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Arbuscular mycorrhizal isolate and phosphogypsum effects on growth and nutrients acquisition of cotton (*Gossypium hirsutum* L.)

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Key words: Arbuscular mycorrhizal fungi, *Gossypium hirsutum*, indigenous, phosphogypsum.

Abstract: Cotton was grown in pots with added phosphogypsum (PG) to evaluate the effect of indigenous arbuscular mycorrhizal fungi (AMF) and phosphogypsum on cotton growth and acquisition of phosphorus (P), potassium (K), calcium (Ca), manganese (Mn), iron (Fe), copper (Cu), and zinc (Zn). AMF isolate was a mixture of *Glomus intraradices*, *Glomus viscosum*, and *Glomus mosseae* previously isolated from a cotton field. Shoot dry biomass was enhanced significantly by both indigenous AMF and PG. Shoot dry biomass and seed cotton yields were enhanced by the AMF and PG combination and even more when PG in compost was added to mycorrhizal plants. P content in AMF with PG and in AMF with PG/compost treated plants was, respectively, 209.3 and 278.7%, significantly higher than control. Acquisition of K, Ca, and micronutrients was significantly enhanced by the combination of AMF and PG. The treatment of AMF with PG/compost induced the highest contents in Mn, Fe, Cu and Zn which were found to be, respectively, 287, 201, 192.8, and 171% higher compared to control. Results indicate that cotton growth responded to indigenous AMF in soils amended with PG. Combination of AMF with PG added in compost can ensure satisfactory benefits for cotton growth in low input, sustainable cropping systems.

1. Introduction

Acquisition of mineral nutrients is important to plant growth and productivity. The ability of plants to acquire nutrients may be associated with root colonization with arbuscular mycorrhizal fungi (AMF) (Clark and Zeto, 1996). AMF are obligatory biotrophic symbionts occurring in nearly all natural and agricultural soils and commonly colonize roots of many plant species (Smith and Read, 1997). Acquisition of mineral nutrients by plants with AMF depends on factors such as soil pH, soil nutrient deficiencies, AMF isolate, and plant species (Sylvia and Williams, 1992). Previous studies showed a positive response of cotton to AMF (Liu *et al.,* 1994; DeFeng *et al.,* 1998; Ibrahim, 2010). In subsistence agriculture systems, it is important to use indigenous AMF that are ecotypically adapted to the site (Davies *et al.,* 2005). Native AMF can grow and function better in soils from which they are isolated, e.g. agricultural systems (Calvente *et al.,* 2004).

Phosphogypsum (PG) is the main by-product of the industrial production of phosphoric acid by treatment of rock phosphate with sulfuric acid. Calcium sulfate is the dominant component in PG. PG contains the radioactive materials ^{226}Ra and ^{210}Po, phosphorus, silicon, Fe, Cu, and F^- (Al-Masri *et al.,* 2004). Studies suggest that PG can be used in the improvement of soil structure, plant growth and agricultural production (Alcordo and Rechcigl, 1993), enhancing seedling emergence (Vyshpolsky *et al.,* 2010), and increasing available S and P (Al-Oudat *et al.,* 1998). The use of PG as a fertilizer in agriculture has been practiced in many parts of the world (Enamorado *et al.,* 2009) without constituting environmental hazards to soil and crop tissue (Al-Oudat *et al.,* 2011).

Application of PG (a poorly soluble source of P) to soil may become available to plants by solubilization from AMF (Al-Karaki and Al-Omoush, 2002). Solubilization of PG might insure a continuous supply of P without inhibiting root AMF colonization (Cui *et al.,* 2014). In addition, enhanced acquisition of nutri-

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ents by AMF plants in combination with PG has been reported (Al-Karaki and Al-Omoush, 2002; Bai *et al.,* 2011).

Compost increases soil organic carbon (El Mrabet *et al.,* 2014) and could increase the release of micronutrients in the soil, making them more available to plants (Habashy *et al.,* 2008; Jan *et al.,* 2014). On the other hand, addition of PG during manure composting decreased the amount of ammonia lost by volatilization (Prochnow *et al.,* 1995). Therefore, addition of compost and the introduction of mycorrhizal technology may become an effective way of applying PG to soils with P deficiency.

The objective of this research was to determine the effects of indigenous AMF, in combination with Syrian PG alone or integrated in compost, on growth and mineral nutrients acquisition of a Syrian cotton variety.

2. Materials and Methods

The experiment was conducted in pots during the summer season (May-September 2013) at Der-Alhajar research Station, located southeast of Damascus, Syria (33°21' N, 36°28' E) at 617 m above sea level. The area is located within an arid region in which the total annual precipitation is 120 mm. Sandy clay loam soil was air dried, sieved to pass a 3 mm screen, and pasteurized at 5 kGy of gamma ray (GR) with $_{60}$Co source using a gamma irradiator (ROBO, Russa).

Phosphogypsum (PG) was previously collected from the area near the phosphoric acid factory in Homs (180 km N of Damascus). A total of 20 composite samples of PG were obtained from the levels corresponding to pile ages of approximately 1, 3-6 and 7-12 years, from the top, middle and bottom layers, respectively (each sample weighed 1 kg). PG samples were ground, homogenized, and sieving through a 0.5 mm sieve. Before planting, soil was mixed with PG alone (at a rate of 30 g kg^{-1} dry soil) and PG integrated in compost (PG/compost mixture was prepared to be applied at a rate of 30 g of compost plus 30 g of PG per kg dry soil). Compost was pasteurized at 120°C for 20 min in the autoclave. Apart from PG, no chemical fertilizers were added during the experiment.

Mycorrhizal inoculum was a mixture of *Glomus intraradices* (Schenck & Smith), *Glomus viscosum* (Nicolson), and *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe. AMF inoculum was previously isolated (Ibrahim, 2010) from a cotton field at Der-Alhajar Research Station and multiplied in pot cultures using onion (*Allium cepa*) as a host. The inoculum consisted of fragments of onion root and spores mixed with soil.

Half of the pots were inoculated with AMF inoculum (100 g per pot) at the time of sowing. Non-mycorrhizal pots were prepared by mixing the same amount of sterilized AMF inoculums.

The treatments were: T1, no AMF inoculation without PG; T2, AMF inoculation without PG; T3, no AMF inoculation with PG; T4, AMF inoculation with PG; T5, no AMF inoculation with PG/compost; and T6, AMF inoculation with PG/compost.

Seeds of cotton (*Gossypium hirsutum* cv. Aleppo 33/1) were sterilized in 20% NaClO for 1 min and subsequently rinsed with sterilized water. They were then sown five per pot and placed to grow under natural conditions. Ten days after emergence, seedlings were thinned to one per pot; the roots of discarded plants were left in the soil to avoid removing the AMF inoculum. Plants were watered with tap water as needed. Growth parameters, such as plant height and fresh weight, were measured at the physiological maturity stage. The total shoot dry weights were measured after oven drying to constant weight at 70°C. Boll weight and number of mature bolls per plant at the first handpicking were recorded. The seed cotton yield and percent lint of each plant was determined at one handpicking for all treatments.

The vegetative portion of the plants was ground to a fine powder (0.5 mm). Nitrogen was determined using the Kjeldahl method and phosphorus was determined colorimetrically using a spectrophotometer (Thermo Spectronic, UK), while determination of Ca, K, Fe, Zn, Cu, Mn was performed by x-ray fluorescence (XRF). Root samples were rinsed free of soil, cut into 1 cm fragments, thoroughly mixed, cleared with KOH and stained with acid fuchsin in lactoglycerol. Percent root colonization and percent root length colonized by AMF were determined microscopically using a gridline intercept method (Giovannetti and Mosse, 1980).

The experimental design was randomized complete blocks with four replications. Data were subjected to analysis of variance by the SAS program (SAS Institut Inc, 2004) and means were compared using the Least Significant Difference (LSD) test at a probability level of $P \leq 0.05$.

3. Results

Addition of PG to the soil significantly decreased pH (Table 1). AMF root colonization was between 22.8 and 30.8% regardless of PG addition; the percent was higher for plants grown without added PG than added PG alone. Adding PG in compost increased the percent of AMF root colonization of cotton plants (Table 1). The percentage of mycorrhizal root length was 66.3, 54.5 and 73.3% in the AMF plants, AMF plus PG, and AMF with PG/compost treated plants, respectively (Table 1). No AMF root colonization was noted for plants grown without AMF.

