar and Kathiria (2010) and Prakash *et al*. (2019) reported similar findings for number of days to flowering in okra.

Selecting okra genotypes with tall plant architecture and short INL might lead to increased number of pods per plant in this study. Although none of the hybrids possessed desirable heterosis for PH and INL, a cross between IK11 × Clemson with significant positive MPH for PH, and IK11 ×LD88, IK11×Iwo Nla, LD88

× Clemson, and LD88 × Iwo Nla with significant and negative MPH and BPH for INL might produce tall multiple hybrids with short INL. Hybrid combinations NH47-4 × Clemson, IK11 × Iwo Nla and LD88 × Iwo Nla and IK11 × Clemson, LD88 × Clemson and Clemson × Iwo Nla displayed significant and positive BPH and MPH respectively for seed-increasing traits NoS, 1000-seed weight and NoR. It looka apparent from the findings of this study that high heterotic effects for measured traits might be due to the dominance nature of genes controlling considered traits. Reddy *et al*. (2012) explained that heterobeltiosis of more than 20% could offset the cost of hybrid seed. Thus, hybrids NH47-4 × Clemson and LD88 × Iwo Nla with BPH of 23.27% and 42.55% respectively for NoS could be useful resources to exploit heterosis for okra hybrid seed production. However, with the exception of NH47-4 × IK11, NH47-4 × LD88, and IK11 × LD88, majority of the hybrids exhibited the potential for use to enhance NoF per plant in okra.

The GYT biplot (Yan and Frégeau-Reid, 2018) used in this study allows genotype evaluation by graphically ranking genotypes based on their level in combining major traits of interest (such as increased number of fruits per plant) with other target traits. The single-arrow line passing through the biplot origin and the average yield-trait combination is called the average tester axis (ATA). The arrow points towards the higher genotype mean values across all NoF-trait combinations and serves the purpose of ranking genotypes based on superiority. The double-arrow line perpendicular to the ATA separates genotypes better than average (on the same side as the ATA arrow) from those poorer than average (on the opposite side of the ATA) and also indicates how balanced the trait profile of a genotype is as well as its strengths and/or weaknesses in terms of adaptation to specific traits. Genotypes placed close to ATA (with short projections to the double-arrowed line) have balanced trait profiles whereas those placed away from the ATA in either direction have obvious strengths and/or weaknesses. The polygon view of the biplot allowed visualization of the trait profiles of the hybrids such that the genotypes placed on a vertex had the largest values for the NoF-trait combinations placed within the corresponding sector.

Hybrid NH47-4 × Iwo Nla had the highest values for NoF-trait combinations indicating that they are top performers in combining NoF with the respective traits. On the other hand, LD88 × Clemson, NH47-4 × Clemson, IK11 × Iwo Nla, and Clemson × Iwo Nla had the highest values for INL showing that these hybrids were the best in combining NoF with the respective traits INL.

The ranking of the hybrids based on the NoF-trait combinations was as followed: NH47-4 × Iwo Nla> LD88 × Iwo Nla> Clemson × Iwo Nla ≥ IK11 × Iwo Nla> LD88 × Clemson> IK11 × Clemson > NH47-4 × Clemson > NH47-4 × IK11> IK11 × LD88> NH47-4 × LD88. The short vector lengths of the hybrids IK11 × LD88, NH47-4 × LD88, NH47-4 × Iwo Nla and IK11 × Clemson implied that these hybrids were the most stable across the various trait combinations.

In conclusion, the pooled analysis of variances successfully identified the extent of genetic variability among parents and hybrids. High significant differences among parents, crosses and parents vs. crosses for most traits indicated sufficient level of variability among the genotypes. The parental genotypes, Clemson and Iwo Nla, are promising sources of genes for earliness while Iwo Nla was promising as a gene donor towards improvement of NoS. The new promising hybrid combinations NH47-4 × LD88, NH47-4 × Clemson and IK11 × LD88 identified from this study could be exploited towards creating early maturing hybrid okra varieties while NH47-4 × Clemson, IK11 × Clemson, IK11 × Iwo Nla, LD88 × Clemson were identified as hybrids with useful heterotic patterns for seed-increasing traits. Promising hybrids NH47-4 × Iwo Nla and IK11 × Clemson that were stable and above-average for NoF-trait combinations and ranked high by the GYT biplot might be useful for okra genetic improvement programmes targeting increased number of fruit per plant over seasons in the region.

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Postharvest quality responses of pomegranate fruit (cv. Shishe-Kab) to ethanol, sodium bicarbonate dips and modified atmosphere packaging

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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Abstract: **Pomegranate fruit is very popular due to its high commercial importance and health benefits. This experiment aimed to evaluate the sensory quality, color, and biochemical properties (TSS, TA, TSS/TA, anthocyanin content and total antioxidant capacity) of pomegranate fruit under post-harvest treatments, included ethanol (EtOH), sodium bicarbonate (SBC), and different packaging. Experimental treatments included: 10% (v/v) EtOH, 1% (w/v) SBC, and the type of packaging (passive-MAP and vacuum). Fruit were then stored at 5±1°C and 90% relative humidity for ten weeks. The peel and aril color evaluations indicate that EtOH treatment and vacuum packaging (VP) improved the quality of pomegranate color by increasing *a** and decreasing *L**. These treatments made the skin color and aril color lighter and redder in pomegranate. In addition, the treatments reduced decay and maintained total soluble solids (TSS), and titratable acidity (TA). Interestingly, EtOH treatment improved fruit nutritional quality as it increased total antioxidant capacity and anthocyanin content by 20% and 50%, respectively, compared to the control. The sensory analysis indicated that treated fruit with EtOH and VP scored higher in taste, color, texture, and appearance, and showed the best acceptability from the panelists' viewpoint. In conclusion, EtOH and VP significantly improved pomegranate fruit quality during cold storage since preserved sensorial quality and bioactive compounds and reduced decay.**

1. Introduction

Pomegranate is mainly confined to the tropics and subtropics and grows well in arid and semi-arid climates. The edible portion of pomegranates (arils) is about 55 to 60% of the total fruit weight, and contains 80% juice and 20% seeds (Erkan and Kader, 2011). The fresh juice contains 85% water and 15% sugars, pectins, ascorbic acid, polyphenolic flavonoids, anthocyanins, and amino acids (Erkan and Kader, 2011). The amount of these compounds vary with pomegranate variety, maturity, and environmental and cultivation conditions. The statistics on acreage and production of pomegranate are not available with Food and

Agriculture Organization at the global level. However, the estimated global cultivated area of pomegranate is around three hundred thousand hectares, with the production of three million tones (Venkitasamy *et al*., 2019).

Pomegranate is classified as a non-climacteric fruit due to low respiration and ethylene production rates after harvest (Kader *et al*., 1984). Despite its non-climacteric nature, the fruit still undergoes both qualitative and quantitative losses during postharvest handling and storage, resulting in chilling injuries, husk scald, weight loss, and decay (Opara *et al*., 2015). Postharvest losses of fresh horticultural products occur from harvest and continue during the handling and storage period, estimated more than 20% of production in developed countries and about 40-50% in developing countries (Watkins, 2020). To reduce quantitative and qualitative losses during the supply chain and increase food availability, postharvest decay control is one of the significant factors to be considered. Because most of the commercial pomegranate cultivars are susceptible to chilling injury when they are stored at low temperatures less than 5°C, commonly held at 5°C or higher temperature (Moradinezhad *et al*., 2020). As a result, postharvest decay occurs by different fungi. Various fungicides have been used traditionally to control postharvest decay in fresh fruits. However, there is public concern about food safety regarding fungicides application in pre and postharvest stages (Tzatzarakis *et al*., 2020).

Chemical treatments should be compounds with known and minimal toxicological effects on mammals and impact on the environment. As substances that will be in contact with fresh produce, they should be affirmed as generally recognized as safe (GRAS) by the United States Food and Drug. Ethanol, carbonates, and bicarbonates belong to the GRAS group (Palou, 2018). Previous reports (Teksur, 2015; Droby *et al*., 2016; Dukare *et al*., 2019) focused on using environmentally friendly, effective, and safe alternative control methods to fungicides to reduce postharvest decay of fresh fruits. Safe inorganic compounds have shown antimicrobial activity (Deliopoulos *et al*., 2010). Successful control of postharvest decay in various fresh fruits was indicated using different safe chemical compounds applications such as ethanol (EtOH), sodium carbonate (SC), sodium bicarbonate (SBC), and ozone (Nunes, 2010). EtOH is a volatile organic compound with antimicrobial potential, widely used as a disinfectant. The positive effects of EtOH application have been indicated in fresh fruits like, grapes, peaches, oranges, nectarines, strawberries, apples, and Chinese bayberries (Dao and Dantigny, 2011). SBC is a common food additive that has been used in the food industry as a safe and effective chemical for controlling fungi growth as a bio fungicide. It is cheap, readily available, and a low risk of injury to the fresh fruit (Vilaplana *et al*., 2018).

The beneficial effects of modified atmosphere packaging (MAP) have been demonstrated on different fruits and vegetables. The results of various studies of MAP in different cultivars of pomegranate showed significant improvement in quality maintenance and extension of the storage life of fruit (Moradinehzad *et al*., 2018; Sahel *et al*., 2018; Venkataramudu *et al*., 2018; Candir *et al*., 2019; Moradinehzad *et al*., 2019, 2020). MAP also reduces the growth of pathogens and consequently postharvest decay (Pareek *et al*., 2015; Teksur, 2015; Rodriguez and Zoffoli, 2016; Ansarifar and Moradinezhad, 2021). Despite MAP has been widely applied to pomegranate; however, the literature review shows that no research focused on the effect of pre-storage EtOH or SBC dips and their combination with modified atmosphere packaging on postharvest decay control and quality attributes of the pomegranate fruit. Therefore, this study aimed to assess the efficacy of pre-storage EtOH or SBC dips and MAP on physiological responses and quality of pomegranate fruit cv. Shishe-Kab during postharvest storage.

2. Materials and Methods

Fruit preparation and treatments

About 200 fully mature pomegranate fruit cv. Shishe-Kab were harvested from a commercial orchard in South Khorasan province, Birjand, Iran, in October 2019. Pomegranate fruits were harvested and placed in carton boxes. A row of fruits was placed in each box so that the fruits would not be damaged. The fruits were transported to the Postharvest Lab of the University of Birjand, Iran, immediately after harvest. Uniform fruits (267-320 g) free of defects were selected and were then dipped in 200 ppm sodium hypochlorite solution for 1 min for surface disinfection. Before applying the treatments on fruits, five fruits were peeled and their juice was taken for initial analysis (Color properties, TSS, TA, TSS/TA, anthocyanin content and total

antioxidant capacity). Thereafter, fruits were dipped in chemical solutions including 10% (v/v) EtOH or 1% (w/v) SBC for 2 minutes. Control fruit were immersed in distilled water (20°C). All the chemicals used in this experiment were obtained from the Merck company (Germany). Fruit were then air-dried and placed into Low-Density Polyethylene (LDPE) bags (Carton Plast Co., Iran) (3 treatments × 2 packages × 3 replications) with 0.05 mm thickness (ten fruit per bag). Bags were sealed after removal of air by a vacuum pump to make vacuum packaging (VP) or sealed without removal of air (passive MAP) and then stored at 5±1°C and 85±5% relative humidity. Physico-chemical and sensory quality attributes of fruits were determined after 10 weeks of cold storage.

Fruit quality assessments

Color attributes. Aril and peel color of fruit were evaluated in all treatments. The color was determined in terms of L^*, a^*, and b^* values using a colorimeter (TES-135 A, country of manufacture Taiwan). Chroma and hue were obtained with the following equations (1 and 2):

$$\text{Chroma} = [a^{(0.5} +)b^{(0.5}]^2 \quad (1)$$

$$\text{Hue angle (h)} = \tan^{-1}(b/a) \quad (2)$$

Measurements were made at three different points on the skin of each fruit. Three fruits in each replicate were used.

Determination of total soluble solids (TSS), titratable acidity (TA), and TSS/TA ratio. To prepare the juice, we first separated the pomegranate arils from fruit' peel. Then pomegranate juice was prepared by hand pressure, and at the end it was filtered using a thin cloth. To prepare juice, three fruits selected from each replication. For higher accuracy in the experiment, the average of three samples was presented as one replication (juice was prepared separately from each fruit).

TSS in the extracted juice of each slice (one center section) was measured by a hand-held refractometer (RF 10, °Brix, 0-32%, Extech Co., USA). To measure titratable acidity, 5 ml of extracted fruit juice titrated with 0.1N sodium hydroxide. The TA was calculated as a percentage of citric acid. The TSS to TA ratio calculated by dividing TSS to TA in each replication of treatments.

Determination of anthocyanin content. The total anthocyanin content of juice determined by the pH-differential method using two buffer systems comprised of potassium chloride (pH 1, 0.025 M) and sodium acetate (pH 4.5, 0.4 M). One ml of juice sample mixed with 10 mL of buffer, and the absorbance (A) measured at 510 and 700 nm using a spectrophotometer (Unico 2100, China) (Wagner, 1979).

*Determination of total antioxidant capacity.*To determine the total antioxidant activity, DPPH radical inhibitor activity method was used. The DPPH radical-scavenging activity of the samples was evaluated according to the method described by Turkmen *et al*. (2005) dissolved in distilled water at different concentrations. Pomegranate juice samples were mixed with 1 mL of a freshly made methanol solution of DPPH radical (100 µM). The contents were vigorously incorporated and incubated at room temperature in the dark for 20 min, and the absorbance was read at 517 nm. Methanol solutions of tested extracts and DPPH were used as blank and control measurements, respectively. All experiments were carried out three times on two separate occasions. The percentage of total antioxidant activity (TAA) of the tested extracts was calculated according to the following equation (3).

$$\% \text{ radical scavenging activity} = [(\text{absorption control} - \text{absorption sample}) / \text{absorption control}] \times 100 \quad (3)$$

Sensory quality and decay percentage. Sensory evaluation of samples was done by a panel of ten trained members, based on a 5-point hedonic scale at the end of the cold storage period. In all treatments, aril was first isolated from the peel and the panelist evaluated and scored three fruits from each treatment. Mean scores was considered as one replication. After tasting each sample by the evaluators, we asked them to drink some water. All the treatments were randomized at room temperature (22°C), and panelists rated the appearance, taste, color, texture, and acceptance of pomegranate arils on a five-point scale, 1= the extremely bad, and 5= extremely good (3≤ acceptable) as described by Moradinezhad *et al*. (2018).

The decay evaluated visually during the storage time. Fruit were examined daily and considered infected when a visible lesion was observed (such as surface mycelia, slimy patches, bruises, and blemishes). Results expressed as the percentage of infected fruits. In fact, upon observing the first effects of decay on the fruit, that fruit considered as a percentage of decay in the whole box. In other words, in a package of ten fruits, if one fruit has decay symp-

toms, 10% of decay is reported for that box.

Statistical analysis

The recorded data were subjected to a two-way analysis of variance (ANOVA) with two factors, pre-storage treatments and packaging methods in three replications using the GenStat program (version 12, 2010, VSN International, Ltd., UK). LSD test at 1% level of probability ($P \leq 0.01$) was used to compare means of different treatments.

3. Results

There were no significant interactive effects of chemical treatments × packaging type on all evaluated traits (data not shown). Therefore, only the simple effects presented in the tables.

Aril and peel color attributes

The aril color analysis showed that the L^*, hue angle ($h°$), and chroma (C) values in all treatments decreased compared to the fruit at harvest, while a^* value increased (Table 1). As shown in Table 1, postharvest application of EtOH and SBC had a significant effect on L^* and a^* of aril. The highest L^* value after 10 weeks of storage was recorded in treated fruit with EtOH (20.2), and the lowest L^* obtained in control fruit (11.6). The highest a^* value (28.1) obtained from EtOH treatment, and the lowest (24.3) observed in control. However, EtOH and SBC treatments had no significant effect on b^*, chroma, and hue parameters. The results showed that treatments had a significant impact on the L^* and a^* parameters of pomegranate peel. In fact, these treatments caused to a lighter and redder both the peel and aril color.

As shown in Table 2, all the color parameters of aril and peel fruit were significantly different compare to fruit at harvest. Most of the parameters (except a^*) were reduced. In addition, the type of packaging had a significant effect on the L^* and a^* values. The highest L^* (18) and a^* (25.7) were obtained in vacuum-packed fruit. However, the type of packaging had no significant effect on b^*, hue

Table 1 - Effect of EtOH and $NaHCO_3$ dipping on color properties of pomegranate fruit (cv. Shishe-Kab) aril and peel after 10 weeks of cold storage at 5°C

Pre-storage treatments	Aril					Peel				
	*L**	*a**	*b**	*h°*	*C**	*L**	*a**	*b**	*h°*	*C**
At harvest	23.2±1.7 a	22.5±1.4 d	6.7±0.1 a	19.2±1.1 a	28.8±2.2 a	44.6±3.1 a	37.4±2.8 c	13.4±1.2 a	19.8±1.4 a	46.7±3.9 a
Control	11.6±0.8 d	24.3±2.0 c	7.6±0.8 a	16.7±0.6 b	26.2±1.7 b	35.4±3.4 c	43.6±2.7 b	15.1±1.9 a	18.7±1.2 a	45.7±3.7 a
Ethanol (10%)	20.2±1.9 b	28.1±1.6 a	7.4±0.8 a	17.7±1.3 b	25.0±1.8 b	39.4±2.9 b	45.4±4.5 a	14.3±1.0 a	18.1±1.9 a	46.4±4.1 a
SBC (1%)	18.7±1.2 c	26.1±0.9 b	6.8±0.4 a	17.9±1.2 b	25.3±1.9 b	39.2±3.1 b	43.2±2.8 a	16.9±1.5 a	19.3±1.1 a	46.9±3.4 a
Level of Sig.	**	**	NS	*	**	*	**	NS	NS	NS
LSD	5.46	6.52	3.62	4.65	6.52	5.04	5.02	5.47	4.21	4.57

Means ± SE followed by different letters in the same column for the same evaluated parameter are significantly different (P≤0.01) according to the LSD test.
SBC= Sodium bicarbonate.

Table 2 - Effect of different MA packaging on color properties of pomegranate fruit (cv. Shishe-Kab) aril and peel after 10 weeks of cold storage at 5°C

Packaging	Aril					Peel				
	*L**	*a**	*b**	*h°*	*C**	*L**	*a**	*b**	*h°*	*C**
At harvest	23.6±1.4 a	23.5±1.8 c	7.5±0.8 a	21.7±1.1 a	27.8±2.0 a	48.0±2.5 a	32.4±2.9 b	16.9±1.8 a	23.1±1.6 a	35.9±3.4 b
Passive MAP	16.3±1.3 c	24.4±1.9 b	6.8±0.5 b	20.5±1.9 b	23.0±1.8 b	40.3±2.8 b	44.2±3.8 a	14.8±0.9 b	20.9±1.9 b	47.8±3.5 a
Vacuum	18.0±2.1 b	25.7±2.1 a	6.1±0.9 b	20.4±1.7 b	25.4±1.4 b	37.7±3.7 b	44.0±3.4 a	15.2±1.5 b	18.7±1.2 b	46.9±3.9 a
Level of Sig.	**	**	*	**	**	**	*	**	**	*
LSD	4.46	5.32	1.45	6.06	5.32	4.12	4.10	4.38	5.95	3.73

Means ± SE followed by different letters in the same column for the same evaluated parameter are significantly different (P ≤ 0.01) according to the LSD test.

angle, and chroma of pomegranate aril (Table 2). After 10 weeks of cold storage, passive MAP and vacuum packaging had no significant effect on the peel color attributes of the pomegranate fruit.