Table 1 - Experimental soil pH, mycorrhizal root colonization, and some growth parameters of cotton plants grown with different treatments of arbuscular mycorrhizal fungi (AMF) and added phosphogypsum (PG)

Treatment	AMF colonization (%)	Mycorrhizal root length (%)	Soil pH	Plant height (cm)	Fresh biomass (g plant^{-1})	Dry biomass (g plant^{-1})
Control	0	0	8.1 a	38.3 e	141.72 c	44.40 e
AMF	24.60 b	66.30 b	8.1 a	55.0 d	256.30 ab	70.38 cd
PG	0	0	7.5 b	58.0 cd	240.86 b	65.79 d
AMF+PG	22.80 bc	54.50 bc	7.5 b	62.0 bc	295.87 a	78.05 b
PG/compost	0	0	7.2 c	64.3 b	255.43 ab	72.97 bc
AMF+PG/compost	30.80 a	73.30 a	7.1 c	73.8 a	316.52 a	96.64 a

Mean values within columns followed by different letters are significantly different at P<0.05.

Significant differences between mycorrhizal and nonmycorrhizal plants were noted for shoot dry biomass regardless of PG addition (Table 1). Shoot dry biomass was significantly enhanced by indigenous AMF and when the mixture of PG/compost was added to soil. Application of PG to soil significantly increased shoot dry biomass for both mycorrhizal and nonmycorrhizal plants. Shoot fresh weight significantly increased in AMF inoculated and PG treated plants in comparison to the control (Table 1). Plant height at harvest was between 38.3 and 73.8 cm, and it was significantly enhanced by AMF inoculation and PG addition compared to control. The maximum plant height was observed for mycorrhizal cotton plants grown with PG/compost mixture.

The growth response of cotton plants to AMF, PG, and AMF plus PG treatments increased by 59.6, 49.4, and 76.3% over control, respectively (Table 2). In addition, the growth response of cotton to the combination of AMF with PG/compost was higher by 118.9% over control (Table 2).

Yield components of cotton under different treatments are shown in Table 2. The number of bolls per plant was significantly increased by both AMF and PG in comparison with control. The plants showed the highest number of bolls when PG/compost mixture was added to AMF plants (T6). The increase in boll number led to an increase in seed cotton yield, which was improved by both AMF and PG. Seed cotton yield varied between 50.83 g plant^{-1} (4574 kg ha^{-1} on the basis of a density of nine plants m^{-2}) and 18.38 g plant^{-1} (1654 kg ha^{-1}). The highest seed yield of cotton was observed in AMF plants with added PG/compost. Boll weight was generally higher in AMF plants and PG treated plants than control and it was significantly higher with the AMF plus PG/compost treatment compared to other treatments. Percent lint varied between 47.3 and 34.3%, and AMF inoculation increased it significantly. In addition, lint percentage was significantly increased by PG and this increase was clearly noted when PG was contained in compost.

Table 2 - Growth response and some yield components of cotton plants grown with different treatments of arbuscular mycorrhizal fungi (AMF) and added phosphogypsum (PG)

Treatment	Growth response (%)	Boll number (per plant)	Boll weight (g)	Seed cotton yield (g plant^{-1})	Lint (%)
Control	0	5.3 c	4.9 c	18.4 d	34.3 f
AMF	59.6 bc	7.8 b	5.1 b	32.9 c	37.9 e
PG	49.4 c	7.3 b	5.2 b	29.8 c	42.3 d
AMF+PG	76.3 b	8.3 b	5.3 b	36.9 b	42.9 c
PG/compost	64.7 bc	8.0 b	5.5 b	36.5 b	46.4 b
AMF+PG/compost	118.9 a	9.5 a	6.1 a	50.8 a	47.3 a

Mean values within columns followed by different letters are significantly different at P<0.05. Values are mean (*N*= 4).
Growth response (%)= (DW_{AMF} - $DW_{control}$) x100/$DW_{control}$.
Lint (%)= (lint weight/seed cotton weight) x 100.
Seed cotton= seed + lint.

N and P concentrations in the vegetative portions of plants were significantly affected by AMF inoculation (Table 3, Fig. 1). The concentrations of N and P

Table 3 - Concentrations of K, Ca, and micronutrients in vegetative portion of cotton plants grown with different treatments of arbuscular mycorrhizal fungi (AMF) and added phosphogypsum (PG)

Treatment	K (mg g^{-1} DM)	Ca (mg g^{-1} DM)	Mn (µg g^{-1} DM)	Fe (µg g^{-1} DM)	Cu (µg g^{-1} DM)	Zn (µg g^{-1} DM)
Control	22.28 c	30.42 e	85.05 c	593.8 d	3.63 d	15.23 d
AMF	28.57 b	33.44 d	127.75 b	676.3 c	4.62 c	18.78 b
PG	23.19 c	34.65 d	104.75 bc	688.3 c	3.78 d	16.28 cd
AMF+PG	29.44 b	40.28 b	154.75 a	797.5 b	4.69 c	20.58 a
PG/compost	29.61 b	36.72 c	116.25 b	766.0 b	5.12 bc	16.68 c
AMF+PG/compost	32.67 a	43.61 a	172.75 a	930.8 a	5.59 a	21.88 a

Mean values within columns followed by different letters are significantly different at P<0.05.

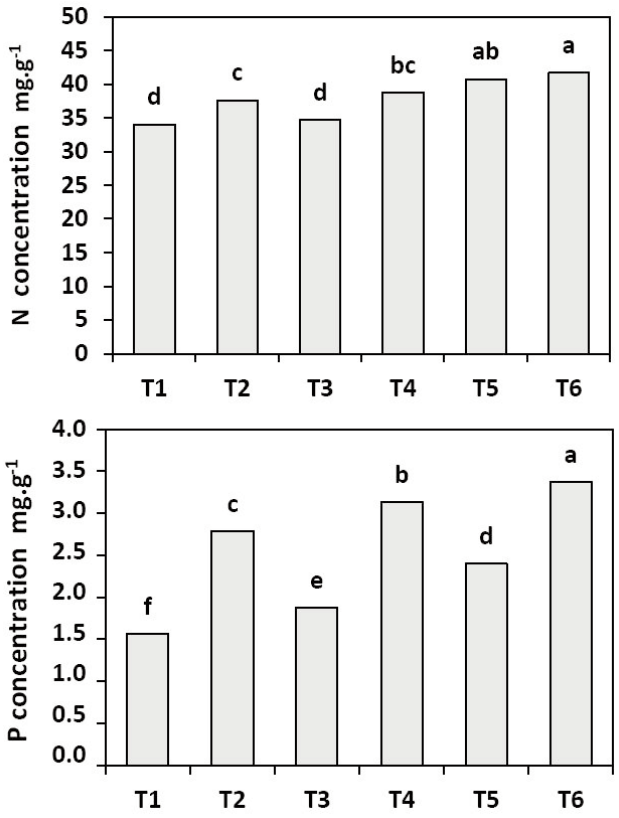

Fig. 1 - Concentrations of N and P in vegetative portion of cotton plants grown at different treatments (T1= no AMF without PG; T2= AMF without PG; T3= no AMF with PG; T4= AMF with PG; T5= no AMF with PG/compost; and T6= AMF with PG/compost).

were generally higher for mycorrhizal than for nonmycorrhizal plants. PG significantly increased N concentration only when combined with compost for nonmycorrhizal (T5) and mycorrhizal plants (T6), while it significantly increased P concentration in nonmycorrhizal and mycorrhizal plants.

The role of the combination of AMF and PG in improving plant P content was noted (Table 4). Data indicate that maximum plant P contents of 107.8 and 131.9 mg plant^{-1} were found in AMF plus PG and AMF with PG/compost treatments, respectively, which was significantly higher ($P \leq 0.05$) by 209.3 and 278.7%, respectively, over control (Table 5).

The concentrations of K and Ca were significantly higher in the vegetative portion of mycorrhizal compared to nonmycorrhizal plants regardless of PG (Table 3). PG significantly increased Ca concentration in nonmycorrhizal and mycorrhizal plants; added alone it had no significant effect on K concentration in either group. The concentrations of both elements increased significantly when PG/compost mixture was added to mycorrhizal plants.

Higher concentrations of Mn, Fe, Cu, and Zn were noted for mycorrhizal than for nonmycorrhizal plants (Table 3) and PG significantly increased Fe concentration. Mn and Zn concentrations were increased by PG only in AMF plants while PG had no significant effect

Table 4 - Contents of nutrients in cotton plants grown with different treatments of arbuscular mycorrhizal fungi (AMF) and added phosphogypsum (PG)

Treatment	Nutrient content (mg plant^{-1})							
	N	P	K	Ca	Mn	Fe	Cu	Zn
Control	708.0 e	35.2 e	465.3 e	634.0 e	1.77 d	12.5 d	0.08 d	0.32 e
AMF	1165.7 c	86.5 c	884.8 c	1036.8 c	3.97 c	21.0 c	0.14 c	0.58 c
PG	890.4 d	45.2 d	595.0 d	887.5 d	2.69 d	17.6 c	0.10 d	0.42 d
AMF+PG	1331.8 b	107.8 b	1015.0 b	1388.5 b	5.35 b	27.5 b	0.16 c	0.71 b
PG/compost	1402.7 b	82.3 c	1013.7 b	1262.0 b	4.02 c	26.5 b	0.18 bc	0.57 c
AMF+PG/compost	1627.8 a	131.9 a	1276.5 a	1705.1 a	6.76 a	36.4 a	0.22 a	0.85 a

Mean values within columns followed by different letters are significantly different at $P<0.05$.

Table 5 - Percentage change in nutrient contents (NC) due to PG amendment and AMF inoculation of cotton plants

Treatment	Nutrient content change (%)							
	N	P	K	Ca	Mn	Fe	Cu	Zn
AMF	65.4 c	148.5 c	91.8 b	65.2 c	128.6 c	73.3 bc	90.6 b	85.0 c
PG	25.9 d	30.0 d	28.2 c	40.6 c	56.2 d	44.4 cd	29.3 c	31.8 d
AMF+PG	89.1 bc	209.3 b	119.1 b	120.9 b	207.3 b	126.3 b	115.6 b	123.9 b
PG/compost	97.7 b	135.6 c	118.5 b	100.0 b	129.2 c	115.3 b	87.4 b	80.4 c
AMF+ PG/compost	130.9 a	278.7 a	177.5 a	171.2 a	287.1 a	201.1 a	192.8 a	171.0 a

Data in the same column followed by the same letter are not significantly different ($P<0.05$).
Nutrient Content (NC) change=(NCAMF - NCnonAMF)x100/NCnonAMF.

on Zn and Mn concentration in nonmycorrhizal plants. Also, PG had no significant effect on Cu concentration in either AMF or non-AMF plants. The highest concentrations of Mn, Fe, Cu, and Zn were observed at AMF plus PG/compost treated plants compared to other treatments.

Plant contents of K, Ca, Mn, Fe, Cu, and Zn were significantly higher for mycorrhizal than for nonmycorrhizal plants (Table 4). The data revealed that maximum plant uptake of K, Ca, Mn, Fe, Cu, and Zn was found in the treatment of indigenous AMF with PG/compost, which was significantly ($P \leq 0.05$) higher by 177.5, 171.2, 287.1, 201.1, 192.8, and 171%, respectively, over control (Table 5).

4. Discussion and Conclusions

The soil P concentration in this study was low (3 mg kg^{-1}), and this nutrient normally has to be added to this soil to provide sufficient P for plant growth. Addition of poorly soluble forms of P, such as phosphogypsum, to soil had no negative effect on AMF root colonization, as was reported by Cui *et al.* (2014). This may be because AMF root colonization often depends on given amounts of soluble P in the soil at the time of root colonization (Stribley *et al.,* 1980). In particular, greater root infection was found with AMF inoculation plus PG/compost treatment and it was possibly due to the improvement of the rooting zone environment which stimulated better root proliferation (Nagahashi *et al.,* 1996; Van der Heijden and Kuyper*,* 2001).

Soil pH affects the availability of nutrients and how the nutrients react with each other. Application of PG to soil lowered soil pH, a result which is in agreement with literature reports of previous studies (Al-Karaki and Al-Omoush, 2002; Lee *et al.*, 2009). The lower soil pH caused by PG might be attributed to the release of phosphoric acid and sulfuric acid contained in PG.

The increase in cotton plant biomass by PG corroborates reports by Zhang *et al.* (2014) who showed that amendment of PG significantly increased shoot biomass in tobacco, regardless of AMF inoculation. According to Quintero *et al.* (2014), increased dry matter of tomato by PG can be ascribed, at least in part, to an increase in water use efficiency. Greater fresh biomass and plant height of inoculated cotton compared to control was noted in this study, which is in accordance with other earlier studies on cotton (Afek *et al.,* 1991; DeFeng *et al.,* 1998).

Enhanced cotton growth with the combination of AMF and PG agrees with previous studies conducted with different plant species such as wheat (Al-Karaki and Al-Omoush, 2002), maize (Bai *et al.,* 2011), tomato (Cui *et al.,* 2014), and shallot (Gu *et al.,* 2012). Improved growth of AMF and PG treated plants may have been due to improved soil P availability. The trend noted for N concentration and biomass of AMF plus PG/compost treated plants might be due to the compost releasing its nitrogen gradually to the soil/crop to produce a greater number of leaves.

Strong mycorrhizal effects on cotton were also observed when looking at the nutrients uptake. Higher K and Ca in mycorrhizal than in nonmycorrhizal cotton is supported by Liu *et al.* (2002) who reported that AMF enhanced acquisition of the nutrients that move mainly by mass flow. PG/compost had a positive effect on Ca and K concentration in mycorrhizal cotton which could be due to an improvement in soil organic matter and exchangeable Ca and K by compost (Adeleye *et al.,* 2010). El Mrabet *et al.* (2014) also showed that bio-compost improved soil K-extractable. Increasing P uptake of plants due to AMF inoculation has been widely reported (Deguchi *et al.* 2007; Sharif *et al.,* 2009; Ibrahim, 2010). In our study, increased P concentration and uptake by PG addition to mycorrhizal cotton is supported by Zhang *et al.* (2015) who reported that PG amendment significantly increased the concentration and absorption of P in mycorrhizal and nonmycorrhizal tobacco plants. Also, Gu *et al.* (2012) found that P concentration in shallot was increased by increasing PG, and the combination of PG and AMF colonization can improve P uptake by shallot to different degrees. Our results show that indigenous AMF increased the concentrations of Cu, Zn, Fe, and Mn in cotton; similar results were obtained in cotton inoculated with different species of AMF (Liu *et al.,* 1994; Ibrahim, 2010). Our result regarding enhanced acquisition of P and micronutrients in AMF cotton grown with PG is supported by Al-Karaki and Al-Omoush (2002) in their work on mycorrhizal wheat grown with PG.

The extension of AMF hyphae, beyond the root zone, provides P and other nutrients to plants during growth stages. The ability of the hyphae to extend the root system should be especially beneficial in the case of cotton because its roots have a low density per unit soil volume (McMichael, 1990). In this case,

AMF likely contributed P (and other mineral nutrients) from soil and PG particles with which roots would not make contact. High absorption of Zn, Cu, and Fe may be due to greater P uptake by AMF plants (Clark and Zeto, 1996; Davies *et al.,* 2005). On the other hand, the positive response of cotton to AMF inoculation for nutrient concentration could be due to the effectiveness of the AMF isolate in improving soil properties and nutrient availability.

Previous reports showed that improvement of plant growth and nutrients acquisition by the combination of PG and AMF depends on compatibility between plant species, the rate of PG added, and the AMF isolate. Bai *et al.* (2011) reported that shoot growth of PG treated maize strain (40 g kg^{-1}) was significantly enhanced when inoculated with *Diversispora spurcum*, but was significantly inhibited when inoculated with *Glomus aggregatum*. Gu *et al.* (2012) reported that the treatment of PG40 addition with *Glomus mosseae* inoculation had a significant effect in improving shallot biomass and P, S uptake. According to Zhang *et al.* (2014), the combination of PG40 and *G. aggregatum* inoculation had the most desirable effects on tobacco growth.

Under the conditions in this study, added PG/compost enhanced P and nutrient concentrations of mycorrhizal plants. Previous reports have shown that PG application induced changes in soil chemical properties (decreased soil pH and enhanced ECe, available P, SO_4, exchangeable K, Ca and Mg) (Al-Oudat *et al.,* 1998; Lee *et al.,* 2009). In addition, organic fertilizers and AMF inoculation could improve soil physico-chemical properties (Warnock *et al.,* 2007). AMF colonization enhances soil aggregation by exuding the glycoprotein, glomalin, from extraradical hyphae (Wright and Upadhyaya, 1998). The improved soil structure enhances air and water percolation, improves root system access to soil water and nutrients, and improves crop production (Celik *et al.,* 2004). Therefore, the increase in nutrients noted in AMF and PG/compost treatments could be due to improved soil structure and to increased release of nutrients in the soil, which become more available to the plant (Habashy *et al.,* 2008; Jan *et al.,* 2014).

Enhanced acquisition of nutrients and plant growth by AMF and PG was reflected by increased yield and yield components of cotton. Previous studies showed that PG increased grain yield of barley, wheat, and cotton (Al-Oudat *et al.,* 2011). Cui *et al.* (2014) reported that tomato yield of AMF or AMF plus PG seedlings were significantly higher than those of the non-mycorrhizal seedlings. Al-Karaki and Al-Omoush (2002) reported that grain yield of wheat was enhanced by PG, and even more so when roots were colonized with AMF.