Total soluble solids (TSS), titratable acidity (TA), TSS/TA ratio

The TSS and TSS/TA ratio in all treatments increased compared to fruit at harvest time. However, TA value in all treatments decreased compared to fruit at harvest (Table 3). Data analysis showed that the highest content of TSS and TSS/TA found in control (18.54 °Brix, and 18.05, respectively), and the lowest content obtained from treated fruit with EtOH (16.31°Brix, and 9.24, respectively). Also, the highest and lowest TA values obtained from EtOH (1.94%) and control (1.02%) respectively. In addition, the evaluation of MA packaging showed that the highest content of TSS and TSS/TA were related to passive MAP (respectively, 18.40 °Brix, and 16.78), and the lowest content obtained from vacuum packaging (respectively, 17.05°Brix, and 9.34). Also, the highest amount of TA observed in vacuum-packed fruit (1.94 %) (Table 4).

Anthocyanin content

According to Table 3, anthocyanin at harvest time was 21.51 mg L^{-1}. After 10 weeks of storage, its value in the control group was 23.19 mg L^{-1} (about 8% more than harvest time). While, anthocyanin content was higher (34.27 mg L^{-1}) in EtOH-treated fruits (about 40% higher than harvest time). Also, anthocyanin in the SBC treatment was significantly higher than in the control. However, the highest amount of anthocyanin was obtained from ethanol treatment after 10 weeks of storage, and the lowest amount was observed in control. As shown in Table 4, the anthocyanin content at harvest time was 22.41 mg L^{-1}. After 10 weeks of storage, the amount of anthocyanin significantly increased than harvest time. However, there was no significant difference between different packaging treatments.

Table 3 - Effect of EtOH and $NaHCO_3$ dipping on biochemical attributes of pomegranate fruit (cv. Shishe-Kab) after 10 weeks of cold storage at 5°C

	TSS (°Brix)	TA (%)	TSS/TA	Anthocyanin (mg l^{-1})	Antioxidant activity (%)
At harvest	15.74±0.98 d	2.42±0.03 a	7.21±1.82 d	21.51±2.03 d	61.7±5.24 c
Control	18.54±1.51 a	1.02±0.01 d	18.05±1.28 a	23.19±1.17 c	68.2±6.50 b
Ethanol (10%)	16.31±0.84 c	1.94±0.01 b	9.24±1.87 c	34.27±1.41 a	77.3±5.74 a
SBC (1%)	17.72±1.22 b	1.26±0.04 c	13.28±1.09 b	26.88±1.26 b	70.9±6.01 b
Level of Sig.	**	**	**	**	**
LSD	0.96	0.12	2.13	4.07	9.42

Means ± SE followed by different letters in the same column for the same evaluated parameter are significantly different (P≤0.01) according to the LSD test.
SBC= Sodium bicarbonate.

Table 4 - Effect of different MA packaging on biochemical attributes of pomegranate fruit (cv. Shishe-Kab) after 10 weeks of cold storage at 5°C

Packaging	TSS (°Brix)	TA (%)	TSS/TA (°Brix/%)	Anthocyanin (mg l^{-1})	Antioxidant activity (%)
At harvest	16.02±1.78 c	2.28±0.01 a	7.13±1.48 c	22.41±2.07 b	63.5±4.54 b
Passive MAP	18.40±1.28 a	1.12±0.04 c	16.78±0.98 a	28.63±1.35 a	79.1±4.91 a
Vacuum	17.05±1.47 b	1.94±0.02 b	9.34±1.04 b	29.84±1.09 a	77.2±5.20 a
Level of Sig.	**	**	**	*	**
LSD	0.78	0.09	1.74	3.33	7.68

Means ± SE followed by different letters in the same column for the same evaluated parameter are significantly different (P≤0.01) according to the LSD test.

The total antioxidant capacity

The antioxidant activity of pomegranate fruit was measured by neutralizing free radicals. In general, in all treatments antioxidant activity was higher compared to fruit at harvest (Tables 3 and 4). The highest and lowest antioxidant activity recorded from EtOH and control treatments, respectively. However, SBC had no significant effect on antioxidant activity. Also, there was no significant difference between vacuum packaging and passive MAP.

Sensorial quality

The appearance of the product is effective in consumer preference. Figure 1 A, and B shows the effect of treatments on the scores of acceptance, taste, texture, appearance, and color of arils during storage at 5°C. EtOH treatment had the best sensory quality as scored higher by panelists. However, SBC treatment also achieved acceptance scores. Besides, sensory properties were improved in vacuum-packed fruit. Interestingly, Passive MAP fruit scored acceptable quality by panelists.

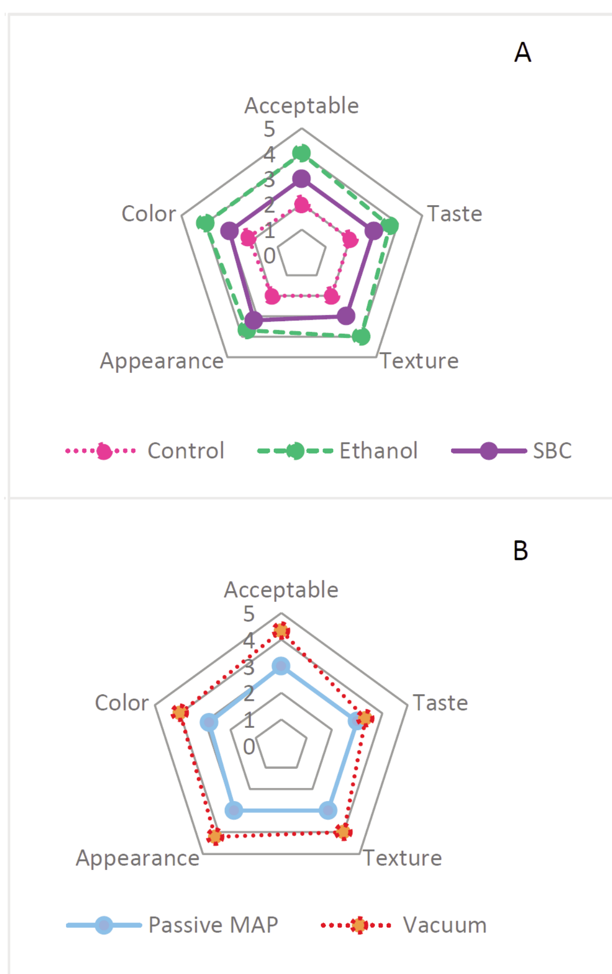

Fig. 1 - Effect of different chemical treatments (A) and MA packaging (B) on sensorial assessments of pomegranate arils (cv. Shishe-Kab) after 10 weeks of cold storage at 5°C. SBC0= Sodium bicarbonate.

Decay

As shown in Figure 2, EtOH treatment significantly controlled fruit decay. The percentage of fruit rot under EtOH treatment was 12.57%, while in control samples was 36.21% (3-fold higher than EtOH). Also, vacuum packaging reduced the fruit decay more effectively than passive MAP.

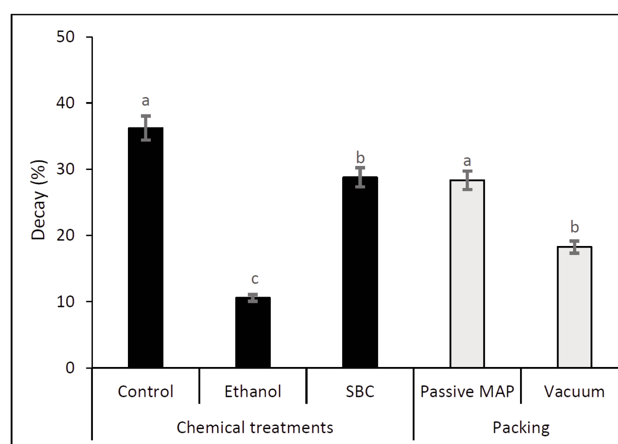

Fig. 2 - Effect of different chemical treatments and MA packaging on decay of pomegranate arils (cv. Shishe-Kab) after 10 weeks of cold storage at 5°C. Error bars represent the error (percentage). Identical letters mean there is no significant difference between them, at P≤0.01 (LSD). SBC= Sodium bicarbonate.

4. Discussion and Conclusions

The L^* represents lightness changes from 0, which has no lightness (absolute black) to 100, which is maximum lightness (absolute white) (Morales *et al.*, 2020). a^* varies between green and red, negative values of a^* indicate green colors, and positive values represent red colors. b^* varies between blue and yellow, negative values of b^* indicate blue colors and positive values represent yellow colors. *Hue* is the color tone, or color name of a color. *Chroma* is the amount of saturation of a color. Colors of high chroma are said to be clear, bright or brilliant. Dull (pastel) colors have a low chroma (Morales *et al.*, 2020).

Jin *et al.* (2013) reported that the application of

EtOH on melon sweet fruit preserves the color and freshness of the fruit. They showed that after 16 days of storage, the highest L^* value related to EtOH 0.5 ml treatment, which is inconsistent with the results of the present report. The results showed that during the storage period, the L^* of fruit peel decreased or darkened, which indicates a decrease in the peel quality compared to harvest time. The researchers showed that the synthesis of ethylene, followed by senescence, causes the enzymes associated with oxidative reactions and ultimately leads to darkening (low L^* value) of the color (Hasan *et al*., 2018; Morales *et al*., 2020). In line with the findings of Abdi *et al*. (1998), our results showed that the highest L^* value obtained in EtOH treatment, maybe due to the inhibition of ethylene biosynthesis which delaying fruit senescence and changes of senescence-related pigments. The results of this study on fruit color characteristics are also in line with the findings of Ponzo *et al*. (2018) on guava fruit. Moradinezhad and Dorostkar (2020) found that fresh jujube fruit under vacuum packaging had a higher L^* value. They stated that vacuum packaging might inhibit enzymatic reactions and delay the darkening of fruit color. Similar results were reported by Moradinezhad *et al*. (2019) on pomegranate fruit. They showed that passive MAP and vacuum packaging did not have a significant effect on the pomegranate peel color attributes. Therefore, it concluded that the treatments used in the present study did not have adverse effects on the peel of pomegranate fruit and did not reduce the marketable value of the fruit.

The increase in a^*, is related to the increased biosynthesis and accumulation of anthocyanin pigments, which are responsible for the intense red color of ripe pomegranate fruit (Lyu *et al*., 2020). Moradinezhad *et al*. (2019) reported that the a^* value of pomegranate peel in vacuum-packed fruit was higher than the control. They stated that vacuum packaging modified the atmosphere around the fruit, extending its shelf-life and improving the color of the fruit, which is similar to the results of the present study. Vacuum-packed fruits had the lowest respiration rate, probably because of reduced O_2 concentration and ethylene removal from the intercellular spaces (Rana *et al*., 2018).

In most fruits and vegetables, sugar makes up the main component of TSS, which is thus a reasonable indicator of the values sugar levels (Huang *et al*., 2021). As the results showed, the TSS content of pomegranate fruit increased slightly after 10 weeks of storage compared to harvest time, because pomegranate fruit is classified as a non-climatic fruit. Generally, most non-climacteric fruits have a minor change on TSS during the storage period, mainly due to low starch accumulation content during growth and development. However, one of the most significant changes that occur during fruit ripening is the hydrolysis of starch to sugar, which changes the taste and texture of the product. Likely, any treatment that delays maturation reduces soluble solids content. Previous studies have shown that EtOH inhibits the production and action of ethylene (Podd and Van Staden, 1998) as a ripening hormone. EtOH prevented the conversion of ACC to ethylene (Podd and Van Staden, 1998). This compound reduces ethylene synthesis by reducing ACC synthase activity (a key enzyme in ethylene synthesis). In the present study, we found that 10% EtOH preserves TSS of the pomegranate fruit. Liu *et al*. (2019) showed that post-harvest application of 50% EtOH in cassava for 12 and 24 hours reduced TSS compared to control samples. This decrease in TSS is probably due to a reduction in the respiration rate. A similar result has been reported on blueberry (Ji *et al*., 2021).

The organic acids present in foods influence the flavor, color, microbial stability keeping quality (Jawad *et al*., 2020). Citric acid is the predominant acid in pomegranate fruit (Tozzi *et al*., 2020). However, inorganic acids such as phosphoric and carbonic acids (arising from carbon dioxide in solution) often play an important and even predominant role in food acidulation (Jawad *et al*., 2020). In plant tissue, EtOH and acetaldehyde can be converted to each other. Therefore, external application of EtOH increases the amount of acetaldehyde inside the fruit tissue, which causes production of more EtOH in the presence of oxygen (Podd and Van Staden, 1998). But, in the process of EtOH production, carbon dioxide is also produced as a byproduct (Podd and Van Staden, 1998). Increased carbon dioxide inhibits ethylene synthesis and reduces the respiration rate (Park *et al*., 2021). As carbon dioxide increases, the synthesis of sugars (especially glucose) also increases, and also organic acids are not consumed in cellular respiration (Park *et al*., 2021). Therefore, the use of EtOH causes the accumulation of organic acids, as a result, increasing TA. According to the proposed mechanism, we found that the post-harvest application of 10% EtOH preserves the TA in pomegranate fruit. Our finding is in consistent with the report of Shao *et al*. (2020) on wampee fruit.

The results also indicated that the lowest TSS/TA ratio obtained from EtOH treatment and vacuum packaging. It can be concluded that these treatments inhibit respiration rate and maintain the acidity of the pomegranate fruit. Selcuk and Erkan (2016) in Turkey found similar results on sweet pomegranate fruit. The reduction of TSS /TA ratio using MAP indicates a delay in the ripening of pomegranate fruit. This results are consistent with the findings of Venkatachalam and Meenune (2015) on longkong fruit.

Anthocyanins are commonly found in plant cell vacuoles in the form of glycosides (i.e., the combination of anthocyanins with simple sugars such as glucose, galactose, etc.) (Podd and Van Staden, 1998). The presence of sugar in the structure of anthocyanins makes them soluble in water. Interestingly, we found that 10% EtOH treatment increased the anthocyanin content by 50% compared to the control. Tzortzakis and Economakis (2007) showed that post-harvest application of EtOH (vapor) to tomatoes increases glucose, fructose and total soluble solids values. They found the increase in sugar was due to the weight loss of the tomatoes. Our findings support the above-mentioned study. Similarly, on bayberries, Wang *et al*. (2010) found that EtOH (22.32 µmol L^{-1}) increased anthocyanins. They stated that this increase in anthocyanin content was related to increased antioxidant activity. Liu *et al*. (2019) showed that treatment of 50% EtOH for 24 hours on cassava was able to increase the anthocyanin content by about 14% compared to control. They stated that this increase in anthocyanin content could be related to the effect of EtOH on reducing free radicals and increasing the activity of antioxidant enzymes. Similar results were reported by El Kereamy *et al*. (2002) on the grapes and Huang *et al*. (2015) on the blueberry. As presented in Table 4, the type of packaging did not affect the anthocyanin content of pomegranate fruit.

An unavoidable consequence of aerobic metabolism is reactive oxygen species (ROS). Environmental stresses lead to enhanced generation of ROS in plants due to disruption of cellular homeostasis (Wang *et al*., 2021). All ROS are highly harmful to organisms at high concentrations. When the level of ROS exceeds the defense mechanisms, a cell is in a state of “oxidative stress” (Tang and Vashisth, 2020). Plant possess complex antioxidative defense systems comprising of non-enzymatic and enzymatic components to scavenge ROS (Wang *et al*., 2021). The antioxidants are substances capable of delaying or inhibiting oxidation processes (Hudson, 2012).

It has been shown that pomegranate juice, and even fermented pomegranate juice have high antioxidant activity (Li *et al*., 2006). These activities may be related to diverse phenolic compounds present in pomegranate juice, including punicalagin isomers, ellagic acid derivatives and anthocyanins (delphinidin, cyanidin and pelargonidin 3-glucosides and 3,5-diglucosides). These compounds are known for their properties in scavenging free radicals and inhibiting lipid oxidation in vitro (Li *et al*., 2006). Our results showed that postharvest use of EtOH maintained the antioxidant activity of pomegranate fruit. In line with these results, Wang *et al*. (2015) showed that EtOH (300 µl L^{-1}) increased H_2O_2 (a type of free radical). They stated that due to the balance between free radicals and antioxidants, the antioxidant content of loquat fruit increased. Natural antioxidants may exhibit one or more of the following roles: free radical scavenger, reducing agent, and quencher of singlet oxygen formation (Kim *et al*., 2007). Moreover, anthocyanidins also have the potent antioxidant capacity and possible protective effects on human health (Kim *et al*., 2007). Anthocyanins have greater antioxidant activity than either vitamin C or E (Kim *et al*., 2007). These reports are in accordance with the results of anthocyanin content in the present study, which indicate the relationship between antioxidant activity and anthocyanin content in pomegranate aril.

Suzuki *et al*. (2004) showed that broccoli packaged with EtOH pads in a perforated film maintained their green color longer than control. Lurie *et al*. (2006) suggested EtOH as a promising alternative to SO_2 in grapes since it prevented the occurrence of quality decay associated with loss of freshness, glossy appearance, and browning. Similar to our results, Liu *et al*. (2012) reported that EtOH (6 ml/kg of fruit weight) improved the sweet melons sensory properties. They stated that the enhanced sweet melons' sensory properties might be more dependent on the EtOH treatment than the process of ripening and senescence. As mentioned before, pomegranate fruits are harvested during commercial maturity, so they have the highest sensory and taste quality at harvest time. According to Table 3, TSS and TSS/TA ration in ethanol treated fruit changed less than harvest time (compared to control and SBC). Therefore, ethanol treatment preserved the taste of treated fruit. In addition, the results of Table 3 in terms of TSS and TSS/TA ratio are consistent with the results of evalua-

tors. Also, as shown in Figure 1, vacuum packaging better retained the taste of pomegranate fruit, which is in line with the results of TSS, and TA in the present study. Similar results have been reported on the effect of vacuum packaging on preserving the sensory properties of jujube (Moradinezhad and Dorostkar, 2020) and litchi (Shah and Nath, 2006) fruit.

Researchers speculate that EtOH could kill the mitochondrial inner membrane of fungal spores, thereby preventing the spread of infection in the fruit (Gabler *et al*., 2004). Similarly, in a recent study by Ji *et al*. (2019) the effect of EtOH on the physical properties of blueberries investigated. They found that EtOH treatment (1000 µl L^{-1}) significantly reduced fruit contamination (rotting was observed about 4% in EtOH treatment and 40% in control). This reduction in EtOH-induced decay may be due to the preservation of cell membranes in treated samples. Since low concentrations of EtOH can lower the temperature at which phospholipids undergo a phase change (Rowe, 1983), the increases in the spore mortality and decay control following the addition of EtOH may have resulted from a lowering of the phase-change temperature of mitochondrial membranes of the spores under these conditions (Margosan *et al*., 1997). Also, possibly external application of EtOH has been shown to increase acetaldehyde produced by the alcohol dehydrogenase. Acetaldehyde directly attacks pathogens. EtOH has also been shown to increase resistance to other environmental stresses (such as chilling in cucumber) in addition to controlling diseases (especially fungal) (Frenkel and Erez, 1996). It had been proven that low oxygen in vacuum treatment significantly decreased ethylene production (Min *et al*., 2019). On the other hand, a treatment that reduces ethylene synthesis has a good effect on infection control (Min *et al*., 2019). Low ethylene production in a vacuum treatment may result in lower cell wall enzyme activity and cell integrity maintenance, considering the fact that cell wall enzymes are activated by ethylene. This will probably help maintain the strength of the cell wall and reduce decay (Ntsoane *et al*., 2019). Similar results were obtained by Moradinezhad and Dorostkar (2020) on fresh jujube fruit.

The results of presented in this report show that the use of EtOH (10%) or vacuum packaging alone minimize decay, and maintain color, total soluble solids and titratable acidity of pomegranate fruit during storage. Also, these treatments increased biochemical properties such as anthocyanin content and antioxidant capacity and improved sensory quality. Overall, it can be concluded that EtOH has the potential to control decay, enhance antioxidant systems, and extend the shelf-life of the pomegranate fruit.