Cotton inoculation with indigenous arbuscular mycorrhizal fungi (AMF) and soil amendment with phosphogypsum (PG) enhanced nutrients acquisition from soil and improved growth and yield of cotton. However, mycorrhizal plants grown with PG and compost mixture had greater growth and yield than plants grown with PG alone. Therefore, the combination of AMF with PG added in compost can ensure satisfactory benefits for cotton growth and yield in low input, sustainable cropping systems.

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Micropropagation of three endemic and endangered fig (*Ficus carica* L.) genotypes

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Key words: explant, rooting, single node, shoot proliferation.

Abstract: In this research, *in vitro* propagation of three endangered and endemic fig genotypes named 'Bargchenari', 'Dehdez' and 'Runu' were investigated. For shoots proliferation, nodal explants on Murashige and Skoog (MS) medium were treated with different concentrations of growth regulators. Rooting of shoots was initiated by the use of MS and MS/2 media and various concentrations of Indole-3-butyric acid (IBA) (0, 0.5, 1 or 1.5 mg l^{-1}). The experiments were conducted in a completely randomized design. Results showed that shoots proliferation improved as subcultures increased. The highest shoot numbers per explant 13.67 and 8.8 were achieved eight weeks after second subculture in 'Runu' and 'Bargchenari' genotypes respectively, when MS medium supplemented with 0.5 mg l^{-1} benzyl adenine (BA) and 0.2 mg l^{-1} N^6-(Δ^2-isopentenyl)adenine (2ip). In 'Dehdez' genotype, the number of shoots was 4.4 per explant, when culture media were supplemented with 6 mg l^{-1} Kinetin (Kn) and 0.2 mg l^{-1} 6-α-naphthaleneacetic acid (NAA). The highest root numbers (2.23) were obtained in 'Bargchenari' genotype on MS/2 medium containing 1.5 mg l^{-1} IBA.

1. Introduction

Figs are one of the earliest cultivated fruit-bearing trees. Iran is one of the major producing countries of fig (*Ficus carica* L.) and it stands fifth after Turkey, Egypt, Algeria and Morocco (FAO, 2012). In Iran, some of the genotypes, including 'Bargchenari', 'Runu' (Faghih and Sabet-Sarvestani, 2001) and 'Dehdez' (Gholami, 2012) are endangered. Compatible old native varieties and their wild relatives as genetic resources are the basis of the breeding programs. In addition, the continuing the search for high yielding varieties with resistance to pests and pathogens ensures the availability and maintenance of a large genetic resources that guarantee accessibility of a useful genetic material at any time (Chawla, 2009).

The number of endangered plant species in the world has increased in recent years due to anthropogenic and environmental pressures. Numerous species are described as endangered, and integrated programs are required to protect and preserve current biodiversity (Sarasan *et al.,* 2006). Special attention must be given to endemic flora, which is a critical target in conservation strategies because it is restricted to small areas (Mallon *et al*., 2008). Genetic conservation of plant can be performed in situ or *in vitro*. Field collections (*in situ*), loose genetic biodiversity because of pests, diseases and adverse weather conditions and their maintenance is labor-intensive and expensive. Recently, the use of *in vitro* tools is an important way of safeguarding the germplasm of endangered plants and become increasingly popular for conservation purposes (Sarasan *et al.,* 2006; Bunn *et al.,* 2007; Mallon *et al.*, 2008; Piovan *et al.,* 2010). *In vitro* techniques can be used for not only the production of large number of plantlets in short period of time, but also for conservation of rare and endangered plant species, including genetic resources of recalcitrant seed and vegetatively propagated of elite genotypes (Engelmann, 2011). Several reports described shoot regeneration and organogenesis from various explants by the use of different culture media, types and concentrations of plant growth regulators in *F. carica* L. (Brum *et al.,* 2001; Fráguas *et al.,* 2004). Somatic embryogenesis from leaf segment of fig (Sultani genotype) was reported on MS medium supplemented with 30 mg l^{-1}

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N6-(Δ2-isopentenyl)adenine (2ip) and 7 mg l^{-1} Thidiazuron (TDZ) and 0.25 mg l^{-1} 6-α-naphthaleneacetic acid (NAA) (Soliman *et al.,* 2010). Dhage *et al.* (2012) obtained shoot regeneration from leaf callus initiated of four fig genotypes on MS medium supplemented with 0.5 mg l^{-1} NAA and 7 mg l^{-1} TDZ. For successful micropropagation axillary buds or shoot tip cultures are preferred as preexisting meristem easily develop into shoots while maintaining clonal fidelity and reduce the risk of somaclonal variability (Anis *et al.,* 2003; Ning *et al.,* 2007).

The present study was undertaken with the aim to evaluate the regeneration potential of three endangered genotypes and to establish an efficient *in vitro* method for their rapid propagation by culture of nodal explants using various plant growth regulators.

2. Materials and Methods

Preparation and decontamination of explants

Shoots with a length of 5-20 cm were collected from mother plants of three different genotypes ('Bargchenari', 'Dehdez' and 'Runu') grown in a greenhouse with 40% relative humidity, day and night average temperatures of 30±2°C and 24±2°C respectively. The shoots were kept for one hour under the running tap water then dipped for 20 min in a solution of 4 g l^{-1} benomyl. Explants were surface sterilized with 70% ethanol for 5 min, then were disinfected with 15% Clorox (commercial bleach, 5.25% sodium hypochlorite) for 15 min and they were rinsed 3-4 times with sterile distilled water. Then, the single-node segments were isolated and inoculated on a proliferation medium (MS) containing 100 mg l^{-1} ceftriaxone (Darusazy Exir, Iran) to control bacterial contamination. For control of phenolic substances 2 g l^{-1} activated charcoal was added in the culture medium.

Shoot proliferation

Based on availability of explants different experiments were conducted as follow:

In the first experiment the effects of different concentrations of Kinetin (kn) (2, 4, 6 and 8 mg l^{-1}) supplemented with 0.2 mg l^{-1} NAA and control (without plant growth regulator) were separately studied for two genotypes 'Bargchenari' and 'Dehdez'. Data were collected after 4 weeks of culture.

The second experiment was carried out on three genotypes of 'Bargchenari', 'Dehdez' and 'Runu'. The explants were sub cultured two times with the interval of 4 weeks and a little change in growth regulators in culture medium as follows:

Culture. The single-node explants cultured on MS (Murashige and Skooge, 1962) medium containing 0.5 mg l^{-1} benzyl adenine (BA).

First subculture. After 4 weeks of culture and data collection, the explants were subcultured into the same medium.

Second subculture. After another 4 weeks and data collection, the explants were subcultured on a new medium with the same growth regulator composition plus 0.2 mg l^{-1} 2ip. In this step, after 4 weeks, the proliferated shoots were compact and short, thus, they were transferred into new medium containing 1 mg l^{-1} gibberellic acid (GA), and data were recorded after another 4 weeks. The experiments were conducted in completely randomized design with 4 replications of at least three explants in each.

Rooting

The produced shoots (above 1 cm length) were used for rooting. In this experiment, the effects of different concentrations of indole-3-butyric acid (IBA; 0, 0.5, and 1.5 mg l^{-1}) and different media (MS and MS/2) on shoot rooting performance were investigated. A factorial test was conducted in a completely randomized design with 8 replications. After 4 weeks, the rooted shoots were transferred to pots containing soil mixture (field soil, leaf mold and sand; 1V:1V:1V).

Data were subjected to analysis of variance by SAS software, version 9.1 (SAS Institude, Cary, NC, USA) and means comparison were done by Duncan Multiple Range test at probability of 5%.

3. Results and Discussion

Shoot proliferation

First experiment. In 'Dehdez' genotype, the results showed that the highest shoots number per nodal segment (4.4 shoots/explant) was obtained at concentration of 6 mg l^{-1} Kn which was significantly higher than the other treatments (Table 1, Fig. 1). However, by increasing of Kn from 6 to 8 mg l^{-1} significantly reduced the number of shoots. The positive effect of Kn in suitable concentration on shoot proliferation have also been reported previously in fig (Fráguas *et al.,* 2004) and strawberry (Balakrishnan *et al.,* 2009).

Table 1 - Effects of different treatments of Kn and NAA on shoot number, shoot length and leaf number per explant in 'Dehdez' genotype

Treatments (mg l^{-1})	Shoot number	Shoot length (mm)	Leaf number
Control	1.67 c	27.67 a	12.67 c
Kn 2 + NAA 0.2	1.67 c	9.78 c	5.33 e
Kn 4 + NAA 0.2	3.00 b	9.96 c	17.86 b
Kn 6 + NAA 0.2	4.40 a	11.59 b	26.40a
Kn 8 + NAA 0.2	2.22 c	7.56 d	9.89 d
Significant	**	**	**

Mean values followed by the same letters are not significantly different according to Duncan's multiple range test (DMRT) at $p<0.05$.

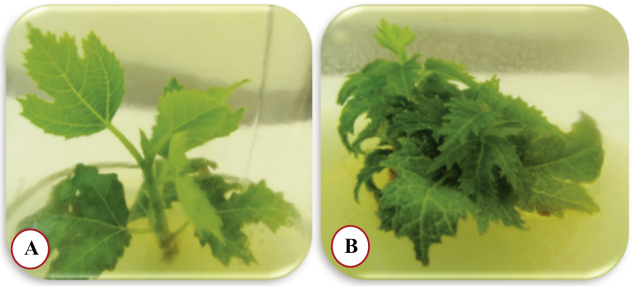

Fig. 1 - Effect of NAA and Kn on nodal explant proliferation in 'Dehdez', A= control, B= 0.2 mg l^{-1} NAA and 6 mg l^{-1} Kn, 8 weeks after culture.

With increasing Kn in culture medium, the shoot length significantly decreased so that the highest shoot length was observed in control (27.67 mm) and the lowest one in 8 mg l^{-1} Kn (7.56 mm). Our results was in agreement with the findings of Fráguas *et al*. (2004), they reported that although the Kn is required for the induction of shoot proliferation, in fig 'Roxo de Valinhos', the supra optimal concentrations can be toxic, and reduce the number and length of shoots. Nevertheless, it was in disagreement with the results reported by Mustafa and Taha (2012) in which 2.5 mg l^{-1} Kn encouraged shoot length. The highest number of leaves (26.4) was gained at the concentration of 6 mg l^{-1} Kn and lowest one (5.33) at 2 mg l^{-1}.

In 'Bargchenari' genotype, the results showed different concentration of Kn had no significant effect on shoots number (Fig. 2 A), although with adding Kn in culture media, shoot number increased. With increasing Kn in medium culture the shoots length decreased (Fig. 2 B). The M2 medium produced the highest numbers of leaf/explant that was significantly higher than control (Fig. 2 C). Shoot length reduction in M1 and M2 media and increment of number of leaves in the same medium is indicative of internodes length reduction (Fig. 2).

Second experiment. The results showed that after 4 weeks of nodal segments culture, there were not significant differences between three genotypes in relation to shoot number, shoot length and leaf number. In the first subculture, the number of shoot (1.78 per explant) and shoot length (18.11 mm) in genotype 'Bargchenari' were significantly higher than 'Dehdez' and 'Runu' (Table 2). In second subculture the maximum shoot numbers was obtained in 'Runu' (13.67 shoots/explant) (Fig. 3, A3) which was not significantly different from 'Bargchenari' (8.08 shoots/explant), and 'Dehdez' (2.8 shoots/explant) produced the lowest number of shoots (Fig. 3, B3). It seems that in the second subculture, the adding of 0.2 mg l^{-1} 2ip in addition to 0.5 mg l^{-1} BA into the medium improved shoot proliferation. Previous reportes, on *Ficus* shoot proliferation with implication of BA and 2ip in culture media, showed BA achieved better than 2ip on shoot proliferation on *Ficus benjamina* (Rzepka-Plevnes and Kurek, 2000) and *Ficus anastasia* (Al Malki and Elmeer, 2010).

It has been also reported that BA (a synthetic

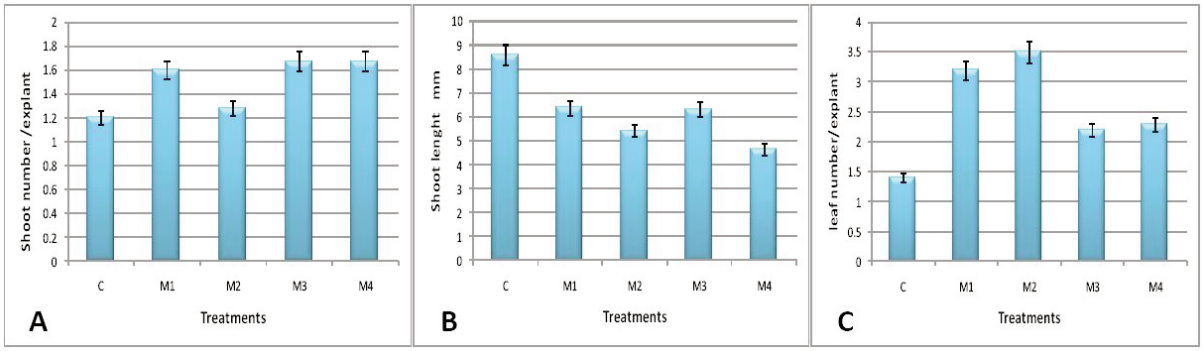

Fig. 2 - Effects of different treatments of Kn and NAA on shoot number (A), shoot length (B) and leaf number (C) per explant in 'Bargchenari' genotype. C= Control (no growth regulator); M1= Kn 2 mg l^{-1}+NAA 0.2 mg l^{-1}; M2= Kn 4 mg l^{-1}+NAA 0.2 mg l^{-1} ; M3= Kn 6 mg l^{-1}+NAA 0.2 mg l^{-1} ; M4= Kn 8 mg l^{-1}+NAA 0.2 mg l^{-1}.

Table 2 - Effects of genotype and subculture on shoot numbers, shoot length and leaf numbers per explants on MS medium

Genotype	Subculture	Shoot number	Shoot length (mm)	Leaf number
'Dehdez'	Culture	1.00 c	9.90 bc	2.50 c
	First subculture	1.00 c	10.30 bc	3.25 c
	Second subculture	2.80 b	6.45 c	10.80 c
'Bargchenari'	Culture	1.00 c	14.89 ab	5.44 c
	First subculture	1.78 b	18.11 a	7.67 c
	Second subculture	8.08 a	17.18 a	38.50 b
'Runu'	Culture	1.00 c	14.20 ab	3.60 c
	First subculture	1.12 c	8.62 c	3.87 c
	Second subculture	13.67 a	13.52 ab	66.67 a

Mean values followed by the same letters are not significantly different according to Duncan's multiple range test (DMRT) at $p<0.05$.

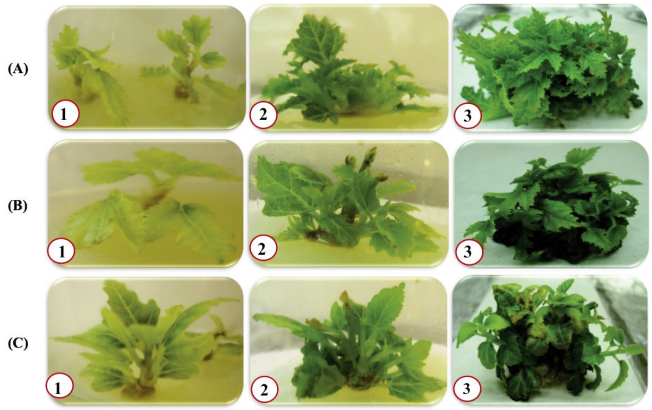

Fig. 3 - Compared growth of nodal explants in 'Runu' (A), 'Dehdez' (B) and 'Bargchenari'(C) genotypes in different cultures, 1= culture, 2= 4 weeks later, first subculture, 3= 8 weeks later, second subculture.

cytokinin) effect is stronger than the other cytokinins on shoot regeneration (Torres, 2013). However, in this study, simultaneous application of 2ip and BA in culture media highly improved shoot proliferation of the three fig genotypes. This indicates that two kinds of cytokinins had synergistic effect and enhanced shoot proliferation. The positive effect of transferring explants to a new medium on explant growth have also been reported by other researchers on different species of *Ficus,* including *carica* (Mustafa and Taha, 2012) and *religiosa* (Hassan *et al.,* 2009; Siwach *et al.,* 2011; Siwach and Gill, 2014).

The results showed in 'Dehdez' and 'Bargchenari', there were no significant differences in shoot length with increasing subcultures. However, the shoot length of 'Bargchenari' was significantly higher than 'Dehdez'. In 'Runu', the shoot length decreased in first subculture and increased in second subculture. As described in Materials and Methods, 4 weeks after the second subculture explants were transferred to a new medium containing GA. The results showed in spite of high shoot proliferation in second subculture, positive effect of GA on stem elongation. It has been reported GA induces internode elongation, meristem and buds growth in *in vitro* culture (Torres, 2013). Fráguas *et al.* (2004) also reported a positive effect of GA on shoot length elongation on fig 'Roxo de Valinhos'.