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Effect of chemical and biological fertilizers on the morphology and yield of safflower and soybean under monoculture and intercropping

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Key words: Biofertilizer, grain yield, number of grains per plant, plant height, urea fertilizer.

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Abstract: Intercropping and biofertilizers application are the most important agricultural methods for moving towards minimizing the risks of agricultural production and increasing production efficiency. Consequently, this experiment was conducted at one year and in 2019. A factorial set of treatments was arranged within randomized complete block design (RCBD) with three replications to investigate the effect of different planting ratios with safflower and soybean (sole cropping, 30:100 soybean to safflower ratio, 60:100 soybean to safflower ratio and 90:100 soybean to safflower ratio) and nutrient levels (100% urea fertilizer, 100% biofertilizer and the combined application of urea and biofertilizer) on growth and yield of these crops. The results of this study indicated that intercropping patterns had the highest plant height, number of grains per plant, biological and grain yields. In addition, the means of the number of heads per plant and the number of grains per head in safflower and the weight of 1000 grains in soybean were increased as intercrops were grown. Maximum of grain number per plant in safflower, leaf number per plan in soybean and biological and grain yields in both crops were attained in urea + biofertilizer. In all of intercropping patterns the values of LER (land equivalent ratio), RVT (relative value total) and RCC (relative crowding coefficient) was more than one, indicating an advantage from intercropping over sole crops.

1. Introduction

Intercropping, as a multiple cropping system, has been used by farmers for many years in various ways and in various countries and has acted as a very significant role in sustainable agriculture (Zhang and Li, 2003) and widely practiced for enhanced production and nutrient acquisition advantages (Ahmed *et al*., 2020). Many studies have shown the effect of legumes on growth increase, potassium (K), phosphorus (P) and nitrogen (N) uptake, and the yield of intercropped plants compared with sole cropped plants (Tosti *et al*., 2010; Piri *et al*., 2011; Raei *et al*., 2020). The

various benefits of an oilseed legume intercropping system-mainly intercropping legumes (soybeans) with oilseeds (safflowers) take in more skillful use of nutrients, available resources and sunlight, enhance yield and the improve land equivalent ratios (Srinivasarao *et al*., 2012).

Soybean (*Glycine max* L. Merill) is one of the most important food legumes in Iran and other parts of the world. It has potential of fixing atmospheric nitrogen besides meeting its own nitrogen requirement and serves as a viable and low cost medium for soil fertility improvement (Muoneke *et al*., 2007). It is considerable as an important edible oil grain for human alimentation and is worldwide planted on approximately 80 million hectares (FAO, 2020). Safflower (*Carthamus tinctorius* L.) is a prospective oilseed crop because it yields 32-40% seed oil. Safflower oil is widely utilized in industry, mainly for edible and dying purposes. Owing to its considerable drought tolerance compared with other oilseed crops, safflower is usually cultivated in Iran where drought stress is a major restriction in the field. Safflower is a deep-rooted annual crop that can be grown in rotation with other species (La Bella, 2019). Nitrogen is very important for its growth and yield (Leghari *et al*., 2016), and the nitrogen supply is therefore essential to produce a high yield from this crop. By contrast, the soybean is a legume that can fix nitrogen and release nitrogen compounds into the soil and so intercropping of these species is probably a suitable field management strategy.

A recent trend in sustainable global production is chemical fertilizers being replaced by biofertilizers. Bio-fertilizer, represent a specific complex of microorganisms which enable the movement of nutrients from soil to plants through biological process such as N fixation and solubilization of rock phosphate (Abou-Khadrah *et al*., 2000). These fertilizers are found to have a positive contribution to soil fertility, resulting in an enhancement in crop yields without causing any environmental, water or soil pollution hazards (Timmusk *et al*., 1999; Daiss *et al*., 2008). This suggested that the yield to components were increasing. It was reported the nitrogen and phosphate biofertilizer applications have many important benefits and decrease the inputs of production because of cost deduction compared to chemical fertilizers which increased biological yield. In some studies, it was clearly revealed that biofertilizer application resulted in high productivity for safflower (Mirzakhani *et al*., 2009; Seyed Sharifi, 2012).

Using biofertilizer and selection of the best microbial strains have vital role when integrating human society with vulnerable ecosystems. Biological fertilizers, which are called biofertilizers, may be used in a way of to maintain soil fertility and guarantee soil improvement. Biofertilizers are products containing living cells of different types of microorganisms, which have the ability to convert important nutritional elements (N, P …) form unavailable to available from through biological process such as nitrogen fixation and solubilization of rock phosphate. Biofertilizers differ from chemical and organic fertilizers in that they do not directly supply any nutrients to crops and are cultures of special bacteria and fungi. Some microorganisms have positive effects on plant growth promotion, including the plant growth promoting rhizobacteria (PGPR) such as *Azospirillum* spp., *Azotobacter* spp., *Pseudomonas fluorescens*, and several Gram positive *Bacillus* spp. (Sivasakthi *et al*., 2014).

Most studies have focused on legume-cereal intercropping as a productive and sustainable system, while intercropping systems such as soybean-safflower have rarely been evaluated. Thus, the present research was carried out to: 1) study the effect of chemical and biological fertilizers on some morphological traits and yields of safflower and soybean mono and intercropping system. 2) to evaluate the influence of cropping system on soybean and safflower performance and finally 3) to investigate the interaction between cropping system and fertilizer.

2. Materials and Methods

Site description

Field experiment was conducted during 2019 growth season at the Heris, East Azerbaijan Province, Iran (Latitude 38°25’ N, Longitude 47°12’ E, Altitude 1850 m above sea level with the mean annual rainfall of 315.2 mm). Some physical and chemical properties of farm soil (0-30 depth) and means of maximum and minimum temperatures and rainfall during the work in 2019 are shown in Table 1.

Experimental design and treatments

In this experiment a factorial set of treatments within randomized complete block design (RCBD) with three replications was arranged. Factors were cropping patterns (sole cropping’s of safflower and soybean, intercropping of safflower/soybean with

Table 1 - Some physical and chemical properties of farm soil (a), and means of maximum and minimum temperatures and rainfall (b) during the work in 2019

Depth (cm)	EC (ds/m)	PH	Organic Carbon (%)	N (%)	P (ppm)	K (ppm)	Sand (%)	Silt (%)	Clay (%)	Soil type
0-35	1.42	8.17	1.29	0.12	51.85	2085	37	50	13	Silty loam

Month	Temperature (°C)	Rainfall (mm)
April	5.9	82.2
May	11.4	28.3
June	18.6	22.5
July	22	0.3
August	22.8	1.7
September	18.6	2.7

the ratios of 30:100, 60:100 and 90:100 soybeans to safflower and nutrient levels (100% recommended urea fertilizer, 100% biofertilizer and 50% biofertilizer + 50% urea. More in details, safflower cultivar Safe and soybean cultivar William were used. The amount of urea given in 100% treatment was 50 kg/ha. The biofertilizer contained Barvar 1 (contains free living nitrogen fixing bacteria) and Barvar 2 (contains phosphate dissolving bacteria) and was used as seed inoculation before the sowing the seeds. The biofertilizers was prepared by Zist Fanavar Sabz company, Iran. Optimum sowing density of soybean and safflower in mono cultures were 50 and 40 seeds per m^2, respectively.

Measurements

Plant height of two crops. At maturity stage, 5 plants of the middle part of each plot were harvested and plant height (by meter) were determined.

Leaf number per plant of safflower and soybean. Leaf number per plant was measured by hand at maturity stage.

Plant biomass of safflower and soybean. To determine plant biomass, 5 plants were harvested from middle part of each plot with considering marginal effect, then were dried in an oven at 75°C for 48 hours. Finally, plant biomass per unit area were determined.

Yield and yield components of safflower and soybean. At final ripening 5 plants were harvested and head number per plant, grain number per head, grain number per plant and 1000 grains weight were determined in safflower plants. For determine the grain yield an area equal to 1 m^2 was harvested from middle part of each plot considering marginal effect grain yields per unit area were determined. Also, in soybean at maturity stage 5 plants from each plot were harvested and pods per plant, grain number per pod, grain number per plant and 1000 grains weight were determined. Grain yield per unit area was determined by threshing all the plants in 1 m^2 of the plots.

Evaluative indices of intercropping

Land equivalent ratio (LER). Land equivalent ratio (LER), as an agronomic index, indicates the efficiency of intercropping in using the environmental resources compared with mono cultures (Mead and Willey, 1980). The value of unity is the critical value. When the LER is greater than one, the intercropping improves the growth and yield of the cultivars. The LER was calculated as:

$$LER = (Ysa_i/Ysa_m) + (Yso_i/Yso_m)$$

Where Ysa_m and Yso_m are the yields of safflower and soybean, respectively, as sole crops and Ysa_i and Yso_i are the yields of safflower and soybean, respectively, as intercrops.

Relative value total (RVT). Relative value total (RVT) as an economic index proposed by Schultz *et al.* (1982). This index is widely used now and has been used by many researchers. The RVT was calculated as:

$$RVT = (aP_1 + bP_2)/aP_1$$

Where a is the key product price, b is a secondary product price, p_1 is the main types yield and p_2 is the secondary species in the mixture. If the RVT is greater than one, it's indicating the intercropping advantage. If this index is smaller than one, it's indicating that monoculture would prefer intercropping. The critical value of RVT is one.

Relative Crowding Coefficient (RCC). RCC is a measure of the relative dominance of one species over the other in a mixture (De Wit, 1960). The RCC was calculated as:

$$RCC = (Ysa_i/Ysa_m)/(Yso_i/Yso_m)$$

Where Ysa_m and Yso_m are the yields of safflower and soybean, respectively, as sole crops and Ysa_i and Yso_i are the yields of safflower and soybean, respectively, as intercrops.

If RCC= 1, the amount of crop in the mixture will be equal to monocropping. Also, if RCC<1 indicates that the amount of the product in the mixture has decreased relative to sole crop and if RCC>1, the yield of the mixture is higher than that of pure stand of crops and the mixing is beneficial.

3. Results

Analyses of variance is shown in Table 2. In safflower plants showed significant effects of cropping pattern on plant height, head number per plant, grain number per head, grain number per plant, 1000 grains weight and biological and grain yields. Also, the effect of fertilizer factor for grain number per plant, 1000 grains weight and biological and grain yields were significant. The interactions of cropping pattern × fertilizer was only significant for 1000 grains weight. For soybean plants, analysis of variance showed significant effects of cropping pattern on plant height, grains number per pod and per plant, 1000 grains weight, biological and grain yields. Also, leaf number per plant, grain number per pod, biological and grain yields were significantly affected by fertilizer factor.

Compared with the sole cropping of safflower and soybean, intercropping patterns had the higher plant height, grain number per plant, biological and grain yields. However, there was not observed significant difference between sole cropping and 90/100 soybean/safflower ratio for soybean traits. Similarly, the means of head number per plant and grain number per head in safflower plants and also 100 grains weight in soybean plants were increased in intercropped patterns compared with pure cultivation (Table 3).

Maximum of grain number per plant in safflower, leaf number per plan in soybean and biological and grain yields in both of these plants were attended in urea + biofertilizer. However, there was no significant differences between 100% biofertilizer and 50% urea + 50% biofertilizer in biological yields of two crops, as same as leaf number per plant in soybean (Table 4).

Maximum of 1000 grains weight of safflower in different cropping patterns was observed in cropping

Table 2 - Analysis of variance of the agronomic traits in safflower and soybean plants under different cropping patterns and fertilizer treatments

Source	df	Mean square							
		Plant height	Leaf number per plant	Head number per plant	Grain number per head	Grain number per plant	1000 grains weight	Biological yield	Grain yield
					Safflower (Carthamus tinctorius L.)				
Replication	2	35426	3326	0.756	0.562	2418347	5477	944302.4	928992
Cropping pattern	3	705.209 *	11.273 NS	3.645 *	18.465 **	12574.412 **	74.811 **	8603907.64 *	5528674.70 **
Fertilizer (F)	2	55.243 NS	22.166 NS	1.441 NS	5.384 NS	7400.101 *	28.189 **	8379561.03 *	3426726.11 **
C × F	6	134.942 NS	7.165 NS	1.109 NS	3.5 NS	1464.802 NS	16.372 **	2104488 NS	722194.6 NS
Error	22	207923	11704	0.946	1.92	1495934	4058	2377343	510591.7
Cv %	-	19.34	18.72	12.27	6.88	14.43	5.98	16.26	19.49
					Soybean (Glycine max L.)				
		Plant height	Leaf number per plant	Pods per plant	Grain number per pod	Grain number per plant	100 grains weight	Biological yield	Grain yield
Replication	2	17606	1245	2462	0.022	6611	0.801	147657.5	34858.45
Cropping pattern	3	76.843 **	8.261 NS	6.223 NS	0.037 *	49.205 *	6.429 **	28566822.68	4661478.68 **
Fertilizer (F)	2	28.492 NS	15.208 *	0.984 NS	0.212 **	44.766 NS	1.672 NS	1547677.03 *	511204.56 **
C × F	6	31.105 NS	0.656 NS	4.983 NS	0.039 **	32.575 NS	1.326 NS	631704 NS	14713.6 NS
Error	22	13331	3369	2584	0.01	13146	1174	414173.6	57485.11
Cv %	-	5.88	15.35	12.52	4.17	12.07	5.84	14.4	12.66

NS, * and **: non-significant and significant at p≤0.05 and p≤0.01, respectively.

Table 3 - Means of the agronomic traits in safflower and soybean plants under different cropping patterns

Traits	Plant height (cm)	Head number per plant	Grain number per head	Grain number per plant	Biological yield (kg/ha)	Grain yield (kg/ha)
			Safflower			
Pure cultivation	63.54 b	7.044 b	18.26 c	217.6 b	8107 b	2697 b
30:100	83.11 a	7.933 ab	21.69 a	295.9 a	10330 a	4423 a
60:100	80.10 a	8.533 a	20.59 ab	296.8 a	9999 a	4166 a
90:100	71.41 ab	8.189 a	20.08 b	261.6 a	9503 ab	3382 b
			Soybean			
Traits	Plant height (cm)		Grain number per plant	100 grains weight (g)	Biological yield (kg/ha)	Grain yield (kg/ha)
Pure cultivation	58.82 b		26.87 b	17.49 b	5654 a	2345 a
30:100	65.87 a		32.46 a	18.71 a	1983 c	912.7 c
60:100	62.51 ab		30.74 a	19.54 a	4379 b	1807 b
90:100	61.38 b		30.07 ab	18.5 ab	5858 a	2510 a

Different letters in each column indicate significant difference at $p \leq 0.05$ (Duncan test).

Table 4 - Means of the agronomic traits in safflower and soybean plants under different fertilizer treatments

Traits	Grain number per plant	Biological yield (kg/ha)	Grain yield (kg/ha)
	Safflower		
100% urea fertilizer	252 b	8831 b	3358 b
100% biofertilizer	255.4 b	9194 ab	3359 b
50% Urea + 50% biofertilizer	296.6 a	10430 a	4284 a
	Soybean		
Traits	Leaf number per plant	Biological yield (kg/ha)	Grain yield (kg/ha)
100% urea fertilizer	10.73 b	4122 b	1706 b
Urea + biofertilizer	12.95 a	4839 a	2115 a

Different letters in each column indicate significant difference at $p \leq 0.05$ (Duncan test).

system of 30/100 (soybean/safflower) with integration application of 50% urea + 50% biofertilizers. Minimum of this trait was related to monoculture and biofertilizer (Fig. 1a). Additionally, it was shown that the entrance of soybean plant to intercropping patterns led to an increase for 1000 grains weight in the ratio. Grain number per pod in soybean plants affected by cropping patterns and fertilizer treatments and significantly, maximum grain number per pod in different cropping patterns was observed in intercropping with 60 to 100 soybean to safflower ratio and urea + biofertilizer treatment (Fig. 1b).

Evaluation of intercropping efficiency of treatments indicated high land equivalent ratio value (LER >1) in all intercropping patterns which indicate those treatments produced biomass more efficiently than monocropping. Maximum of LER, relative value total (RVT), and relative crowding coefficient (RCC) were achieved in 60/100 soybean/safflower ratio (Table 5).

4. Discussion and Conclusions

Some important benefits of intercropping in this research are increasing in plant performance such as plant height, grain number, 1000 grains weight, biological yield and production of grain yield per unit area compared to sole cropping (Table 3), due to the effective use of resources, including water, nutrients and solar energy (Nasri *et al*., 2014). This is probably due to the fact that in intercropping system plants can achieve a better absorption. Intercropping is pre-

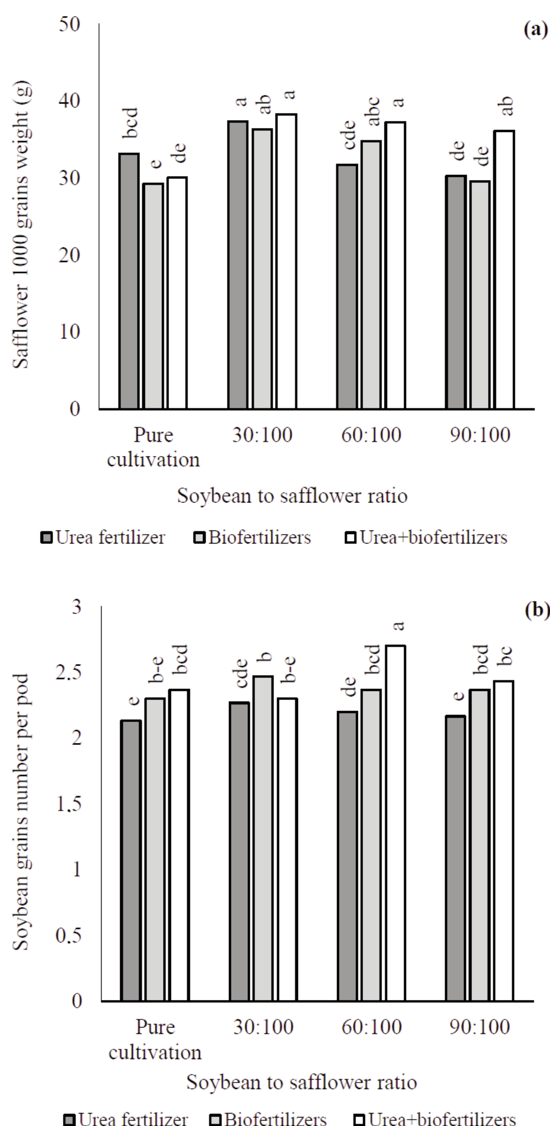

Fig. 1 - Means of safflower 1000 grains weight (a) and soybean grains number per pod (b) for interaction of cropping pattern × fertilizer treatments.

ferred to sole cropping as a result of superior yield due to better absorption of resources, and this is especially realized when legumes are used (Sachan and Uttam, 1992), because they improve soil fertility due to increased nitrogen fixation (Manna *et al.*, 2003). Intercropping of legumes (soybean) and Asteraceae (safflower) families results in increased crop yield (Table 3), maximized resource consumption and enhanced productivity of cultivation system (Singh Rajesh *et al.*, 2010). Interspecific interaction between species in the rhizosphere can also affect the nutrient availability and uptake in intercropping (Li *et al.*, 2010). Light, water and nutrients may be more completely absorbed and converted to crop biomass by intercropping. This is a result of differences in the competitive ability for growth factors between intercrop components (Amini *et al.*, 2013).

Combined application of urea + biofertilizer significantly improved the grain number per plant in safflower and leaf number per plant in soybean plants. Also, biological and grain yields of two crops were significantly increased by this treatment (Table 4). Nitrogen is a chemical fertilizer that has an important role in enhancing the growth and yield of plants (Kulekci *et al.*, 2009). However, intensive utilization of chemical fertilizers entails several ecological issues and increases the production costs and food insecurity. Integrated plant nutrient management and irrigation are practically two elements of crop production. The application of biofertilizers is critical in the agricultural sector for sustainability of soil fertility, plant growth and development, and final yield performance (Bhardwaj *et al.*, 2014). Biofertilizers contain living cells or efficient strains of symbiotic and non-symbiotic microorganisms. These beneficial bacterial or fungal inoculants accelerate the uptake of nutrients in the rhizosphere once applied over seed and soil. Various studies have documented that plant growth-promoting rhizobacteria can promote plant growth by various mechanisms such as fixation of atmospheric nitrogen, production of siderophores that chelate metal elements and make them accessible to plant roots, solubilization of minerals such as phosphorus, and synthesis of phytohormones (Gusain *et al.*, 2015).

Results indicated that the interaction effect of cropping pattern × fertilizer treatments was significant. The introduction of soybean plants to cropping patterns resulted in the significant increase for safflower 1000 grains weight and soybean grains number per pod as integrated nutrition was applied (Fig. 1a). Intercropping with soybean can improve available nitrogen through supplementary in nutrient resources achieved from N_2 fixation (Agegnehu *et al.*, 2006). This nitrogen resource is anticipated to: (i) alleviate interference between safflower and soybean for nitrogen absorption and; (ii) increase the available nitrogen for the next crops by improving the nitrogen content of the soil after the decomposition of the leguminous debris (Hauggaard-Nielsen *et al.*, 2008).

In this study, the values of LER, as the most common agronomic index was more than one (Table 5) and used for suitability intercropping evaluate. It can be attributed to differences in traits such as rooting

depth, maximum absorption for nutrient elements and especially no significant competition for resource such as nitrogen on the basis of complementary resources due to dispute in space, time and form. Another indicator used in assessment of intercropping is RVT, which evaluate intercropping in terms of economic value. By placing the numbers associated with each parameter in the formula of this index, the economic value of each treatments of intercropping can be calculated and interpreted. In calculations of this research, the daily price of two crops was used. The value of RVT in all of intercropping patterns is more than one indicating economic superiority of intercropping over monocropping. The highest value was obtained in 60 to 100 soybean to safflower ratio (Table 5). Relative crowding coefficient (RCC) is the ability of a species to use limited resources in intercropping relative to its ability to gain the same resource in monocropping system by using yield comparing. It shows the competitive advantage of intercropping components (Snaydon, 1991) and RCC of 60 to 100 soybean to safflower ratio was higher than those of other intercropping patterns (Table 5).

Table 5 - Evaluation of intercropping efficiency of treatments

Soybean to safflower ratio	Land equivalent ratio (LER)	Relative value total (RVT)	Relative crowding coefficient (RCC)
30:100	2.02	1.95	2.9
60:100	2.31	2.17	17.4
90:100	2.18	2.13	10.16

Agronomic traits in safflower and soybean plants showed intercropping patterns and urea + biofertilizers were superiority treatments compared to other treatments. Evaluation of different treatments of intercropping by LER and RVT showed that in all the treatments the value of LER and RVT was more than one. This is due to high density of vegetation and better use of environmental resource. These results are referred to a one-time trial, therefore would need confirmations and more in deep investigation.

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AHS
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Effect of different packaging materials on shelf life and postharvest quality of tomato (*Lycopersicum esculentum* var. Srijana)

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Key words: HDPE, LDPE, modified atmosphere, permeability.

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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Abstract: Tomatoes, being highly perishable, experience extreme post-harvest losses due to improper packaging materials. Experimentation was done to investigate the effect of different packaging materials on shelf life and quality traits of tomato var. Srijana at the horticulture laboratory of the Institute of Agriculture and Animal Science, Lamjung Campus under a completely randomized design. Seven treatments viz. no packaging (control), unperforated low-density polyethylene (LDPE) bag, perforated (4 holes of 2 mm) LDPE bag, unperforated high-density polyethylene (HDPE) bag, perforated HDPE bag, unperforated non-woven fabric bag, and perforated non-woven fabric bag with 3 replications were used. Tomatoes were evaluated for weight loss, color development, total soluble solids, titratable acidity, pH, and shelf life. Among the treatments, the lowest percentage of weight loss (0.66%) was observed on tomatoes packed in an unperforated HDPE bag, however, it had a higher fungus attack. No packaging group showed rapid shriveling of fruits with the highest percentage of weight loss (14.70%). Although packaging in a non-woven fabric bag was better than control, it showed a higher percentage of weight loss than plastic packaging due to its high permeability to gases and water vapor. The TSS and pH values were found to be higher and TA to be lower in no packaging compared to other packagings. The longest shelf life of tomatoes was observed in perforated LDPE (24 days), followed by HDPE (23 days) whereas the lowest was observed in control (16 days). Overall, the perforated plastic packaging was found best among all treatments with no significant variation among perforated HDPE and perforated LDPE for maintaining qualities of tomatoes and longer shelf life.

1. Introduction

Tomato (*Lycopersicon esculentum* Mill.) is considered as one of the widely grown horticultural crops across the world that ranks second in importance to potato. (FAO, 1989). The area under tomato cultivation in

Nepal occupies about 21,747 ha giving a total production of 413,761 mt (MOALD, 2019/2020). Despite this production, post-harvest loss in tomatoes accounts for about 30-33% of total production. (Tiwari *et al*., 2020). This loss has been attributed to a broad number of factors among which improper packaging, storage facilities, and poor means of transportation and roads constitute a major part. Reduction of post-harvest losses of any perishables is of utmost importance as it is hard to increase a 10% production than to reduce a 10% loss without laying additional land for cultivation (Bhattarai and Gautam, 2006).

Post-harvest quality maintenance is of great challenge in developing countries like Nepal where post-harvest technologies are not so far developed or available in every part of the country. Even the availabilities of some costly technologies are not affordable to smallholder consumers, sellers and farmers. So, there is a need to explore every possible cost-effective way to minimize the prevailing post-harvest losses. One of the better alternative and viable options for improving shelf life and reducing quality degradation of the produce inexpensively is Modified Atmospheric Packaging (MAP) (Kader *et al*., 1989). MAP is achieved by using various packaging materials like LDPE, HDPE that result in alteration of the gaseous environment inside the packages by manipulating the levels of O_2, CO_2, N_2, and C_2H_4. The permeability of film decides the level of O_2 and CO_2 inside it. If the film is of correct permeability, a preferable equilibrium modified atmosphere can be entrenched where the O_2 and CO_2 transmission rate via package can balance the product's respiration (Day, 2001). Reduced O_2 and/or elevated CO_2 levels can reduce respiration, retard ethylene production and ripening, impede textural softening and slow down biochemical changes associated with ripening and thus ultimately resulting in an extension of shelf life (Farber, 1991).

Low-density polyethylene (LDPE) and High-density polyethylene (HDPE) are the materials that are commonly used for MAP. The non-woven fabric bag is the packaging material that has recently evolved in the Nepalese market and has been replacing plastic packages. These packaging materials are easily available and purchasable in the Nepalese market. Different studies suggest that the use of improper packaging and storage has significantly shortened the shelf-life of tomatoes. Hence, to extend the storage life coupled with quality maintenance cost-effectively, this research is focused on identifying the most suitable and effective packaging for tomatoes.

2. Materials and Methods

Description of the study area

The research was conducted in the horticultural laboratory of the Institute of Agriculture and Animal Science (IAAS), Lamjung Campus located in Lamjung, Nepal. It lies at an elevation of 800 meters with 28.127°N latitude and 84.4167°E longitude. The mean annual rainfall of the area is 700 mm. Within a year, the average maximum temperature occurs during June at around 35.7°C whereas the average minimum temperature occurs during January at around 14.5°C.

Experimental materials

Breaker stage defect-free tomato fruits of Srijana variety were harvested from the field of lamjung campus during morning time and taken to the horticultural lab of lamjung campus. The harvesting was done by handpicking with leaving a small pedicel above the fruit. The fruits were free from defects such as sun scorch, bruises, and pest or disease damage. Then, these tomatoes were cleaned, washed, and dried before preparing each sample.

Environmental parameters

The maximum temperature of the experimental lab varied from 33-28°C, minimum temperature from 17-22°C, and relative humidity from 48-75% (Fig. 1).

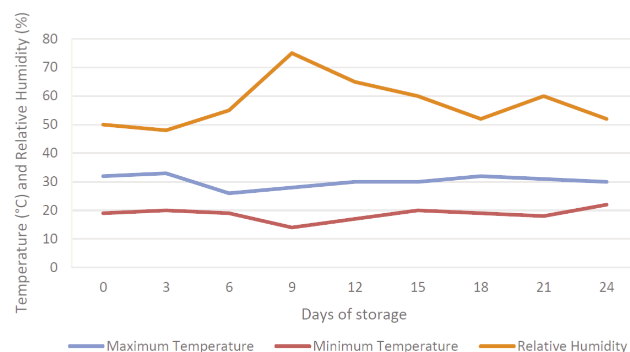

Fig. 1 - Graphical representation of temperature and relative humidity during storage period of tomato.

Treatments and experimental design

The experiment was carried out with seven treatments elaborated be laid in a completely randomized

design with three replications. Thus, the overall experimental units were 21. The washed tomato fruits were divided into 21 groups each with a half kilogram then subjected to various treatments and kept in the ambient environment for its post-harvest quality assessment and physiological weight loss. Each plastic bag was of 25-micron thickness and 20 x 15 cm size while the non-woven fabric bag was single-layered spun-bond polypropylene non-woven fabric with 50 gsm and 0.520 mm thickness. The rubber band was used for sealing the mouth of packages each of the same size. 4 perforations of 2 mm diameter were made in perforated packages with a heated wall nail.

Treatments detail:
T1= No packaging or control;
T2= Unperforated Low-Density Polyethylene (LDPE);
T3= Perforated Low-Density Polyethylene;
T4= Unperforated High-Density Polyethylene (HDPE);
T5= Perforated High-Density Polyethylene;
T6= Unperforated Non-woven fabric bag (NW fabric);
T7= Perforated Non-woven fabric bag.

Data collection

Data for physiological weight loss was observed at every three days of storage whereas the qualitative data was observed every six days.

Weight loss (%)

The fruit was weighed using the electronic digital balance on successive intervals and the loss in weight at each interval was calculated by using the following formula:

Weight loss, % = [(initial weight - final weight)/Initial weight] x 100

Color development

The color development was observed visually. USDA standard color chart (Fig. 2) was used for observation of various maturity stages of tomatoes.
Mature Green = Complete green color on the surface of the tomato;
Breaker = Distinct break in color from green to tarnish-yellow, pink or red on not more than 10% of the surface;
Turning = Change in color from green to tannish-yellow, pink, red up to 10-30% of the surface;
Pink= Pink or red color on 30% to 60% of the surface.
Light red = Pinkish red or red color on 60% to 90% of the surface;
Red = Red color on more than 90% of the surface.

The color changes were determined by using a numerical rating scale from 1-7 where 1= green, 2= breaker, 3= pink, 4= turning, 5= light red, 6=red, and 7= deep red.

Fig. 2 - Standard tomato ripening color chart.

Total soluble solids (°Brix)

To determine the total soluble solids, tomato fruit was squeezed and a few drops of juice were added onto the prism plate of the refractometer (ERMA INC-JAPAN) and TSS (°Brix) was recorded. The prism plate of the refractometer was cleaned after each test with distilled water and soaked up with soft cotton.

Titratable acidity (%)

TA was determined by titrating the 10 ml of tomato juice with 0.1% NaOH which was placed in the burette. A few drops of phenolphthalein were added to the juice as the indicator. The mouth of the burette was opened to allow NaOH to drop down until the color of the juice changed to pink for about 10 seconds. The volume of NaOH required was recorded and titratable acidity was calculated by using the following formula that is expressed as % of citric acid.

$$TA (\%) = [(N_B \times V_B \times M_{eq.} \text{ of acid})/\text{volume of sample}] \times d.f. \times 100$$

Where, N_B = Normality of base (NaOH), V_B= Volume of the base, d.f. = Dilution factor, and M_{eq}.= Milliequivalent weight of predominant acid i.e. citric acid = 0.064.

The pH of the juice

The pH reading was measured by using a digital pH meter from the juice extracted for titration. The pH meter was placed in the juice and left for a certain time until the reading become stable and the stable pH value was noted.

Shelf life

The shelf life of fruits was recorded by judging the non-marketability parameters such as damage by fungus attack, shriveling, etc. It was detected when 50% of tomatoes of each treatment were non-marketable.

Data analysis

The collected data entry and analysis on various parameters were done using the computer software package, Microsoft Excel (2016) and R (agricolae v.1.3-2). The analyzed data were subjected to LSD for mean comparison.

3. Results

Weight loss

Statistically, significant variation was found concerning the weight loss of tomatoes among different treatments. The higher rate of weight loss was observed in no packaging (open) and the lower was in unperforated HDPE that was statistically at par with unperforated LDPE at all days of the storage.

The perforated packages showed a higher weight loss than any unperforated packaging material (Table 1).

One of the important factors that determine the shelf life of the tomato is weight loss and it was found to increase with the increase in permeability of the packaging material. The non-woven fabric bag has a high permeability to gases and water vapor as compared to the plastic film resulting in higher weight loss.

Color development

The color development was observed to be rapid with the increase in permeability of the packaging material. Extremely slow development of the color was observed in unperforated polyethylene storage and rapid in open tray storage (Table 2).

Total soluble solids

The TSS of the tomato increased under all the packaging material up to 18 days of storage. There was no significant difference in the TSS value of the tomato under different packaging materials on the 6th day of storage. However, the significant difference among the treatments began to appear from the 12th day of storage. The highest TSS was observed in no

Table 2 - Effect of packaging materials on color development

Treatments	Color development		
	6DAS	12DAS	18DAS
T1	4.17 a	6.33 a	6.80 a
T2	2.17 a	3.00 d	4.33 d
T3	3.33 cd	5.33 c	6.10 c
T4	2.10 e	3.16 d	4.16 d
T5	3.17 d	5.10c	6.13 c
T6	3.5 c	5.76 b	6.46 b
T7	3.93 b	6.03 b	6.60 ab
Grand Mean	3.19	4.96	5.80
LSD	0.17 ***	0.29 ***	0.31 ***
CV	3.05%	3.38%	3.01%

Means in the column followed by similar letters are not statistically different at p=0.05 by LSD.
Color Score (1 Green, 2 Breakers, 3 Turning, 4 Pink, 5 Light Red, 6 Red, 7 Deep Red).
DAS=Days after Storage, LSD=Least significant difference, CV=Coefficient of variance, NS=Non significant, * significant at 5%, ** significant at 1%, ***significant at 0.1% level of significance.

Table 1 - Effect of packaging materials on physiological weight loss (%)

Treatments	Weight loss (%)					
	3DAS	6DAS	9DAS	12DAS	15DAS	18DAS
T1	2.67 a	3.08 a	4.95 a	8.12 a	12.18 a	14.70 a
T2	0.09 d	0.21 e	0.30 e	0.37 e	0.50 e	0.81 e
T3	0.41 c	0.82 d	1.29 d	1.62 d	2.07 d	2.77 d
T4	0.04 d	0.19 e	0.26 e	0.30 e	0.40 e	0.66 e
T5	0.038 c	0.78 d	1.22 d	1.37 d	1.85 d	2.58 d
T6	1.84 b	2.34 c	3.47 c	6.08 c	9.07 c	11.54 c
7	1.94 b	2.62 b	3.86 b	6.55 b	9.87 b	12.37 b
Grand Mean	0.994	1.435	2.198	3.488	5.134	6.49
LSD	0.1397 ***	0.2256 ***	0.3190 ***	0.3588 ***	0.4662 ***	0.7020 ***
CV%	8.02%	8.97%	8.32%	5.87%	5.18%	6.18%

Means in the column followed by similar letters are not statistically different at p=0.05 by LSD.
DAS= Days after Storage, LSD = Least significant difference, CV = Coefficient of variance, * significant at 5%, ** significant at 1%, *** significant at 0.1% level of significance.

packaging on all days of the storage and the lowest was in unperforated HDPE (Table 3).

Table 3 - Effect of different packaging materials on TSS content of tomato

Treatments	TSS of tomato		
	6DAS	12DAS	18DAS
T1	4.016 a	4.533 a	5.100 a
T2	3.850 a	4.067 d	4.301 d
T3	3.867 a	4.183 bc	4.550 c
T4	3.850 a	4.083 cd	4.301 d
T5	3.867 a	4.177 bcd	4.533 c
T6	3.867 a	4.233 b	4.750 b
T7	3.933 a	4.277 b	4.77 b
Grand mean	3.893	4.222	4.614
LSD	NS	0.1106 ***	0.1079 ***
CV(%)	2.64%	1.49%	1.33%

Means in the column followed by similar letters are not significantly different at p=0.05 by LSD.
DAS=Days after Storage, LSD=Least significant difference, CV=Coefficient of variance, NS= Non significant, * significant at 5%, ** significant at 1%, ***significant at 0.1% level of significance.

Titratable acidity (TA)

TA of the tomato decreased with an increase in the period of storage in all the packaging materials used. The lowest TA was observed in no packaging storage while the unperforated plastic packaging showed significantly higher TA (Table 4).

Table 4 - Effect of different packaging materials on TA content of tomato

Treatments	TA of tomato		
	6 DAS	12DAS	18DAS
T1	0.697 e	0.613 e	0.303 d
T2	0.987 a	0.863 a	0.563 a
T3	0.750 c	0.640 cd	0.367 b
T4	0.990 a	0.853 a	0.550 a
T5	0.767 b	0.670 b	0.353 b
T6	0.713 d	0.647 c	0.326 c
T7	0.703 de	0.627 de	0.323 c
Grand mean	0.80	0.701	0.398
LSD	0.013 ***	0.016 ***	0.016 ***
CV(%)	0.9%	1.28%	2.33%

Means in the column followed by similar letters are not statistically different at p=0.05 by LSD.
DAS=Days after Storage, LSD = Least significant difference, CV = Coefficient of variance, * significant at 5%, ** significant at 1%, ***significant at 0.1% level of significance.

The pH of the juice

The pH of the tomato increased with the increase in the storage days for all the packaging material. The pH was found more in open tray conditions and low in unperforated polyethylene. The higher the barrier to the gases through the packaging film, the lower was the PH (Table 5).

Shelf life

The longest shelf life was observed in perforated LDPE (24 days) and the shortest in no packaging (16 days) (Fig. 3).

4. Discussion and Conclusions

The weight loss of the tomatoes is primarily due to the loss of water from transpiration and respiration (Singh, 2010). Lower weight loss in all the pack-

Table 5 - Effect of different packaging materials on PH content of tomato

Treatments	PH of tomato		
	6DAS	12DAS	18DAS
T1	4.13 a	4.30 a	4.53 a
T2	3.80 d	4.03 c	4.20 d
T3	3.95 b	4.17 b	4.30 bc
T4	3.83 cd	4.00 c	4.20 d
T5	3.93 b	4.17 b	4.26 cd
T6	3.90 bc	4.00 c	4.33 bc
T7	4.10 a	4.12 ab	4.37 b
Grand mean	3.95	4.12	4.31
LSD	0.074 ***	0.114 ***	0.076 ***
CV(%)	1.07%	1.59%	1.01%

Means in the column followed by similar letters are not statistically different at p=0.05 by LSD.
DAS= Days after Storage, LSD= Least significant difference, CV= Coefficient of variance, * significant at 5%, ** significant at 1%, *** significant at 0.1% level of significance.