Eight weeks after second subculture the highest number of leaves (66.67) was observed in 'Runu' and the lowest number (10.8) in 'Dehdez' genotype. The results showed that all measured parameters, especially shoot proliferation are genotype dependent. In other species such as *Prunu mume* (Ning *et al.*, 2007), pomegranate (Al-Wasel, 1999; Naik *et al*., 1999) the effect of genotype on shoot proliferation rate has been reported.

Rooting

The highest rooting percentage (84.61), root number (2.23) and root length (1.51 cm) per explant were observed in MS/2 medium containing of 1.5 mg l^{-1} IBA which were significantly higher than their controls (Table 3). On MS/2 medium supplemented with 0.5 mg l^{-1} IBA did not produce any roots (Fig. 4).

Table 3 - Effects of different concentrations of IBA and media (MS and MS/2) on rooting characteristics in 'Bargchenari' genotype

Medium	IBA (mg l^{-1})	Rooting (%)	Root number	Root length (cm)
MS	0	18.18 c	0.45 bc	0.11 b
	0.5	71.43 ab	1.14 ab	0.30 b
	1	16.67 c	0.33 bc	0.08 b
	1.5	28.57 bc	0.71 bc	0.09 b
MS 1/2	0	30.77 bc	0.92 bc	0.10 b
	0.5	0.00 c	0.00 c	0.00 b
	1	28.57 bc	0.28 bc	0.43 b
	1.5	84.61 a	2.23 a	1.51 a
Significant		*	**	**

Mean values followed by the same letters are not significantly different according to Duncan's multiple range test (DMRT) at $p<0.05$.

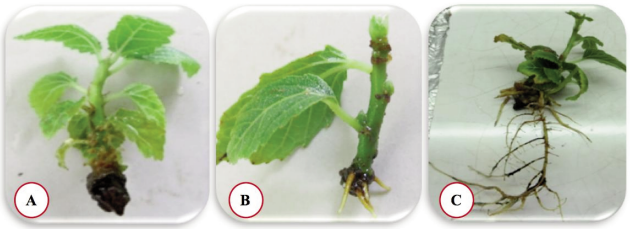

Fig. 4 - Effects of IBA on rooting of shoots in 'Bargchenari' genotype in *in vitro* condition, A= control, B= 0.5 mg l^{-1} IBA on MS medium, C= 1.5 mg l^{-1} IBA on MS 1/2 medium.

The results showed a high interaction between rooting media and different concentrations of IBA on rooting percentage and root number, such that in MS/2, the best results was obtained with 1.5 mg l^{-1} IBA, where in MS medium it was 0.5 mg l^{-1} IBA that produced the best result. Hepaksoy and Aksoy (2006) also reported that IBA is necessary for *in vitro* rooting of fig, Sarilop cultivar, that is in consistent with our results. The obtained results are in disagreement with those reported by Brum *et al*. (2001) and Fráguas *et al.* (2004) who found IBA are not essential for *in vitro* rooting of fig cv. Roxo de Valinhos. In this experiment, the best rooting occurred on MS/2 medium. Dhage *et al.* (2012) also introduced MS half strength medium for *in vitro* fig rooting, while Yakushiji *et al.* (2003) and Kim *et al.* (2007) reported MS full strength was the best medium for *in vitro* rooting of fig cultivars.

Adventitious root formation on proliferated shoots in a commercial propagation is very important. The ability of plant tissue to form adventitious roots depends on the interaction of many different endogenous and exogenous factors. The role of auxins in root development was reviewed, and it is a well-established fact that auxins are the main factors involved in the root formation (Németh, 1986).

4. Conclusions

Simultaneous application of 2ip and BA in culture media highly improved shoot proliferation of the three fig genotypes. This indicates that two kinds of cytokinins had synergistic effect and enhanced shoots proliferation. The highest shoot numbers per explant 13.67 was achieved eight weeks after 2nd subculture in 'Runu' genotype. The highest root number (2.23) was obtained in 'Bargchenari' genotype on MS/2 medium containing 1.5 mg l^{-1} IBA.

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Insight on trans-plasma membrane behavior of virus-infected plant cells

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Key words: grapevine, potassium channel, tobacco, TMV.

Abstract: Little is known about the ion fluxes generated during plant-virus interactions, despite significant losses caused by viruses to agricultural crops. Changes in average ion currents were identifying an early event in the signal transduction pathway related to virus/host interaction. While significant decrease in the average inward currents, mainly due to Ca^{2+} moving into the cell was observed, the role of potassium may be significant. Host specific K^+ efflux with a concomitant decrease in the intracellular K^+ was observed in tobacco plants during the early minutes after infection, suggesting many hypothesis about the role of potassium in host-virus interaction. In the last years, trans-plasma membrane potential was evaluated for some viruses, observing as effect on membrane was different in relation to virus infection and host. Conversely, settle virus infection generally lead to an increase of activity in trans-plasma membrane electron transport.

1. Introduction

In animal cells, the role of plasma membrane behavior is deeply investigated, and it may reflect health status. Cellular defense against stress is a key function in which the plasma membrane redox system is involved. Moreover, some of the plasma membrane redox system enzymes produce reactive oxygen species as well as play a protective role against them. Moreover, evidences underline as redox state of the cell and, accordingly, plasma membrane electron transport contribute to control cell growth, development and apoptosis (Ly and Lawen, 2003). In animal cells, virus infection can lead to variation in membrane potential (Akeson *et al.*, 1992). These effects may occur even during early pathogen recognition events in plant-microbe interaction. As reviewed by Elmore and Coaker (2011), plasma membrane H^+-ATPases are dynamically regulated during plant immune responses and quantitative proteomics studies suggest complex spatial and temporal modulation of plasma membrane H^+-ATPase activity during early pathogen recognition events, even if no data relative to virus were available. Anyway, an important role is played by H^+-ATPases in bacterial infection, where the enzyme cooperate with plant immune signaling protein (namely RIN4) to regulate stomatal apertures during pest invasion of leaf tissue. These enzymes are ubiquitous and are involved in plant immune responses. Moreover, they may are targeted by pathogens to increase plant susceptibility, even if these evidences are related to fungi or bacteria. Thus Shabala *et al.* (2010) affirm that little is known about the ion fluxes generated during plant-virus interactions, despite significant losses caused by viruses to agricultural crops. Viruses represent a major threat to agricultural production, mainly due to the lack of effectiveness treatments, and preventative measures represent the main tools available to protect plants. While it is widely believed that in many seed-propagated crops virus the threat is of limited importance, the health status of plants subjected to vegetative propagation is critical. Although the viruses that infect woody plants raised more

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health concerns, trials are longer and more difficult to manage compared to test with herbaceous plants.

2. Viruses and hosts: a poorly investigated binomial in trans-plasma membrane events

Few study investigated the trans-plasma membrane behavior of virus-infected plants, thus few viruses were evaluated in trials, mainly focused on herbaceous plant-related virus. *Tobacco mosaic virus* (TMV) is a widespread virus difficult to eradicate or control from plant and soils (Luvisi *et al*., 2012 a; Panattoni *et al*., 2013 a; Luvisi *et al*., 2015 a). TMV was frequently chosen as "model virus" in host-virus interaction tests. It was also chosen in first studies in trans-plasma membrane behavior of plants (Schvarzstein, 1997). *Cucumber mosaic virus* (CMV) (Rinaldelli *et al*., 2012), *Papaya mosaic virus* (PMV) (Schvarzstein, 1997), *Potato virus X* (PVX) (Shabala *et al.*, 2010) and *Tobacco ringspot virus* (TRSV) (Stack and Tattar, 1978) were also investigated.

Among virus affecting woody plants, grapevine viruses (Rizzo *et al.*, 2012, 2015) were the most investigated. *Grapevine leafroll associated virus* 1 or 3 (GLRaV-1, GLRaV-3), *Grapevine fanleaf nepovirus* (GFLV), *Grapevine fleck maculavirus* (GFkV), *Grapevine vitivirus A* (GVA) and *Arabis mosaic virus* (ArMV) were evaluated during antiviral tests or in settle infections (Panattoni *et al.*, 2013 b; Rinaldelli *et al.*, 2014).

3. Trans-plasma membrane behavior in early virus infection events

Few data are available for early events in plant-virus interaction. Stack and Tattar (1978) observed as TRSV-infected cells of cowpea showed altered trans-membrane electropotentials and responded differently from healthy cells when subjected to the metabolic inhibitor sodium azide in their ability to recover. Changes in average ion currents were also observed in protoplast of *Gomphrena globosa* artificially infected by PMV and TMV, identifying an early event in the signal transduction pathway related to virus/host interaction (Schvarzstein, 1997). In *G. globosa*, necrotic lesions surrounded by a chlorotic ring developed in inoculated leaves are thought to be the results of a non-host hypersensitive response (HR) that ultimately defend the plants from virus infection. Schvarzstein (1997), using patch-clamp techniques, elucidated the early events occurring during PMV infection. Whole cell recording indicate a significant decrease in the average inward currents, mainly due to Ca^{2+} moving into the cell or gluconate moving out of the cell. In the same time, an increase in the outward currents was also observed, due to Cl^- moving into the cell and K^+ moving out of the cell. The outward currents were correlated to PMV concentration, and a similar behavior was observed during TMV infection. The studies of Schvarzstein were the first one in which ion fluxes were characterized during virus-plant interaction and unfortunately, very few studies following.