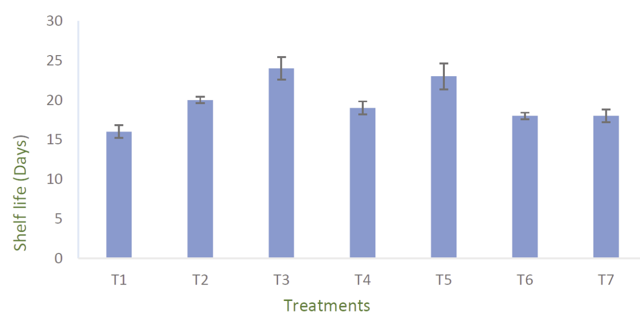

Fig. 3 - Effect of different packaging material on shelf life of tomato.

aged fruits as compared to control could be due to a lower rate of transpiration and counteraction of excessive moisture loss which was similar to the results presented by Gonzalez *et al*. (1990) and Nath *et al.* (2011). The difference in weight losses of fruits among different films could also be largely due to differences in transmission rates of water vapor through the packaging film (Batu and Thompson, 1998). Here the lower transmission rate of polyethylene packages might contribute to the development of higher relative humidity inside the package thereby reducing weight loss (Thompson, 2001). Batu and Thompson (1998) also found less weight loss in sealed packed tomatoes after 60 days of storage at 13°C. A modified atmosphere is created around the fruits due to the permeable nature of the film. During the storage, the CO_2 concentration accumulates inside MAP restricting the respiration of the produces thereby reducing weight loss and prolonging the shelf life (Selçuk *et al*., 2020). Similarly, higher weight loss was observed in macro-perforated packages than any of the unperforated packages due to higher permeability to gases and water vapor (Van Der Steen *et al*., 2002). Similar might be the case for non-woven packages where the bag itself has a low barrier to the exchange of gases even without any perforations resulting the higher weight loss.

Lycopene pigment is the primary reason for the development of the color in tomatoes. As the ripening proceeds, lycopene content increases. However, in presence of low oxygen, the formation of lycopene is inhibited resulting in the slow development of color. Due to low O_2, high CO_2, or the suitable combination of these two gases, there could be retardation of color change in tomato fruits (Kidd and West, 1930). Yang and Chinnan (1987) also found that there Is less accumulation of lycopene in sealed packaged tomatoes with fewer chroma values than that of control treatment. Lycopene formation was found to be completely inhibited at 1% O_2 and 99% N2 for 50 days of storage (Yang *et al*., 1987). Ethylene is responsible for triggering the ripening of tomatoes and it is associated with a sudden change in the physiology of tomatoes at the onset of ripening. CO_2 concentration affected the development of color in tomatoes by suppression of ethylene production (Kubo and Inaba, 1989). While exposing the tomatoes to high levels (20, 40, and 60%) of CO_2, color development was inhibited in tomatoes (Buescher, 1979).

The TSS content of fruit determines its overall taste (Baldwin *et al*., 1998). Getinet *et al*. (2008) have reported a low total soluble solid at the color breaker stage but higher when tomato fruits were harvested at the pink mature stage. The increase in TSS with the increase in maturity could be attributed to the breakdown of starch to simple sugars or the hydrolysis of cell wall polysaccharides (Crouch, 2003). The lower TSS in packed tomatoes as compared to the open storage could be due to a slower rate of respiration and metabolic activities that slow down the ripening process in packed tomatoes (Gharezi *et al*., 2012). However, the higher TSS observed in non-woven treatment might be attributed to the relatively faster rate of respiration resulting by the comparatively higher air permeability of the bag. The more TSS is related to more ripen of fruit (Dhakal *et al*., 2020). However, the rapid increment in TSS is not desirable as it causes rapid shriveling and decreases the shelf life.

Priyankara *et al*. (2017) also found the titratable acidity reaching the peak at the color breaker stage and decreasing along with the advancement of fruit ripening. Since the acidity of the fruit is due to the presence of various organic acids, the amount of organic acid is usually found decreasing during maturity as being a substrate of respiration (Albertini *et al*., 2006). The slow rate of decrement of TA in unperforated plastic packaging could be attributed to the reduced O_2 and increased CO_2 inside the packages that result in the slow rate of respiration (Mathooko, 2003); thus, it may impede the loss of organic acids (Wang, 1990). Even so, the rapid decrement of TA in non-woven packages could be ascribed to the exchange of gases good enough to deplete the organic acid. Every factor that is responsible for reducing cellular respiration and catabolism prevent the reduction of organic acid in the product (Feizi *et al.*, 2020). De Castro *et al*. (2005) also reported the decrement in acidity with maturity evolution.

The difference in the pH and TA in different packages is attributed to the variations in respiration rate and enzyme activities (Feizi *et al*., 2020). Organic acid being an intermediate of carbon metabolism increase the pH of the produce. The higher pH under the open tray could be associated with the faster utilization of acids for sugar catabolism. For a similar reason, pH is higher in the non-woven bag as compared to others owing to increased O_2 inside the packages engendered by the air enterable nature of the bag. The significantly lower pH values of unperforated packaged fruits could be explained by the relatively reduced respiration rate due to reduced O_2 inside the packages. The increase in the PH of the fruits during storage was also observed by Batu and Thompson

(1998).

The difference in the shelf life was due to the difference in marketability of fruits due to the decaying of fruits by fungus or shriveling of the fruits unacceptably. Unperforated plastic packaging showed the highest percentage of fungus attacks that could be probably due to higher relative humidity inside the packages. The non-woven fabric bag allows access to air sufficient enough to escape the modified atmospheric condition resulting relatively higher shriveling and weight loss ensued from faster ripening and transpiration and ultimately over-ripening.

The beneficial effect of perforated plastic packaging could be attributed to the well-modified atmosphere created inside the package along with the reduction in water loss. Lower rate of respiration and ethylene production inhibited ethylene action, delayed ripening and senescence, impeded growth of decay-causing pathogens and insects due to gaseous modification inside the package could be the probable reason to extend the shelf life of fruits (Kader and Rolle, 2004). Ben-Yenonshuna (1985) also reported the delayed ripening and softening in the case of packaging of climacteric fruits in low-density polyethylene bags and hence improving marketability.

Thus, packaging significantly affected various quantitative and qualitative post-harvest properties. All the packaging system was found to be better than open tray storage. The permeability of packaging material had a huge influence on the composition of the internal atmosphere and the creation of optimal storage conditions. Very low permeability would induce fungal growth due to high relative humidity and high permeability would result in higher weight loss due to faster respiration. Therefore, it is necessary to establish an optimal condition that may be an equilibrium-modified atmosphere that avoids both of these problems. Based on the result of this experiment, perforated plastic bag (both HDPE and LDPE) was discovered to be best among all packaging treatments for reducing weight loss, avoiding fungal growth, and maintaining a better quality of tomatoes for a longer duration.

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Maintaining physicochemical and sensory properties of guava var. Getas Merah using alginate and *Cyclea barbata* leaves powder as edible coating

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Key words: Postharvest treatment, preservation, *Psidium guajava*, shelf-life.

Abstract: Indonesia is one of the major countries which contributes the world's guava production. Guava var. Getas Merah is commonly found in Indonesia. Guava has a short shelf-life as it rapidly goes under postharvest ripening. This leads to a faster deterioration of physicochemical and sensorial properties of guava. A generally used method to extends shelf-life is by edible coating. In this study, a combination of alginate and *Cyclea barbata* leaves powder (CBLP) was investigated as a potential edible coating. The analysis of firmness, total soluble solids, total reducing sugar, total titratable acidity and organoleptic tests were conducted to evaluate the quality of guava fruits stored for 20 d at 14°C. A split plot design study was used and four different treatments with different CBLP concentrations were applied. The samples treated with 2% alginate and 0.8% CBLP showed the lowest total dissolved solids, total reducing sugar, and total titratable acidity. Moreover, the samples were reported with the highest score on color, taste, and texture parameters. The firmness test showed that samples treated with 2% alginate and 0.2% CBLP had the lowest firmness loss and highest score for aroma. In summary longer quality retention of guava fruits was found after the addition of CBLP in alginate-based edible coating.

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All relevant data are within the paper and its Supporting Information files.

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1. Introduction

As a tropical country, Indonesia is rich in horticultural products, especially fruits. Guava (*Psidium guajava L.*) var. Getas Merah is one of many fruits that are highly produced and consumed in Indonesia. Although it has high content of vitamin C, dietary fiber, antioxidants, and minerals, guava is a perishable food product and has a relatively short shelf life (Kumar *et al.*, 2001; Naaseer *et al.*, 2018). During its ripening stage, guava undergoes physicochemical changes such as total soluble solid, pectin content, total titratable acidity, total sugar content, and total anthocyanin content (Dube and Singh, 2015). Total soluble solids of guava cv. Apple

Colour were found to be increasing rapidly between 75 to 150 days. Whereas its maximum acidity of 0.41% was reached after 105 days. Following the increase of total titratable acidity during immature and intermediate stage of maturation, the value will be decreased during maturity stage. On the contrary, total reducing sugar increased during late maturity. These changes happen very fast; therefore, fully ripe guava can easily bruise and considered as highly perishable (Fabi *et al.*, 2010). By reducing the rate of this physicochemical and sensory properties to sustain the quality of guava, shelf-life extension can be achieved.

The storage of guava with chemicals such as boric acid and naphthalene acetic acid (NAA) at ambient temperature is commercially available and feasible (Singh *et al.*, 2017). Although this technique has been proven to effectively maintain the quality of guava, the use of chemicals may affect those potential consumers that have a negative perception of chemical preservatives. The use of plastic packaging for individual packaging with clink and shrink film was reported (Rana *et al.*, 2015). By individually wrapping guava fruits, changes of total soluble solids slowed down and extending the shelf life for 10 days. However, the use of plastic is not a sustainable choice.

The preservation technique by edible coating can be an option for a more sustainable choice. It is applied by adding a thin layer to the fruit to prevent gas release. The edible coating must be able to form a layer that acts as a respiration and transpiration inhibitor by reducing moisture and solute migration and gas exchange. Another requirement for edible coating material is that it is safe to consume and has the ability to enhance shelf-life (Vaishali *et al.*, 2019). Compared to the synthetic coatings, edible films can act as both gas and moisture barrier which results in a modified atmosphere to enhance the shelf life of fruits (Kocira *et al.*, 2021). Polysaccharides such as alginate, chitosan, and pectin, are the most frequent polymer used for edible coatings. Alginate is derived from brown sea algae and is an example of hydrocolloids, film-forming biopolymers (Parreidt *et al.*, 2018). The application of alginate-based edible coatings has been conducted with the addition of various ingredients to extend the shelf life of guava. The application of alginate and betel-essential oil on rose apple resulted in quality retention until nine days (Setiawan *et al.,* 2019). The effect of both chitosan and alginate edible coating with the addition of pomegranate peel extract were reported (Nair *et al.*, 2018). The study reported significant retention of quality deterioration in guava after the edible coating's application and storage at 10°C. Another study by showed that the addition of ZnO nanoparticles in chitosan and alginate effectively inhibits rot in guavas, indicating their antimicrobial action (Arroyo *et al.*, 2020).

In this study, *Cyclea barbata* leaves, which also has potential as an edible coating, is economically feasible, and is more available in Indonesia, was included to the alginate coating. *Cyclea barbata* is a well-known plant that is widely used by Indonesian people as food and medicine. It is rich in carbohydrates, polyphenols, saponin, calcium, phosphor, and vitamins A2 and B (Acamovic and Brooker, 2005). The main component of *Cyclea barbata* leaves extract that forms a gel is pectin polysaccharide with low methoxy content belongs to the gel-forming hydrocolloid group. The gel formed by pectin will be highly adhesive ŧand transparent and consequently has potential as an edible coating material (Rachmawati *et al.*, 2010). Thus, this study investigated the addition effect of *Cyclea barbata* leaves powder (CBLP) in alginate-based coating as edible coating to maintain physicochemical parameters and sensory quality of guava.

2. Materials and Methods

Study design

This study was conducted using a split plot design study. This study was carried out in 2018 and used four treatments with a variation of CBLP concentrations. A total of 135 whole guava fruits were used during this study. Each treatments used 27 guava that were grouped for each three guavas to be stored in a polystyrene board with plastic wrap after coating as one set. These three pieces of guava acted as triplicates of biological replicates. During the 20 days of storage, physicochemical and sensory analyses were done every four days. During this days, one set of fruits were randomly taken to be analyzed further for its physicochemical properties. Then, one fruit were selected randomly for sensory analyses.

Plant materials

This study used guava from an orchard in Sleman Regency, Yogyakarta, Indonesia. The guava used in this study was harvested 109-114 days after the fruit

bloomed. According to a previous study, guava can be harvested after optimum maturity by observation of color attained or between 105-135 days after the fruit sets (Dube and Singh, 2015). This range of day is suitable for distant transportation to refrain from harvest losses. However, the length of the distance from this study was not mentioned. Therefore, this study used a more restricted range of harvesting days.

The fruits were selected by visual observation to remove the defected fruits and choose the fruits with the same size. Then, fruits with a weight of 170 g were selected. From the orchard, selected fruits were transported to Faculty of Agriculture, Universitas Muhammadiyah Yogyakarta, Indonesia. The fruits were stored in the refrigerator at 14°C for one day; then they were washed by submerging in 0.05% sodium benzoate solution for 10 min and dried to prevent the growth of bacteria and molds during storage (Masamba Mndalira, 2016).

Preparation of CBLP and alginate edible coating

The coating used in this study was produced according to a previous study (Olivas *et al*., 2007), with some modifications. The CBLP was made by cleaning fresh *Cyclea barbata* leaves with water followed by drying (oven-dried) at 50°C for 18 h. The dried leaves were ground and sieved using a sifter (0.5 mm mesh) to produce a powder. Alginate was made by dissolving 10 g alginate in 500 mL water (to make 2% alginate solution) and heating with a water bath at 80°C for 30 min while stirring until the solution became transparent. A total of 2.5% glycerol as a plasticizer was added to the edible coating solution.

The treatments with different formulation used was as below:

- No coating applied (control)
- 2% alginate + 0.2% CBLP
- 2% alginate + 0.4% CBLP
- 2% alginate + 0.6% CBLP
- 2% alginate + 0.8% CBLP

Application of edible coating solution

Selected fruits that had been washed were soaked in the edible coating solution for 3 min, followed by dipping into a 2% $CaCl_2$ solution for 15 min until it formed a layer. The samples were dried at room temperature. Each three samples were stored in a polystyrene board wrapped with plastic. The storage temperature was kept at 14°C and 95% RH.

Analyses of physicochemical parameter

To investigate the ability of shelf-life extension by adding CBLP to alginate coating, the samples were measured for their physical (firmness), chemical (total soluble solids, total reducing sugar, and titratable acidity), and organoleptic qualities. The parameters were measured every four days within 20 d of storage time.

Firmness. The analysis of firmness was conducted by measuring the samples chosen for the weight loss measurements using a penetrometer. The samples with the skin still intact were stabbed with the device in three different locations using 250 g of pressure on the scale of 1/10 mm for 10 s. The results obtained were averaged and expressed in kg/cm^2.

Total soluble solids. The samples for the analysis of total soluble solids were selected by choosing one random guava in a box of nine fruits. The chosen sample was extracted by mashing the fruit and pressing to obtain the juice. About two to three droplets of the juice were inserted into a handheld digital refractometer (ATAGO, Tokyo, Japan). The samples were measured three times for replication and then the average value was taken. The results were expressed in % Brix unit.

Total reducing sugar. The reducing sugar analysis was carried out according to Somogyi (1937) as modified by Nelson (1944). One milliliter of the juice extracted for the total soluble solid analysis was added to a test tube that contained 25 mL Nelson A, 1 mL Nelson B, and 1 mL cupric reagent solution. The test tubes were heated in a water bath (Memmert, Schwabach, Germany) for 20 min and cooled. Afterward, 2 mL arsenic-molybdic reagent was added to the tube and mixed until homogeneous before adding 7 mL of distilled water. The samples' absorbance was read using a spectrophotometer (λ = 540 mm) (Thermo Fischer Scientific, Massachusetts, America). The results were reported as the percentage of reducing sugars (%).

Total titratable acid. The total titratable acid analysis was conducted according to Ranganna (1986). From a box of nine guava fruits, one was selected randomly to be measured. The chosen sample was mashed, and 10 g were taken to be added into a 100 mL volumetric flask. After that, distilled water was added until it reached the 100 mL mark. The solution was then mixed until homogeneous and filtered using filter paper. Then, 10 mL of the filtrate was transferred to an Erlenmeyer. Two to three drops of the indicator of 1% phenolphthalein were

added. Titration was conducted using NaOH 0.1 N until the solution turned a pinkish color. The volume of NaOH 0.1 N used for the titration was recorded to be calculated and converted to the total titratable acidity value. The total titratable acidity (%TA) was calculated with the equation

$$\%TA = (V \times N \times MW \times df \times 100\%)/(m \times 1000 \times v)$$

where V = volume of NaOH 0.1 N used (mL), N = normality of NaOH used, MW = molecular weight of dominating acid (molecular weight of citric acid = 192), df = dilution factor, m = mass of sample (g), and v = valence of dominant acid (valence of citric acid = 3). The results were reported as percentages (%)

Organoleptic tests. In this study, the organoleptic test used a hedonic test where the perception of the sample's color, aroma, taste, texture, and overall acceptability was measured. The measurement used a ranking test with a 9-point scale (Meilgaard *et al.*, 2006) where a score of 1 is the lowest score (dislike extremely) and 9 is the highest (like extremely). The hedonic test was done with 50 panelists (ratio male: female is 1:1) from academic staff and students of Universitas Muhammadiyah Yogyakarta. The panelists were aged between 18 and 60 years old.

Statistical analysis

The results of firmness, total soluble solids, total reduced, sugar and total titratable acid were analyzed with one-way ANOVA. Means were separated and analyzed using the Duncan multiple range test (DMRT, α = 5%) using Statistic Analytical Software (SAS) version 9.4.

3. Results and Discussions

Firmness

The firmness analysis of guava treated with alginate-based edible coating and different concentrations of CBLP is exhibited in Table 1. All samples show declining trends during the 20 days of observation. A sudden drop can be seen in samples coated with alginate but no addition of CBLP after four days of storage and the firmness continues to decrease. Compared to these samples, the samples treated with the addition of CBLP in alginate shows slower decrease of firmness. The different values between control and treated samples indicated that the edible coating could prevent firmness loss during storage.

In this study, samples coated with the addition of the lowest CBLP concentration shows the highest firmness after 20 days of storage. Similar result was also seen in the application of aloe vera and sage essential oil in tomatoes (Tzortzakis *et al.*, 2019). After 7 days of storage, tomato with aloe vera and 0.1% sage essential oil had lower softening compared to the addition of 0.5% sage essential in aloe vera. Another study reported that apples coated with higher concentration of lemongrass oil and oregano oil showed sudden firmness drop (Rojas-Graü *et al.*, 2007). A possible explanation of this result is the low pH of coating solution due to the higher concentration of essential oil was added.

This result occurred because the sample coated with alginate and CBLP had a more effective gas diffusion barrier than the control sample, therefore, enzymes that were involved in respiration and tissue softening will be less active. Firmness and juiciness of fruit is an important parameter that also relates to the marketability. The firmness of fruit is correlated to the pectin content on the cell walls. Due to pectin solubilization, the texture of fruits will be softer and resulted in firmness loss. This pectin solubilizations can be caused by several reasons, such as the depolymerization of pectin by pectinase enzymes (i.e., polygalacturonase, cellulase and pectin methyl esterase)

Table 1 - Result of firmness in kg/cm^2 of guava treated with alginate-based edible coating enriched with different concentration of *Cyclea barbata* leaves powder (CBLP)

Treatments	Observation days					
	0	4	8	12	16	20
Control	3.58 ± 0.13 a	0.60 ± 0.09 c	0.58 ± 0.02 d	0.28 ± 0.01 c	0.12 ± 0.01 c	0.08 ± 0.02 d
2% alginate + 0.2% CBLP	3.56 ± 0.07 a	2.81 ± 0.18 a	2.58 ± 0.13 ab	2.35 ± 0.23 a	1.99 ± 0.14 a	1.76 ± 0.05 a
2% alginate + 0.4% CBLP	3.05 ± 0.05 c	2.43 ± 0.05 b	2.34 ± 0.13 bc	2.29 ± 0.13 a	1.99 ± 0.17 a	1.58 ± 0.10 b
2% alginate + 0.6% CBLP	3.03 ± 0.22 b	2.83 ± 0.06 a	2.23 ± 0.15 c	1.97 ± 0.15 c	1.65 ± 0.14 b	1.39 ± 0.09 c
2% alginate + 0.8% CBLP	3.34 ± 0.27 ab	2.87 ± 0.11 a	2.72 ± 0.17 a	2.22 ± 0.08 b	1.95 ± 0.07 a	1.55 ± 0.03 b

Results followed by the same letter(s) imply that there is no significant difference between results according to the Duncan multiple range test (p>0.05).

and the loss of neutral chains (arabinan and galactan) which binds pectin to the cell wall via glycans or cellulose matrix (Paniagua *et al.*, 2014). By adding edible coating, the pectinase enzymes activity is lower and firmness loss is hindered (Zhou *et al.*, 2010).

Another factor of the firmness is the transpiration of fruit. The balance between water loss and water uptake highly affected mechanical properties of the skin by controlling cell turgor. When turgor is removed by membrane matrix disassemble (i.e., solubilization of pectin during ripening), water can move freely which results in cell walls relaxation (Brüggenwirth and Knoche, 2016). Fruit transpiration is highly correlated with the water vapor pressure of airspace inside of the fruit and the air directly outside as the driving force (Montanaro *et al.*, 2012). Even after harvesting, detached fruit has sufficient water content to support an additional of 10 hours transpiration without moisture supply from xylem/phloem supply. The application of edible coating can generate a layer to delay transpiration (Kocira *et al.*, 2021). Thus, a possible mechanism of alginate and CBLP as edible coating is by generating a water vapor barrier to lower water content loss of fruit.

Total soluble solids

Table 2 exhibits the results of total soluble solids with different CBLP concentrations. The result of total soluble solids indicates the total sugars in the sample. All samples had an increase at the beginning of storage time followed by a decline until the last observation day. This increase of total soluble solids during the ripening stage in guava was previously reported (Patel *et al.*, 2014). It was also explained that the increase can be from depolymerization of polysaccharides and conversion of fruit starch to sugars.

However, all samples showed a decline in total dissolved solids after 20 days due to the senescence phase. A similar trend of total soluble solids was also reported in the application naphthalene acetic acid (NAA) and potassium nitrate as pre-harvest treatments on the storage quality of winter guava (Mandal *et al.*, 2012). At the beginning of storage, hydrolysis of insoluble polysaccharides to simple sugars resulted in the increase of total soluble solids (Brothakar *et al.*, 2002). Within time, all the insoluble polysaccharides will be completely hydrolyzed while soluble solids and organic acids will be used for respiration. This leads to the decrease of total soluble solid.

The fall of total soluble solids content in mature fruit towards senescence has also been reported in mango fruits. The same study investigated the effect of different concentrations of Bavistin DF solution to extend shelf-life as a postharvest treatment on mango fruits. The trend of total soluble solids observed in the control samples indicated that they underwent a faster maturation stage and headed towards senescence (Islam *et al.*, 2013). On day 20, samples coated with 2% alginate and 0.8% CBLP had the lowest level of total dissolved solids. The higher the amount of CBLP, the lower the total dissolved solids were.

The mechanism of edible coating in inhibiting starch degradation which leads to the increase of total soluble solids during fruit ripening is by the decrease of respiration rate. The degradation of cell walls and starch to soluble sugars and organic acids act as a supply of carbons during climacteric respiration of fruit (Colombié *et al.*, 2016). Guava is considered climacteric fruit, indicated by the inclined ethylene production at the peak of ripening (Ishartani *et al.,* 2018). The edible coating of alginate and CBLP in guava created an additionally layer that can hinder the respiration rate; thus, lowering the conversion rate of starch to total soluble solids.

Table 2 - Result of total soluble solids in % Brix unit of guava treated with alginate-based edible coating enriched with different concentration of *Cyclea barbata* leaves powder (CBLP)

Treatments	Observation days					
	0	4	8	12	16	20
Control	7.27 ± 0.06 a	7.30 ± 0.10 a	7.47 ± 0.15 a	7.88 ± 0.07 a	8.19 ± 0.03 a	7.03 ± 0.06 a
2% alginate + 0.2% CBLP	6.53 ± 0.07 b	6.90 ± 0.10 b	7.03 ± 0.06 b	7.13 ± 0.04 b	7.15 ± 0.05 c	6.73 ± 0.12 b
2% alginate + 0.4% CBLP	5.36 ± 0.14 c	6.20 ± 0.20 d	6.82 ± 0.04 c	7.10 ± 0.00 b	7.23 ± 0.00 b	6.71 ± 0.10 b
2% alginate + 0.6% CBLP	5.27 ± 0.06 c	7.00 ± 0.10 b	7.03 ± 0.00 b	7.15 ± 0.05 b	7.23 ± 0.06 b	6.39 ± 0.13 c
2% alginate + 0.8% CBLP	5.42 ± 0.25 c	6.62 ± 0.08 c	6.85 ± 0.12 c	7.07 ± 0.06 b	7.05 ± 0.02 d	6.17 ± 0.06 d

Results followed by the same letter(s) imply that there is no significant difference between results according to the Duncan multiple range test ($p>0.05$).

Total reducing sugar

The results of total reducing sugar analysis with different edible coating concentrations on guava fruits are shown in Table 3.

All samples show an increase in sugar reduction throughout the observation time. These results are similar to those of a previous study, where different varieties of guava were investigated for their total reducing sugars after 5 months of storage time at ambient temperature (Choudhary *et al*., 2008). The reducing sugars may be increased in the senescence phase of climacteric fruits due to starch hydrolysis to glucose, fructose, and sucrose (Patel *et al*., 2013). A high level of reducing sugars indicates rapid starch hydrolysis in fruit.

The reducing sugar content in guava is varied within different varieties (Choudhary *et al*., 2008). The highest level of reducing sugar is fructose, followed by glucose (Mowlah and Itoo, 1982). After the ripening stage, reducing sugar content of guava were found to be decreasing in overripe fruits (Bashir and Abu-Goukh, 2003). However, a clear explanation about this decrease has not been fully understood due to the complex process of starch breakdown, sugar synthesis and sugar metabolism in fruits are very complex which involves not only by ethylene but also an abundant of hormones and enzymes (Cordenunsi-Lysenko *et al*., 2019). In addition to this complexity, different fruits may possess different metabolic pathway of sugar. Previously, a study was conducted to investigate the sugar metabolism of peach during fruit development (Desnoues *et al*., 2014). The proposed pathway mentioned a conversion of fructose and glucose to fructose 1,6-bisphosphate (F16BP) which involved in the glycolysis and respiration flux.

On the last observation day, samples with 2% alginate and 0.8% CBLP showed the lowest reducing sugar level. This implies that the addition of 0.8% CBLP mixed with alginate could suppress starch hydrolysis into glucose, sucrose, and fructose. The edible coating with polysaccharide base and CBLP acted as a selectively permeable membrane towards CO_2 and O_2 gases' diffusion. This characteristic may extend shelf-life, as fruit's respiration rate became slower (Kocira *et al*., 2021). CBLP has an alcohol content that could inhibit fruit senescence when added in alginate (Viña *et al*., 2000).

Total titratable acid

The analysis of total titratable acid in guava fruits treated with different concentrations of the edible coating was conducted. The results are exhibited in Table 4.

Table 3 - Result of total reducing sugar in percentage (%) of guava treated with alginate-based edible coating enriched with different concentration of *Cyclea barbata* leaves powder (CBLP)

Treatments	Observation days					
	0	4	8	12	16	20
Control	9.33 ± 0.06 b	9.94 ± 0.02 b	12.16 ± 0.05 a	12.34 ± 0.00 b	13.59 ± 0.00 a	15.29± 0.02 a
2% alginate + 0.2% CBLP	9.76 ± 0.05 a	10.13 ± 0.02 a	10.14 ± 0.03 e	11.50 ± 0.06 c	12.21 ± 0.13 a	13.37 ± 0.00 d
2% alginate + 0.4% CBLP	8.98 ± 0.13 c	9.46 ± 0.03 d	10.30 ± 0.06 d	13.00 ± 0.04 a	13.45 ± 0.21 a	13.57 ± 0.02 b
2% alginate + 0.6% CBLP	8.69 ± 0.03 d	9.76 ± 0.07 c	11.38 ± 0.04 b	12.97 ± 0.05 a	13.03 ± 0.38 a	13.30 ± 0.03 e
2% alginate + 0.8% CBLP	8.24 ± 0.06 e	9.70 ± 0.07 c	10.55 ± 0.11 e	10.98 ± 0.06 d	12.03 ± 0.08 c	13.42 ± 0.04 c

Results followed by the same letter(s) imply that there is no significant difference between results according to the Duncan multiple range test (p>0.05).

Table 4 - Result of total titratable acid in percentage (%) of guava treated with alginate-based edible coating enriched with different concentration of *Cyclea barbata* leaves powder (CBLP)

Treatments	Observation days					
	0	4	8	12	16	20
Control	0.67 ± 0 b	0.67 ± 0 b	0.67 ± 0 b	1.11 ± 0.003 a	1.78 ± 0.003 a	2.01 ± 0 a
2% alginate + 0.2% CBLP	0.67 ± 0 b	0.67 ± 0 b	0.67 ± 0 b	0.81 ± 0.001 ab	1.13 ± 0.003 ab	1.56 ± 0 b
2% alginate + 0.4% CBLP	0.67 ± 0 b	0.67 ± 0 b	0.67 ± 0 b	0.67 ± 0 b	1.13 ± 0.003 ab	1.34 ± 0 c
2% alginate + 0.6% CBLP	0.67 ± 0 b	0.67 ± 0 b	0.67 ± 0 b	0.67 ± 0 b	1.13 ± 0.003 ab	1.34 ± 0c
2% alginate + 0.8% CBLP	0.67 ± 0 b	0.67 ± 0 b	0.67 ± 0 b	0.67 ± 0 b	0.67± 0 b	0.67 ± 0 b

Results followed by the same letter(s) imply that there is no significant difference between results according to the Duncan multiple range test (p>0.05).

As a climacteric fruit, guava will have a sudden increase in respiration along with or before the senescence stage due to increases in CO_2 and ethylene after the climacteric phase (Tripathi *et al*., 2016). Therefore, the level of total titratable acid has frequently been used to indicate the shelf-life of fruit. According to the results, most of the samples had an increase in total titratable acid starting from day 12, except the samples coated with 2% alginate + 0.8% CBLP. This result is different from a previous study of guava (cv. Sardar), in which titratable acidity decreased after 40 days of storage (Mandal *et al.,* 2012). This reduction of total titratable acid level during storage was due to guava, as climacteric fruit, during the peak of ripening resulted in the rise of malic enzyme activity and pyruvate decarboxylation. Therefore, respiration rate and metabolic activity such as degradation of organic acids.

However, similar to the results in this study an increase of total titratable acidity level in guava fruits was also observed (Dube *et al*., 2015). In their study, the total titratable acidity increased until 120 days of storage, after which it declined. The increase of total titratable acidity is correlated to the undissociated organic acids level in the maturation of fruit which is stored in the vacuole of cell plants. When ripening stage begin, organic acids will be depleted as substrates in respiration. Therefore, this indicate that the samples were not fully ripen during harvesting.

Total titratable acid in guava coated with alginate and CBLP tended to have a lower rate of increase. This indicates that the edible coating with alginate and CBLP could decrease the respiration rate and suppress the use of organic acid, followed by maintaining the amount of total acid in guava throughout the storage time. According to Paul and Pandey (2014), fruit's deterioration rate is affected by O_2 and CO_2 gases' diffusion through the lenticel on the fruit's surface (Paul and Pandey, 2014). The O_2 gas entering the sample will increase the respiration rate, causing further deterioration. The sturdy edible coating of 2% alginate + 0.8% CBLP on the fruit's surface prevented gas diffusion, which resulted in no significant increase of total titratable acidity.

Organoleptic tests

The visual appearance of samples before and after 20 days of storage can be seen in figure 1. From the appearance after 20 days, samples with no edible coating applied showed yellowish color and went into deterioration stage. In contrast, samples with edible coating were visibly similar in appearance with light green color.

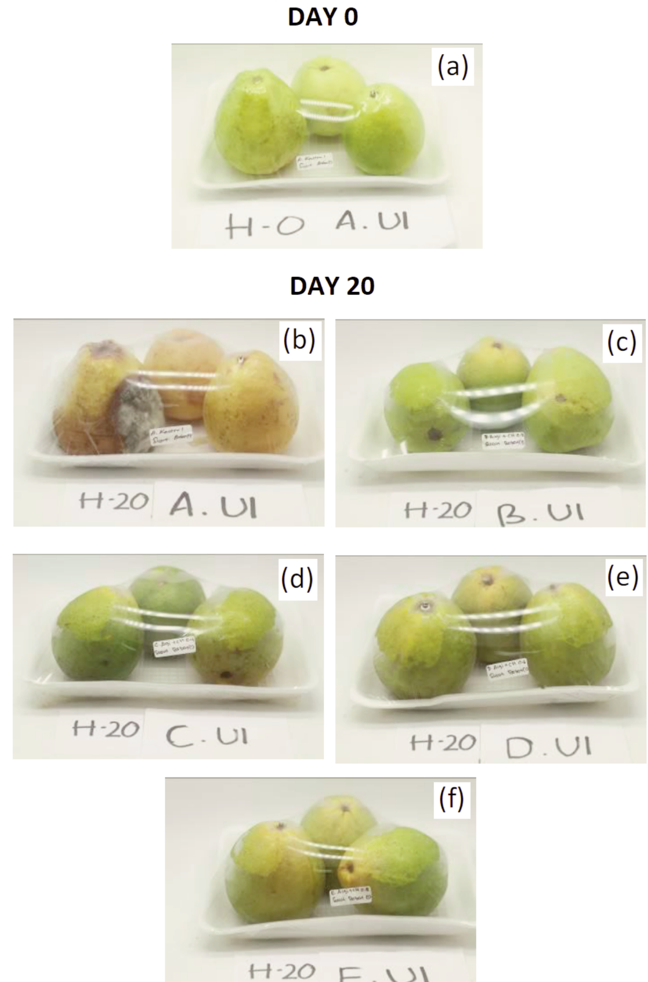

Fig. 1 - Visual appearance of guava fruit on day 0 (a) and after 20 days of storage with: no edible coating (b); 0.2% alginate + 0.2% Cyclea barabata leaves powder (CBLP) (c); 0.2% alginate + 0.4% CBLP (d); 0.2% alginate + 0.6% CBLP (e); 0.2% alginate + 0.8% CBLP (f).

For organoleptic testing, a hedonic test was conducted to evaluate the sensory qualities of guava fruits treated with different edible coating concentrations. The results of the analysis are shown in figure 2. Based on the organoleptic test, samples coated with 2% alginate + 0.8% CBLP had the best score in the color, taste, and texture parameters. This is because a higher CBLP concentration in the edible coating would further inhibit the respiration and transpiration rates. The best score for the aroma parameter was obtained by an edible coating with 0.2% CBLP. This is because the alcohol level due to fermentation in CBLP gave an unpleasant odor; thus, the lowest concentration gave the best score of

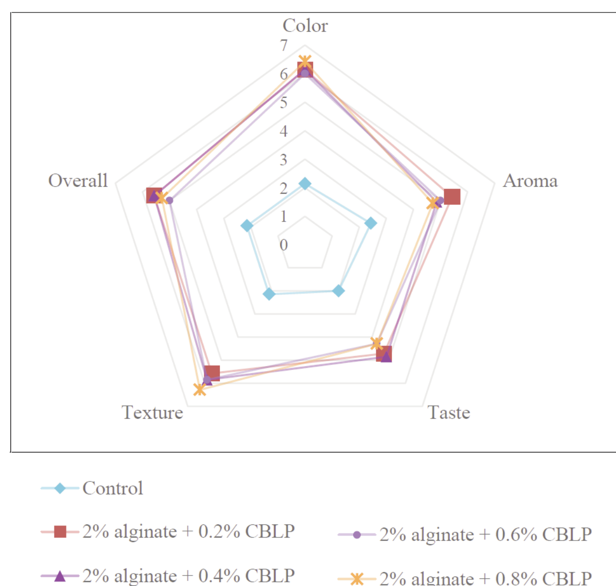

Fig. 2 - Result of organoleptic tests of guava treated with alginate-based edible coating enriched with different concentration of *Cyclea barabata* leaves powder (CBLP).

aroma quality. The highest overall score was obtained by two different concentrations of CBLP: 0.2% and 0.4%.

Moreover, edible coating with 0.4% CBLP had the highest score of taste.

In general, samples coated with alginate and CBLP had a higher score for sensory analysis compared to control samples. Similar results were obtained from fresh-cut mango coated with polysaccharide-based edible coatings: samples coated with alginate had the highest consumer acceptance due to their ability to prevent firmness loss (Salinas-Roca *et al.*, 2018). The maintenance of sensory quality by alginate-based edible coating on fruits were also found on strawberry, water apples and apple fresh-cut (Guerreiro *et al.*, 2015; Utama *et al.*, 2020; Marghmaleki *et al.*, 2020).

4. Conclusions

Different concentrations of CBLP in an alginate-based edible coating to extend the shelf-life of guava fruit were evaluated. The samples treated with 2% alginate and 0.8% CBLP had the significantly lowest level of total dissolved solids, total reducing sugar, and total titratable acidity and the highest scores on color, taste, and texture parameters in the organoleptic tests. From the analysis of firmness and organoleptic tests, the lowest firmness loss and highest score for aroma parameters were observed in samples treated with 2% alginate and 0.2% CBLP. Therefore, these results imply that the application of an edible coating based on alginate with the addition of CBLP can maintain the physicochemical and sensory properties of guava fruits.

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Salinity effects on growth, chlorophyll content, total phenols, and antioxidant activity in *Salvia lavandulifolia* Vahl.

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Key words: Biomass, electrolyte leakage, total flavonoids, water content.

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All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no competing interests.

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Abstract: Although the effect of salinity stress on some species of *Salvia* has been studied, so far no research has been done on *S. lavandulifolia* species. Therefore, a greenhouse pot experiment was carried out to investigate the impacts of salt stress on vegetative parameters, chlorophyll content, and antioxidants activity in *Salvia lavandulifolia* Vahl. Treatments included different irrigation water salinity levels (S0=1.3, S1=3.3, S2=5.3, S3=7.3, S4=9.3, S5=11.3, and S6=13.3 dS m^{-1}) which were arranged in a completely randomized design. The results showed that salinity treatments significantly affected the plant growth attributes. The lowest plant height, leaf number, leaf length, and shoot dry weight was recorded in the S6 treated plants with 62%, 41%, 44%, and 82% decrease compared to the control, respectively. Treatment of *S. lavandulifolia* plants with the highest salinity level (S6) decreased the content of chlorophyll a, chlorophyll b, and total chlorophyll by 57%, 53%, and 54% compared to the control, respectively. Salt stress at all levels increased the total phenolic content, and the highest value was obtained in the S6 treated plants. Free radical scavenging capacity was significantly increased by all the levels of salinity stress, and the highest (85.14%) value was obtained in the S6 treated plants. In general, *S. lavandulifolia* can be classified as a species-sensitive plant.