The phenomenon of rapid alteration of ion fluxes in host cells during the early phase of viral infection was reported in *Chlorella* (Neupartl *et al*., 2008). *Chlorella* viruses tests suggested a key-role of potassium, due to an observed host specific K^+ efflux with a concomitant decrease in the intracellular K^+. The use of blockers of the viral-encoded K(+) channel (Kcv) reduced this K^+ efflux, suggesting as depolarization and K^+ efflux are at least partially mediated by Kcv. The K^+ efflux seems virus-triggered and it occurs in the same time frame as host cell wall degradation and ejection of viral DNA. Therefore, Neupartl *et al.* (2008) supposed that loss of K^+ and associated water fluxes from the host lower the pressure barrier to aid ejection of DNA from the virus particles into the host. Even if *Chlorella* shared some metabolic pathway with plants, this interesting findings cannot directly transferred to plants without further studies. Moreover, DNA-based viruses are uncommon pathogens in plants. Anyway, the key-role of potassium in plant-virus interaction was confirmed by Shabala *et al.* (2010), moving the focus from calcium. The role of ion fluxes in plant defense signaling is well documented and elicitor-induced transient Ca^{2+} influx from the external environment into the cytosol has always been named as a key element of the signaling cascade and appears to be crucial in the induction of plant defense against pathogens (Scheel, 1998; Zimmermann *et al*., 1999; Blume *et al*., 2000; Grant *et al.*, 2000), but this studies are not related to viruses. Shabala *et al*. (2010) observed as an addition of the purified PVX to the mesophyll tissue of tobacco plants caused no changes in the rate of Ca^{2+} transport across the plasma membrane. These evidences were supported by no significant changes in concentration of Ca^{2+} in cytosol for at least 50 min after PVX treatment. This behavior indicated that Ca^{2+} release

from internal stores was also not a part of the signal transduction mechanism in plant-viral interaction. Authors suggest that, contrary to bacterial pathogens, rapid Ca^{2+} signaling may not be essential for the viral perception and initiation of downstream transduction pathway. Conversely, massive K^+ efflux was measured as early as 10 min after virus inoculation and this behavior in host-related. Prolonged exposure virus caused lower concentration of Ca^{2+} in cytosol compared with control plants, suggesting of the role of Ca^{2+} efflux systems in downstream cascades of the plant responses to viral infection. K^+ efflux was partially reduced by blockers of Kcv channels such as Cs^+ (Shabala *et al*., 2010), similarly to *Chlorella* tests. The antiviral drug mycophenolic acid (MPA) (Panattoni *et al.*, 2014; Guazzelli *et al*., 2015) was also indicated as interferent with K_{ATP} channel activity in grapevine, as well as inhibiting activity of the inward-rectifier potassium ion channel which could be mediated by guanosine depletion induced by MPA (Luvisi *et al.*, 2015 b).

Nowadays, the physiological role of the observed viral-induced K^+ efflux was not known. Neupartl *et al*. (2008) suggest that the efflux of K^+ and the associated water efflux from the *Chlorella* cell may be needed to reduce turgor and lower the pressure barrier, to aid ejection of DNA from the virus particles into the host, but adding several known blockers of K^+ efflux channels to the buffer media during PVX inoculation in tobacco plants did not ameliorate infection symptoms, probably because the blocking effect of pharmacological agents was only partial (Shabala *et al.*, 2010). Further hypothesis may be related to role of potassium homeostasis in plant adaptive responses to environment (Shabala *et al.*, 2007), including programmed cell death (Shabala, 2009).

Interestingly, the observed viral-induced activation of K^+ efflux systems appears to be a highly host-specific process, indicating that the observed K^+ fluxes may be linked to the plant's ability to recognize compatible viral infection and activate defense pathways (Shabala *et al*., 2010).

4. Trans-plasma membrane behavior in settle virus infection

Membrane potential

Membrane potential of grapevine cells were monitored during antiviral drugs treatments (Luvisi *et al.*, 2012 b). The complex of results obtained in this research highlights, first of all, that membrane electrical response of the tested antiviral drugs is supported by the metabolism of plant cell, and no differences in Δ Em were found GLRaV-1-infected grapevine leaves compared to virus-free leaves. However the behavior of membrane of plants treated with antiviral drugs was different in other hosts, as reported in tobacco plants infected by CMV, where trans-plasma membrane potential activity seemed to be influenced by virus presence, acting differently in infected or healthy samples during drug uptake by cells (Rinaldelli *et al*., 2012).

Anyway, drugs may hindered or enhance virus effects of membrane behavior and specific trials in settle virus infection were carried out in grapevine (Rinaldelli *et al*., 2014). Samples infected by leafroll viruses showed no difference in membrane potential values compared to healthy samples, confirming the similar behavior of GLRaV-1 or -3 infected samples and healthy ones observed during antiviral drug treatments. GFLV, or ArMV infected tissues led to plasma membrane hyperpolarization with higher values compared to healthy samples, while GFkV and GVA infected tissues showed plasma membrane depolarization significantly lower than the control (Rinaldelli *et al*., 2014). According to the role of H^+-ATPase activity in generating trans-plasma membrane potential gradient (Sondergaard *et al*., 2004), GFLV and ArMV infected cells could be considered more energized compared to others. Conversely, GFkV and GVA infection was also associated to increasing difficulty of cell membrane measurements, as occurs under stress conditions (Rawyler *et al*., 2002).

Electron transport

Rubinstein and Luster (1993) indicated that that trans-plasma membrane electron transport (t-PMET) occurs in all types of organisms, including plants. t-PMET allows reduction of extracellular oxidants at the expense of intracellular reducing equivalents that may derive from NADH or NADPH (Del Principe *et al*., 2011). The t-PMET enzymes have been investigated based on their ability to reduce external artificial impermeant electron acceptors. Ferricyanide (Fe^{3+}) has been commonly used as electron acceptor in assays performed with intact cells; ferricyanide is converted to ferrocyanide (Fe^{2+}), and the rate of this reduction can be monitored. Using a carbon fibre microelectrode (CFME) it is possible to map oxidoreductase activity using impermeant electron acceptors or donors (Taylor and Chow, 2001). This techniques

were also used to evaluate *in vivo* estimation of the inosine monophosphate dehydrogenase inhibition caused by antiviral drugs (Panattoni *et al.*, 2015).

The t-PMET was observed during antiviral treatments in CMV-infected tobacco plants (Rinaldelli *et al*., 2012). Infected samples were less sensitive to antiviral treatments considering t-PMET. This effect may be due to the concurrent entry of drug within the symplast that, as indicated by membrane potential, was lower in infected samples and that can lead to lower inhibition of NAD^+/NADH conversion by drug and to the following increase of Fe^{3+} conversion.

This virus-related behavior was confirmed in GLRaV-1 and -3 tests (Panattoni *et al.*, 2013 b). Virus-infected samples exhibited elevated t-PMET activity compared to healthy samples. The [Fe^{2+}] in healthy samples was set at 26 µM, while GLRaV-1- and GLRaV-3-infected samples showed 34-49 µM [Fe^{2+}]. These data can be linked to the NADH content in GLRaV-1 and GLRaV-3 samples, that was set at 1.2 times higher than healthy samples. This trans-plasma membrane behavior was partially confirmed for other grapevine viruses (Rinaldelli *et al.*, 2014). In virus-infected samples, while the [Fe^{2+}] produced by GFkV and GVA infected tissues were similar to healthy tissues, the samples infected by GFLV, ArMV and leafroll viruses showed higher t-PMET activity. The higher NADH content due to virus infection was used differently by infected samples during their t-PMET activity. Samples whose infectious status did not interfere negatively with membrane potential, such as ArMV, GFLV, GLRaV-1, and -3 showed higher t-PMET activity compared to healthy samples, in agreement with the higher NADH availability.