1. Introduction

The genus *Salvia*, belonging to the Lamiaceae family, has about 1000 species worldwide (Walker *et al*., 2004; Will and Claßen-Bockhoff, 2017). Different species of *Salvia* have various applications in the pharmaceutical and therapeutic industries due to their antibacterial, antifungal, antitumor, and antioxidant properties. It is traditionally used to treat bronchitis, colds, sore throats, gastrointestinal disorders, eczema, and tuberculosis (Li *et al*., 2013; Bahadori *et al*., 2015). Terpenoids and phenolic compounds are the main secondary metabolites of the genus *Salvia* (Lu and Foo, 2002). *Salvia lavandulifolia* Vahl. is a perennial herbaceous plant native to South France, Spain, and Northwest Africa. This species is well adapted to the semi-arid Mediterranean climate and grows up to 100 cm height and has opposite green or gray-white leaves. Several secondary

metabolites including polyphenolics, flavonoids, triterpenes and monoterpnes have been extracted from the aerial parts *S. lavandulifolia* (Amalia and Kintzios, 2005).

Salinity stress is considered one of the most significant environmental stresses that restrict the growth and yield of plants, especially in arid and semi-arid areas (Deng *et al*., 2015). In these areas, low rainfall, high evaporation, and poor drainage increase salt concentration in the soil and create salinity stress (Abdel Latef, 2010). Due to the scarcity or low quality (saline waters) of water resources worldwide, the management of crop production in saline conditions is critical. Salinity stress occurs with the accumulation of salts, especially sodium chloride, in the root zone. It causes disturbances in vital plant processes such as nutrient uptake and transport, transpiration, photosynthesis, and biosynthesis of primary and secondary metabolites (Valifard *et al*., 2014; Ahanger and Agarwal, 2017). Salinity stress impairs plant growth and development by increasing the osmotic potential of the soil solution, disturbing the nutrient balance, and the toxicity caused by the accumulation of sodium (Na^+) and chlorine (Cl^-) ions (Rehman *et al*., 2019). Salinity stress increases reactive oxygen species (ROS) in the cells that damage nucleic acids, proteins, and membrane lipids (Foyer, 2018). The decrease in growth, dry matter production, and yield were reported in most plants such as *Salvia hispanica,* feverfew (*Tanacetum parthenium* L.), and *Salvia splendens* due to salinity stress (Raimondi *et al*., 2017; Mallahi *et al*., 2018; Karimian *et al*., 2019). Karimian *et al*. (2019) reported that salt stress treatments (0, 20, 40, 60, and 80 mM NaCl) caused the decrease in growth parameters, relative water content, chlorophyll content and increase electrolyte leakage, total phenols and total soluble sugars in *Salvia splendens*. Gengmao *et al*. (2014) demonstrated that salt treatments less than 100 mM NaCl had no effect on growth parameters of *Salvia miltiorrhiza*, but significantly decreased the accumulation of dry matter. In *Salvia officinalis*, the decrease in plant height, chlorophyll content, and essential oil content were reported due to salinity stress (150 mM NaCl) (Es-sbihi *et al*., 2021).

Plants have developed different physiological, biochemical, and molecular mechanisms to deal with salinity stress (Zhao *et al*., 2020). Osmotic regulation is one of the mechanisms for maintaining cellular turgidity and membrane stability. In the osmotic regulation process, cellular concentrations of osmotically compatible solutes such as sugars increased (Chakhchar *et al*., 2015). Moreover, plants to deal with oxidative stresses enhance enzymatic and non-enzymatic antioxidant activities to reduce the deleterious effects of the ROS (Acosta-Motos *et al.*, 2017; Bayat and Moghadam, 2019).

The increasing population of the world coupled with the depletion of freshwater resources and the salinization of agricultural lands necessitates further studies on plants resistant to adverse environmental conditions. Although the effect of salinity stress on some species of *Salvia* has been studied (Valifard *et al*., 2014; Raimondi *et al*., 2017; Karimian *et al*., 2019), so far no research has been done on *S. lavandulifolia* species. Considering the medicinal importance of *Salvia lavandulifolia*, it is necessary to investigate the tolerance to salt stress. Hence, this study was aimed to study the effects of salt stress on vegetative and physiological indices and some secondary metabolites in *Salvia lavandulifolia*.

2. Materials and Methods

Plant materials and experimental design

This study was carried out in Research Greenhouse, Faculty of Agriculture, University of Birjand, Iran. *Salvia lavandulifolia* var. Lavandulifolia seeds were purchased from Jelitto Seed Co (Germany) and sown in 105 cell seedling trays in April 2017. Coco peat and peat with a ratio of 1:1 were used for the substrate. Irrigation was done daily during the seedling emergence and growth. After forty days, the seedlings were transplanted into 3-liter plastic pots at the 6-8 leaf stage. The physiochemical characteristics of the soil are given in Table 1 (Sparks, 1996). Organic matter (OM) was determined by the Walkley-Black method, and soil texture was mea-

Table 1 - Some physicochemical characteristics of the experimental soil sample

Texture	pH	EC dS m^{-1}	Organic matter	Field capacity (FC) (%)	N	K meq lit^{-1}	Ca meq lit^{-1}	Na meq lit^{-1}	Cl meq lit^{-1}	Mg meq lit^{-1}	Sodium adsorption ratio (SAR)
Sandy loam	7.9	1.3	0.3	17.8	0.02	8.27	8.61	23.1	25.3	1.49	10.28

sured by the hydrometer method. Soil pH and electrical conductivity (EC) were measured with pH meter (HANNA HI2211-02, USA) and EC meter (Jenway EC meter, Germany), respectively. Phosphorus (P), potassium (K), copper (Cu), zinc (Zn), iron (Fe), and manganese (Mn) were extracted by the Mehlich 1 extracting solution. Sodium and potassium concentrations were measured by a flame photometer. Phosphorus was determined colorimetrically, and Cu, Zn, Fe, and Mn were measured by atomic absorption spectroscopy. Calcium and Mg were extracted with 1 M potassium chloride and determined by titration with ethylenediaminetetraacetic acid (EDTA). Chlorine was determined by titration method. The SAR was calculated by computing Na^+, Ca^{2+} and Mg^{2+} concentrations (in meq/L) from the saturation extract. The experiment was conducted under greenhouse conditions at temperatures of 25/20°C and relative humidity of 50-60%.

The salinity stress started four weeks after the transplantation of seedlings into the pots. A completely randomized design with four replications was used to compare seven different irrigation water salinity treatments (S0=1.3, S1=3.3, S2=5.3, S3=7.3, S4=9.3, S5=11.3, and S6=13.3 dS m^{-1}). To prepare solutions S1, S2, S3, S4, S5, and S6, sodium chloride (NaCl) was dissolved in irrigation water in the amounts of 1.14, 2.18, 3.27, 4.43, 5.49, and 6.65 g, respectively. Some physicochemical parameters of control water (S0) were: EC= 1.3 dS m^{-1}, pH= 7.79, Na= 5.6 meq l^{-1}, Cl= 6.8 meq l^{-1}, and K= 0.35 meq l^{-1}. The pots were irrigated twice a week with saline water based on the field capacity by pot weighting. Salinity treatments were applied for one month, and then the traits were measured.

Growth indices

Plant height, leaf number, leaf length, leaf width, and maximum root length were measured. To determine the dry weight of shoots and roots, the samples were dried in an oven for 48 hours (78°C) (Bayat *et al*., 2016).

Relative water content (RWC) and electrolyte leakage (EL)

The leaf RWC was measured using the method reported by Gonzalez and Gonzalez-Vilar (2003) and calculated according to the formula:

$$RWC = (\text{Fresh weight} - \text{Dry weight}) / (\text{Turgid weight} - \text{Dry weight}) \times 100$$

Leaf dry weight was measured after oven-drying of the samples for 48 h (78°C). Turgid weight was determined after soaking leaves in distilled water in the refrigerator for 6 h.

Electrolyte leakage (EL) of the leaf was measured based on the method reported by Lutts *et al*. (1996) and calculated according to the formula:

$$EL = (EC1/EC2) \times 100$$

where EC1 and EC2 are the primary and secondary electrical conductivities, respectively. Fresh leaves (0.5 g) were dispensed with distilled water (10 ml) in test tubes and then were shaken for 24 hours (24°C). The EC1 was measured by the EC meter. The test tubes were then transferred to an autoclave (121°C) for 15 minutes and EC2 was determined.

Chlorophyll content and total soluble sugars

The pigments of fresh leaves (0.1 g) were extracted by 5 ml of acetone 80%. The amount of chlorophyll a and b were measured by a spectrophotometer (Model Unico 2100, China) at 645 and 663 nm (Arnon, 1949).

The content of leaf total soluble sugars was measured according to the anthrone method (Irigoyen *et al.*, 1992). For this purpose, 0.1 g of dried leaves was extracted with 1 ml of ethanol. Leaf sampling was performed at 10:00 AM.

Total phenols, total flavonoids, and free radical scavenging capacity (FRSC)

Fresh leaves (1 g) were homogenized in methanol for 24 h and then centrifuged at 6000 rpm for 15 min. The Folin-Ciocalteu method was used to measure the total phenolic content (Singleton and Rossi, 1965). Total flavonoids were determined based on the method of Yoo *et al.* (2008). The FRSC was determined using the method reported by Koleva *et al*. (2002) and calculated according to the formula:

$$FRSC = 1 - A\ \text{Sample}\ (517\ nm) / A\ \text{Control}\ (517\ nm) \times 100$$

Data analysis

The JMP 13 statistical software (SAS Campus, Cary, NC, USA) was subjected to analysis of variance of the data. The means were separated by the least significant difference (LSD) test at the 5% significance level.

3. Results

Growth attributes

The results demonstrated that the plant growth traits were significantly affected by increasing the

salinity of irrigation water. The lowest plant height, leaf number, leaf length, and leaf width values were recorded in the S6 treated plants by 62%, 41%, 44%, and 46% decrease compared to the control, respectively (Table 2). Salt stress affected the root length of *S. lavandulifolia* plants. Increasing salinity to S3 level had no significant effect on the root length, but its amount decreased with increasing salinity to S6 level (Table 2). Biomass production was significantly influenced by salt treatments. Increasing salt stress to S2 level had no significant effect on the root dry weight. However, with increasing salinity to S6 level, its values significantly decreased (Table 2). All salinity levels significantly reduced the shoot dry weight, and the lowest value was obtained from S6 treated plants with an 82% decrease compared to the control (Table 2). With increasing salt levels, total dry weight decreased significantly. Treatment of *S. lavandulia* plants with S6 decreased total dry weight by 78% compared to the control (Table 2). Salinity stress significantly affected shoot/root dry weight ratio of *S. lavandulifolia* plants. The highest and the lowest values of shoot/root dry weight ratio were obtained from the S4 and S6 treated plants, respectively.

The leaf RWC and EL

Salinity stress decreased the leaf RWC of *S. lavandulifolia* plants. The lowest leaf RWC was achieved in S6 treated plants by a 68% decrease compared to the control (Fig. 1A). The leaf EL significantly increased with increasing salinity stress levels. The lowest (19.34%) and the highest (86.15%) leaf El values were obtained from the S0 and S6 treated plants, respectively (Fig. 1B).

Chlorophyll content and total soluble sugars

The salinity effect was significant on the content of photosynthetic pigments. Treatment of *S. lavandulifolia* plants with the highest salinity level (S6)

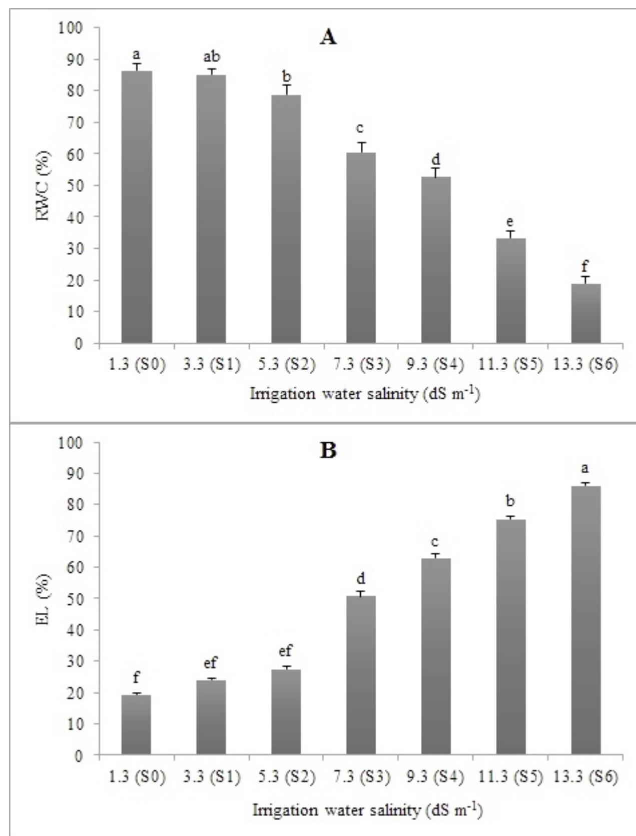

Fig. 1 - Effects of salinity stress on the leaf relative water content (RWC) and electrolyte leakage (EL) in *S. lavandulifolia*. Different letters indicate significant differences according to least significant difference (LSD) test at P<0.05. Values are mean ± standard error (SE).

Table 2 - Effects of different levels of irrigation water salinity on the plant height, number of leaves per plant, leaf length, leaf width, root length, root, shoot, and total dry weight, and shoot/root dry weight ratio of *S. lavandulifolia*

Irrigation water salinity (dS m^{-1})	Plant height (cm)	Number of leave per plant	Leaf length (cm)	Leaf width (cm)	Root length (mm)	Root dry weight (g. $plant^{-1}$)	Shoot dry weight (g. $plant^{-1}$)	Total dry weight (g. $plant^{-1}$)	Shoot/root dry weight ratio
1.3 (S0)	9.87 ± 0.31 a	52.50 ± 1.44 ab	5.32 ± 0.04 a	2.07 ± 0.04 a	35.51 ± 2.46 a	0.328 ± 0.02 a	0.45 ± 0.01 a	0.78 ± 0.01 a	1.42 ± 0.16 bc
3.3 (S1)	7.25 ± 0.25 b	52.76 ± 2.09 ab	4.92 ± 0.04 b	1.80 ± 0.07 b	33.00 ± 1.35 a	0.291 ± 0.01 a	0.33 ±0.01 b	0.62 ± 0.01 b	1.16 ± 0.06 bcd
5.3 (S2)	6.87 ± 0.42 b	57.25 ± 1.65 a	4.45 ± 0.18 c	1.77 ± 0.02 b	32.25 ± 0.75 a	0.284 ± 0.01 a	0.29 ± 0.02 c	0.57 ± 0.03 b	1.10 ± 0.05 cd
7.3 (S3)	6.62 ± 0.23 b	48.25 ± 1.10 b	4.27 ± 0.04 c	1.55 ± 0.06 c	31.50 ± 1.19 ab	0.164 ± 0.01 b	0.25 ± 0.01 d	0.41 ± 0.04 c	1.56 ± 0.14 ab
9.3 (S4)	5.37 ± 0.12 c	36.25 ± 1.18 c	3.12 ± 0.04 d	1.21 ± 0.04 d	26.51 ± 2.17 bc	0.113 ± 0.01 cd	0.21 ± 0.008 e	0.32 ± 0.007 d	1.91 ± 0.21 a
11.3 (S5)	4.62 ± 0.31 c	32.75 ± 4.30 c	3.02 ± 0.02 d	1.17 ± 0.04 d	25.01 ± 0.70 c	0.147 ± 0.01 bc	0.17 ± 0.004 f	0.31 ± 0.01 d	1.19 ± 0.11 bcd
13.3 (S6)	3.66 ± 0.16 d	30.50 ± 0.28 c	2.95 ± 0.05 d	1.10 ± 0.05 d	23.78 ± 2.47 c	0.094 ± 0.008 d	0.08 ± 0.004 g	0.17 ± 0.009 e	0.90 ± 0.14 d
Significance	**	**	**	**	**	**	**	**	**

Different letters indicate significant differences according to least significant difference (LSD) test at P<0.05.
** represent significant at 1% level of probability. Values are mean ± standard error (SE).

decreased the amount of chlorophyll a and b, and total chlorophyll by 57%, 53%, and 54% compared to the control, respectively (Table 3). Total soluble sugars were significantly affected by salt stress. By increasing the level of salt stress, the content of total soluble sugars increased upwards. The highest total soluble sugars were achieved by S6 treated plants with a 2.8 times increase compared to the control (Table 3).

Total phenols, total flavonoids, and the FRSC

Irrigation with saline water significantly affected the total phenols and total flavonoids of the leaves. All the levels of salt stress increased the total phenols, and the highest value was obtained in the S6 treated plants (Fig. 2A). The lowest total flavonoid content was obtained in the S1 treated plants (Fig. 2B). The FRSC was significantly increased by all the levels of salinity stress. The lowest (68.16%) and the highest (85.14%) leaf FRSC values were obtained in control and S6 treated plants, respectively (Fig. 3).

4. Discussion and Conclusions

The present results demonstrated that salt stress influenced the vegetative parameters in *S. lavandulifolia*. The negative impacts of salt stress on plant growth have been reported in *Salvia hispanica* (Raimondi *et al*., 2017), in *Salvia splendens* (Karimian *et al*., 2019), and *Salvia officinalis* L. (Es-sbihi *et al*., 2021). The decrease in growth parameters under salinity stress can be related to the reduction of soil water potential and toxicity of Na^+ and Cl^- ions, which leads to a nutritional imbalance (Kasrati *et al*., 2014; Es-sbihi *et al*., 2021). Salinity stress reduces cell division and elongation, thereby reducing plant growth (Netondo *et al*., 2004; Kamran *et al*., 2020). Moreover, the decrease in plant growth under salinity stress can be due to the reduction of photosynthesis and energy reserves. Usually, in saline conditions, the leaf stomata are closed, and the photosynthesis rate decreases due to reduced gas exchange (Chaves

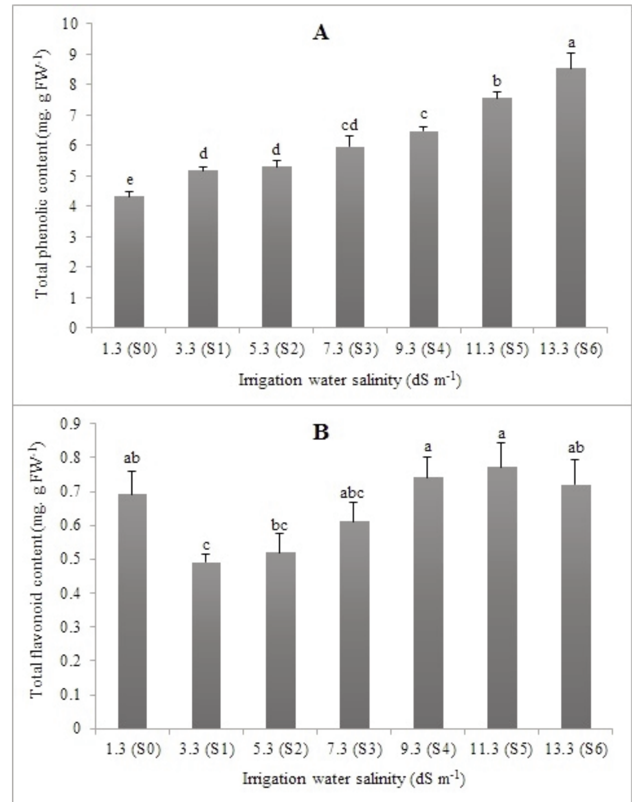

Fig. 2 - Effects of salinity stress on the leaf total phenolic and flavonoid content in *S. lavandulifolia*. Different letters indicate significant differences according to least significant difference (LSD) test at P<0.05. Values are mean ± standard error (SE).

Table 3 - Effects of different levels of irrigation water salinity on the chlorophyll a, chlorophyll b, total chlorophyll, and total soluble sugars in *S. lavandulifolia*

Irrigation water salinity (dS m^{-1})	Chlorophyll a (mg. g FW^{-1})	Chlorophyll b (mg. g FW^{-1})	Total chlorophyll (mg. g FW^{-1})	Total soluble sugars (mg. g DW^{-1})
1.3 (S0)	1.14 ± 0.05 a	0.58 ± 0.04 a	1.71 ± 0.09 a	7.62 ± 0.55 d
3.3 (S1)	0.79 ± 0.04 b	0.41 ± 0.03 b	1.21 ± 0.06 b	8.33 ± 0.91 d
5.3 (S2)	0.70 ± 0.05 bc	0.33 ± 0.01 bc	1.00 ± 0.04 cd	10.39 ± 1.11 cd
7.3 (S3)	0.70 ± 0.02 bc	0.35 ± 0.02 bc	1.05 ± 0.06 bc	11.69 ± 1.37 bc
9.3 (S4)	0.64 ± 0.03 c	0.30 ± 0.05 c	0.94 ± 0.06 cde	12.85 ± 0.48 bc
11.3 (S5)	0.51 ± 0.04 d	0.30 ± 0.03 c	0.79 ± 0.05 de	13.84 ± 0.85 b
13.3 (S6)	0.49 ± 0.01 d	0.27 ± 0.01 c	0.78 ± 0.11 e	21.61 ± 1.39 a
Significance	**	**	**	**

Different letters indicate significant differences according to least significant difference (LSD) test at P<0.05.
**= represent significant at 1% level of probability. Values are mean ± standard error (SE).

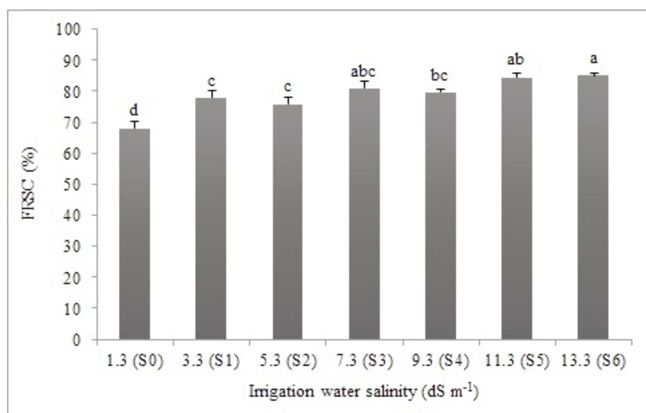

Fig. 3 - Effects of salinity stress on the leaf free radical scavenging capacity (FRSC) in *S. lavandulifolia*. Different letters indicate significant differences according to least significant difference (LSD) test at P < 0.05. Values are mean ± standard error (SE).

et al., 2009). Salinity can also inhibit root growth, thereby reducing the absorption and transport capacity of water to the shoot (Acosta-Motos *et al*., 2017).

Irrigation with saline water decreased the RWC of *S. lavandulifolia* leaves (except S1). The leaf RWC is commonly used to estimate the water status of plants under stress conditions (Parida and Das, 2005). Salinity decreases the leaf RWC due to a reduced availability of water from the soil solution as a result of lowered osmotic potential triggered by the toxic effects of the Na^+ and Cl^- ions (Munns, 2005; Álvarez *et al.*, 2012; Bayat *et al.*, 2012).

In this study, the EL increased with increasing salinity levels (except S1). Increased leaf EL under salinity stress has been reported in different crops (Bayat *et al*., 2013; Hniličková *et al*., 2019; Karimian *et al*., 2019). Electrolyte leakage is one of the standard parameters for examining salinity tolerance in plants. Salinity stress causes inefficiency of the leaf cell membrane and consequently increases membrane permeability for ions (Zhao *et al*., 2020).

In this experiment, the content of chlorophylls decreased with increasing salinity stress levels. Reduced leaf chlorophyll content under salt stress conditions has been reported in various crops (Taïbi *et al*., 2016; Rahneshan *et al*., 2018; Es-sbihi *et al*., 2021). Valifard *et al.* (2019) reported that photosynthetic pigments in *Salvia mirzayanii* leaves were decreased by increasing salinity stress. The decrease in chlorophyll content may be related to the toxicity effects of Na^+ and Cl^- ions, which prevent the formation of pigments (Yang *et al*., 2011). Decreased photosynthetic pigments under salinity stress can be mainly due to the destruction of their structure with the ROS and inhibition of biosynthesis of new chlorophylls (Ashraf, 2003; Yang *et al*., 2020).

In this study, irrigation with saline water enhanced the content of leaf total soluble sugars in *S. lavandulifolia*. Accumulation of leaf soluble sugars under salinity stress has been reported in sunflower (Zheng *et al*., 2010), in *Salvia miltiorrhiza* L. (Gengmao *et al*., 2014), and cotton (Peng *et al*., 2016). Karimian *et al*. (2019) reported that salt stress significantly increased total soluble sugars in the leaves of *Salvia splendens.* Increased the content of soluble sugars is an indicator for osmotic regulation under stress conditions, to maintain cell turgor and continued water influx (Mittal *et al.*, 2012). Soluble sugars were accumulated under salinity stress and protect plants through osmotic regulation, maintenance of turgor pressure, and preservation of membrane and protein stability (Bayat *et al*., 2013; Nounjan *et al*., 2018). The increase in concentration of soluble sugars under stress conditions is due to the higher activity of enzymes such as phosphorylase starch and sucrose phosphate synthase (Peng *et al*., 2016).

Salinity stress significantly affected the total phenols, total flavonoids, and the FRSC of *S. lavandulifolia* leaves. Various studies have reported the increment in total phenols, total flavonoids, and the FRSC in response to salt stress (Karimian *et al*., 2019; Sirin and Aslım, 2019). Valifard *et al*. (2014) reported the total phenols and antioxidant activity in *Salvia mirzayanii* were increased by salinity stress. Salt stress causes the production of ROS, which damages proteins, lipids, and nucleic acids (Foyer, 2018). Plants use antioxidant defense systems to scavenge and detoxify these compounds from the cell surface, which leads to increased plant antioxidant activity (Rezayian *et al*., 2018; Bayat and Moghadam, 2019). Phenols and flavonoids are secondary metabolites that act as potent antioxidants against oxidative stress. These non-enzymatic antioxidants protect plants by increasing their osmotic potential and thereby avoiding the dehydration of cells or regulating the redox potential, and depleting the ROS (Bautista *et al*., 2016; Yan *et al*., 2017).

Although the effect of salinity stress on some species of *Salvia* has been studied, so far no research has been done on *S. lavandulifolia* species. The results demonstrated that salt stress had adverse effects on the growth parameters, photosynthetic pigments,

and cell membrane stability of the *S. lavandulifolia* plant. However, the total phenolic content and antioxidant activity of the leaves were increased under salinity stress conditions. In general, *S. lavandulifolia* can be classified as a species-sensitive plant. However, further experiments are needed to investigate other mechanisms of salt stress tolerance.

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Short note

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Observation of unexpected neo like-fruit development from *Cakile maritima* calli

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Key words: Cell differentiation, *in vitro* culture, totipotency.

Abstract: Parthenocarpy, the ability of some plants to undergo fruit growth in absence of fertilization, is an important question of basic science and the subject of much interest due to its possible agricultural benefits. In the context of our cellular biology studies on a halophyte of interest, *Cakile maritima,* we generated calli, pluripotent cell masses, that unexpectedly allowed the appearance of parthenocarpic fruits without any floral tissues. These observations raise the hope to develop an *in vitro* model to study parthenocarpic fruit development.

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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1. Introduction

For several years, we are studying *Cakile maritima*, a promising model of halophyte in a worldwide context of increasing salinization of lands due to climate change (Arbelet-Bonnin *et al*., 2019). In addition to whole plant studies (Debez *et al*., 2006; Ellouzi *et al*., 2014; Ben Hamed-Louati *et al*., 2016 b; Arbelet-Bonnin *et al*., 2020) we underwent cellular biology studies (Ben Hamed-Laouti *et al*., 2016 a; Arbelet-Bonnin *et al*., 2018) for which we have developed a *C. maritima* cultured cell suspension that starts by calli generation as a first step (Ben Hamed *et al*., 2014). It is now admitted that *in vitro* cultured cells do not undergo a full dedifferentiation but rather a transdifferentiation, leading to increased developmental potency and/or cell proliferation (Sugimoto *et al*., 2011; Fehér, 2019). The supposed totipotency capacity of plant cells (Haberlandt, 1902) is largely operated for the *in vitro* culture of plants. Calli are thus frequently considered as transient tissue dedicated to somatic embryogenesis allowing the plant regeneration (Sugimoto *et al*., 2011; Fehér, 2019). Interestingly, hormonal balances play an important role in many developmental processes in plant (Molesini *et al*., 2020), and in particular the balance between auxin and cytokine seems to be crucial during *in vivo* parthenocarpic fruit development (Pandolfini, 2009; Joldersma and Liu, 2018; An *et al*., 2020; Sharif *et al*., 2022).

Parthenocarpy is the ability of some plants to undergo fruit growth in absence of fertilization. Parthenocarpy has contributed to some of humanity's domestication of plants such as breadfruit or banana (Zerega *et al*., 2004; Kislev *et al*., 2006; Sardos *et al*., 2016). Furthermore, it represents a highly desirable trait in agronomy since seedless fruits are highly

appreciated by consumers and fruit set is less affected by environmental factors in absence of fertilization (Ruan *et al*., 2012). Therefore, parthenocarpy is the subject of much interest and leads to many studies (Sharif *et al.*, 2022). However, to our knowledge, all studies published so far have been conducted in whole plants but not at the level of cell cultures so far.

We report here the first appearance of neo-like fruits directly on calli obtained and long maintained on callus-inducing medium (CIM). These observations raise the hope to develop a cellular model that will allow *in vitro* studies of parthenocarpic fruit development.

2. Materials and Methods

Plant material and establishment of calli

Cakile maritima seeds used in this study come from Raoued (North of Tunisia). The seeds cultivation *in vitro* conditions were established as described by Ben Hamed *et al*. (2014). Briefly, bleached seeds were placed in petri-dishes containing Murashige and Skoog medium (1962) hormone-free (MS, Sigma), supplemented with 30 g.L^{-1} sucrose, 8 g.L^{-1} agar and the pH was adjusted to 5.8. This medium induced seed germination under a light cycle of 12 h light and 12 h dark with 40 µE m^2 s^{-1} at 22°C. The stems of 14-days-old seedlings were then chopped finely and placed on an agar-callus-induced medium (CIM) (Valvekens *et al.*, 1988) and then put in a growth chamber, in the same conditions that for seed germination. CIM medium contain 6.2 g.L^{-1} Gamborg B5 (Gamborg *et al.*, 1968) from Sigma supplemented with 20 g.L^{-1} glucose, 8 g.L^{-1} agar and phytohormones : cytokinin and auxin (Valvekens *et al*., 1988; Akama *et al*., 1992). The precise hormone balance depends of plant species (Pacheco *et al.*, 2012; Thomas and Hoshino, 2015). For *C. maritima* we used 9.06 µM of 2,4-dichlorophenoxyacetic acid (2.4 D) and 0.46 µM of kinetin (Kn) that were shown to be efficient (Ben Hamed *et al*., 2014). The pH was adjusted to 5.7. After two weeks, calli were formed from the fragments of seedlings tissues. When the size of the calli reached 1 cm in length, they were subcultured on a fresh CIM medium.

For some experiments, the hormonal balance was modified with 9 µM of Kn and 2-4D, or 0.46 µM of Kn and 2-4D and calli were grown during two months. To assess the putative role of volatile organic compounds (VOCs) emitted from mature fruit, a surface sterilized fruit of *C. maritima* was added in petri-dishes with calli during 2 months.

3. Results and Discussion

Cakile maritima calli were subcultured every four weeks on the CIM medium, otherwise they began to brownish and lose their ability to be sub-cultured. During a callus subculture, a spontaneous green excrescence from calli easily visible (Fig. 1a) was observed. As the subcultures went along on fresh medium CIM, green excrescences grew on calli and more excrescences appeared on different calli (Fig. 1b and 1c). Each appeared excrescence grew all-long the time (Fig. 1d).

After 58 days, numerous green structures resembling *C. maritima* differentiated tissues arise (Fig. 2). 40% of them are clearly green differentiated tissue although we cannot identify specific organ structures (Fig. 2A, B). On the contrary, the other 60 % of green differentiated structures resemble *neo* like-fruits (Fig. 2C-E). Indeed, they present two asymmetric segments reminiscent of the typical dimorphic fruit that

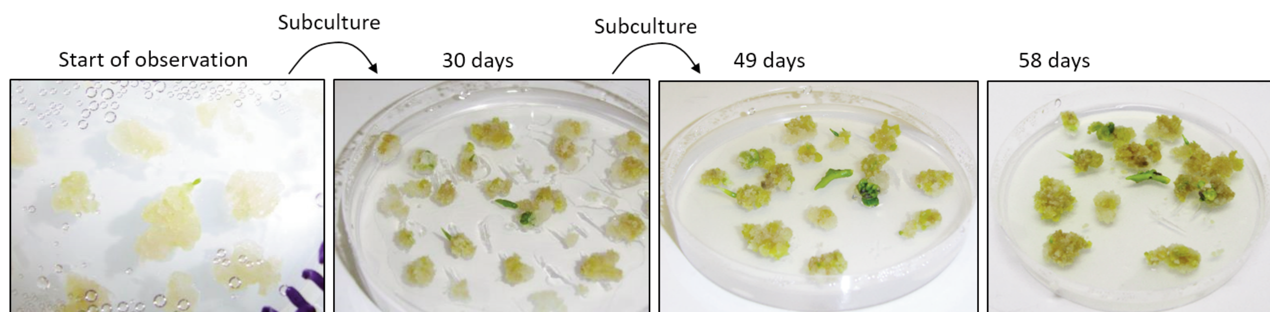

Fig. 1 - Emergence and development of parthenocarpic fruits on *Cakile maritima* callus. First observation (a), observations after 30 days (b); 49 days (c) and 58 days (d).

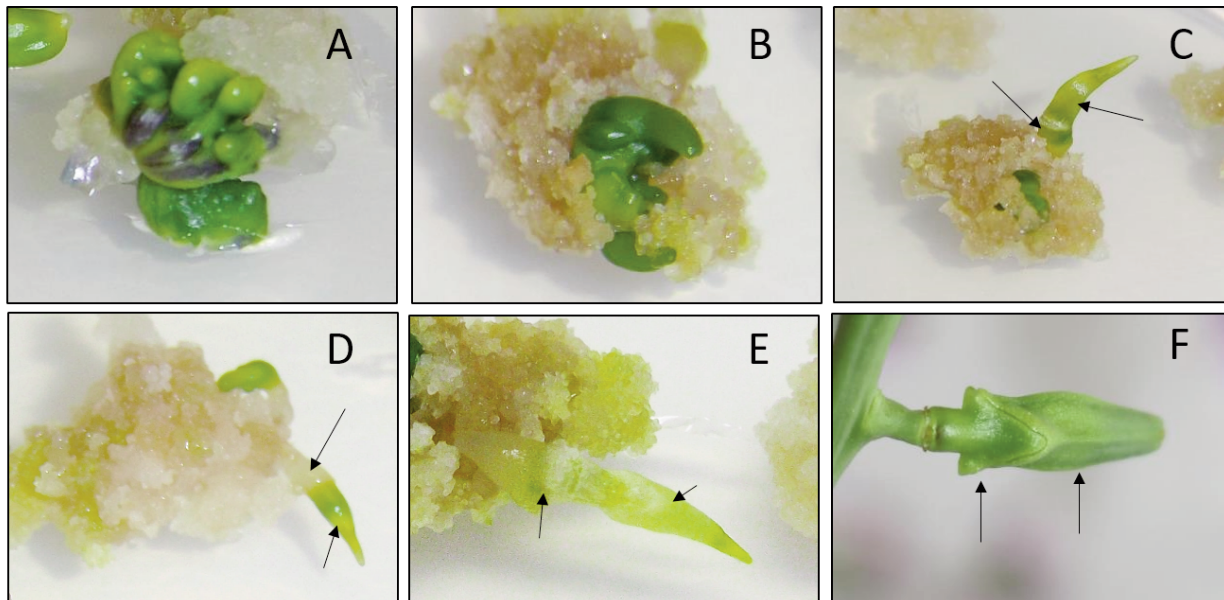

Fig. 2 - Various green structures observed on *Cakile maritima* callus. A-B. Undefined differentiated green structures. C-E. Typical dimorphic fruit like structures. F. Typical dimorphic fruit developed on a whole plant of *C. maritima* (arrows indicated the two asymmetric segments).

develops on whole plant *of C. maritima* (Fig. 2F). These *neo* like-fruits did not contain seeds and are thus parthenocarpic. Such parthenocarpic fruits have never been reported in *C. maritima* in the literature. More interestingly, this represents not only the first report of parthenocarpic fruits forming from calli, but also forming from non-floral tissues. These parthenocarpic fruit appeared without any change in the hormone balance, a mean generally used to triggered shoot or root regeneration *in vitro* in the context of somatic embryogenesis and plant multiplication (Thomas and Hoshino, 2015; Das *et al.*, 2018; Shin *et al.*, 2020).

Parthenocarpy can be artificially obtained by applying synthetic growth factors to unpollinated ovaries (Pandolfini, 2009; Molesini *et al*., 2020). Auxins seem to play a prominent role in triggering and coordinating the transition from flower to fruit, and exogenous supplies of auxins to unpollinated flowers could induce fruit growth in various plants, suggesting that these hormones can replace the signals provided by pollination and fertilization (Pandolfini, 2009; Molesini *et al*., 2020). Cytokinin could also induced parthenocarpy but probably through modulation of auxin metabolism (Molesini *et al*., 2020; Sharif *et al.*, 2022). We tried to modify the hormones concentrations and the balance between Kn and 2-4D by using 0.46 µM or 9 µM of both of these hormones but unfortunately, no new spontaneous green excrescences development were observed on the 20 calli present on each petri dish (n=2 per conditions). Since we observed a multiplication of these parthenocarpic fruits in the same petri-dishes, we asked ourselves if this phenomenon could be reminiscent of fruit ripening triggered by volatiles organic compounds (VOCs) such as ethylene released from already developed fruits (Tohge *et al*., 2014). Even if the role of ethylene still appeared unclear (Sharif *et al.*, 2022), ethylene responses could also lead to parthenocarpic fruit development (Pandolfini, 2009). We thus put a mature *C. maritima* fruit harvested from a fully developed plant in a petri-dish with calli, as a putative VOCs furnisher to calli. One more time no excrescences appeared on the 20 different calli present in the petri dish. Although we have no clear explanation at the moment, the question of genetic homogeneity of callus cells is not solved and only certain cells of a callus could be regarded as totipotent and thus involved in organ regeneration (Fehér, 2019). Moreover, this ability to develop parthenocarpic fruits disappeared after three months and more subcultures. This suggests that only freshly prepared calli are able to develop parthenogenetic fruits. Accordingly, if callus tissue can express a wide variety of genes especially at the early phase of their development, their transcriptome seems to be homogenize along time (Fehér, 2019).

Although we cannot control the appearance and development of parthenocarpic fruits on calli at this time, these data certainly, deserve more investigations. Recent genomic studies have greatly contributed to elucidate the role of phytohormones in regulating fruit initiation, providing at the same time genetic methods for introducing seedlessness in horticultural plants. Moreover, that some plants may produce fruit without the need for fertilization by the male gamete remains an important question of basic science. Therefore, the development of an *in vitro* model of parthenocarpic fruits will certainly be an essential tool to understand how a fruit could form without the need of fertilization nor floral tissues.

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