5. Discussion

The high level of specialization attained by many viruses due to their replication and pathogenetic mechanisms towards the host make them an extremely variable and complex target. This complexity is the background upon which a defensive strategy can be optimized. Therefore, the outcome of therapeutic action is strongly influenced by the ontological properties of the virus to be eliminated as well as the characteristics expressed by the plant as well as the trans-membrane transport of drugs. In human pathology, trans-membrane alteration may represent signalling systems for regulating cellular metabolism able to interfere with distinct cellular functions such as redox homeostasis and pathogens defense (Herst and Berridge, 2006), but few data are available in plants. For systemic viruses such as TMV, movement of viral particles occurs by infected cell to nearby healthy ones, and subsequent systemic transport is governed by a series of mechanisms involving various virus and plant factors (Scholthof, 2005), interacting with the host cell membrane, binding to some cytoskeletal proteins which could include changes in ion fluxes and signals in the transduction pathway (Atkinson *et al.*, 1996), thus further studies in trans-plasma membrane behavior of virus-infected cells are desirable

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Morpho-physiological alteration in common bermudagrass [*Cynodon dactylon* (L.) Pers.] subjected to limited irrigation and light condition

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Key words: antioxidative enzymes, irrigation, photoperiod, turfgrass.

Abstract: Bermudagrass (*Cynodon* spp.) is the most popular warm-season turfgrass used in warm climatic regions of the world due to its recuperative ability, high traffic tolerance, heat tolerance, and relative drought and salt tolerance. However, shade is a microenvironment in which bermudagrass performs poorly. In order to evaluate the interaction of photoperiod and irrigation on [*Cynodon dactylon* (L.) Pers. California Origin], a greenhouse experiment was conducted at the Research Greenhouse of the Department of Horticultural Sciences, College of Agriculture, Shiraz University, Shiraz, Iran. The experiment was conducted with four field capacity regimes (25%, 50%, 75% and 100%) and three light durations (8, 12 and 16 h) in a completely randomized design factorial arrangements with four replications. Results showed that decreasing field capacity and photoperiod decreased fresh and dry weights shoot and root, chlorophyll and starch contents and superoxide dismutase, catalase and ascorbate peroxidase activities. Decreasing the field capacity and light duration increased proline content. Reducing sugars and peroxidase enzyme in leaves increased with decreasing field capacity. Shoot height and leaf area increased by shortening the photoperiod. In overall, results showed that, the increase in irrigation alleviates the destructive effects of reduced day lengths and vice versa. Further studies are needed to clarify more the interaction between irrigation and light treatments at structural and ultrastructural levels, in common bermudagrass.

1. Introduction

Bermudagrass is a warm-season, C4, perennial grass. It has short, grey-green blades with rough edges, stems of 1 to 30 cm in length and a deep root system that can penetrate 2 m into the ground; however, most of the root mass is less than 60 cm deep (Xu *et al*., 2011). Among the many advantages of turfgrass areas are erosion and dust control, aquifer recharge and protection from pollutants, heat reduction in urban environments, reduction of noise and pollution, and providing human health and aesthetic benefits (Stier *et al*., 2013). Water scarcity is an increasing challenge to the turfgrass industry and may result in irrigation restrictions being imposed without regard for damage to turfgrass (Beard and Kenna, 2008). For turf managers, thriving in an industry where turf quality is of utmost importance is difficult when water is limiting. Therefore, researches investigating turfgrass resistance to drought stress have become increasingly important (Fry and Huang, 2004). Fu and Huang (2001) investigated the effects of drought stress on two cool-season turfgrasses and found that moderate drought stress had not effects on morphological and physiological characteristics, however in intensive drought stress, antioxidant enzyme activities, chlorophyll content, relative water content and shoot dry weight were decreased. In addition to limited amounts of water, turfgrasses are impacted by low-light environments. Shade is more problematic for warm-season turfgrasses to maintain quality given their higher light saturation point compared to cool-season turfgrasses (Fry and Huang, 2004). Turfgrasses perform poorly in reduced light environments due to high traffic rate, daily mowing, and reduced photosynthesis. In shade, increased disease presence adversely affects cool-season turfgrass development, while morphological limitations, such

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as reduced lateral stem growth, inhibits warm-season turfgrass development (Beard, 1972). Variations of shade responses among species and cultivars (Jiang *et al*., 2004; Trenholm and Nagata, 2005; Sladek *et al*., 2009) make it possible to select turfgrasses with superior shade tolerance. Identifying morphological characteristics that are associated with superior shade performance based on genetic variation would add value to germplasm screening for shade tolerant species and cultivars. Esmaili and Salehi (2012) noted in bermudagrass that were treated with short photoperiod duration, verdure fresh and dry weight, shoot height, tiller density, leaf area and chlorophyll and relative water contents were decreased, however electrolyte leakage and proline content were increased. Although bermudagrass, the most widely grown C4 turfgrass on an international basis (Shearman, 2006), has been extensively studied, many challenges and questions still remain when light is a limiting growth factor. The main objective of the present study was to investigate the effects of both irrigation interval and light duration on growth and quality of common bermudagrass.

2. Materials and Methods

Plant material and experimental conditions

This experiment was conducted at the Research Greenhouse of the Department of Horticultural Sciences, College of Agriculture, Shiraz University, Shiraz, Iran (52°32′E and 29°36′N, 1810 m asl). Seeds of common bermudagrass (*Cynodon dactylon* [L.] Pers. California Origin) were weighed and cultured in plastic pots with 19 cm in diameter and 25 cm in height, without drainage (0.25 g pot^{-1}) filled with 4 kg clay-loam soil with permanent wilting point (PWP) of 19% and field capacity (FC) 29%. Watering was carried out daily prior to beginning of treatments. Plants were kept in a greenhouse with 31/25°C (day/night) temperature and 35% relative humidity for one month before the beginning of treatments. Treatments were conducted at four irrigation levels (25%, 50%, 75% and 100% FC) and three photoperiod duration [8, 12 and 16 h as short day length (SDL), intermediate day length (IDL) and long day length (LDL)]. Watering was carried out daily before seed germination and after turf establishment. Then, the turves were watered equally when required. Established turves were clipped from 3 cm above soil by a hand mower and were transferred to a covered frame which temperature, light (intensity and length) and relative humidity were controlled with digital sensors. The environmental condition of covered frame was 31°C, white and creamy fluorescent lamps one m above the pots with a constant light intensity of 3000 lux, and 35% relative humidity for applying simultaneous irrigation and photoperiod treatments. Pots were weighed daily and set to different irrigation treatments (25, 50, 75 and 100% FC), during the whole of experiment**.** After three months, plants were harvested in order to measure morphological and biochemical traits.

Growth parameters

Growth parameters including, shoot height (cm), leaf area (cm^2) and fresh and dry weights of shoot and root (g) were measured. Dry weights were measured when the materials dried at 60°C for 48 h.

Chlorophyll content

Chlorophyll content was measured according to the method of Saini *et al*. (2001) using the following formula:

$$\text{Chlorophyll (mg/g f.w.)} = [20.2(\text{OD } 645 \text{ nm}) + 8.02(\text{OD } 663 \text{ nm}) \times V/ (\text{f.w.} \times 1000)]$$

Where: OD is optical density, V is the final solution volume in ml and f.w. is tissue fresh weight in mg.

Proline content

Proline was determined according to the method described by Bates *et al*. (1973). Using spectrophotometer (Biowave II, England) at 520 nm wavelength, appropriate proline standards were included in calculation of its content in samples.

Total soluble sugars and starch analysis

The total soluble sugars were measured using the method as previously described by Dubois *et al.* (1956). The total soluble sugar content of samples was measured at 490 nm of absorbance and glucose solution was used at different concentrations for standard curve drawing. The starch content was quantified using the Bradford method (McCready *et al*., 1950). The starch content was measured at absorbance of 630 nm and calculated using the standard curve of glucose and multiplying it by 0.92.

Antioxidant analysis

Fresh samples were homogenized in extraction buffer (0.1 M phosphate buffer pH 6.8) with mortar and pestle on ice. The homogenate was then centrifuged at 12,000 g for 15 min at 4°C and the supernatant was used as the crude extract for the superox-

ide dismutase (SOD), guaiacol peroxidase (POD), ascorbate peroxidase (APX) and catalase (CAT). The SOD, POD, APX and CAT enzymes were estimated using the methods previously described by Beauchamp and Fridovich (1971), Chance and Maehly (1995), Nakano and Asada (1981) and Dhindsa *et al.* (1981), respectively.

Experimental design and data analysis

This study was conducted in a completely randomized design with factorial arrangements and two factors: field capacity and photoperiod with four replicates. Data were analyzed using statistical software (SAS Software) and mean comparisons were performed using LSD test at 5% level.

3. Results and Discussion

Results of analysis of variance (Tables 1 and 2) showed that photoperiod (except for soluble sugar) and irrigation had significantly influenced the measured traits and also the interaction of photoperiod and irrigation had a significant effect on fresh and dry weights of shoot, proline content and the level of activity of superoxide dismutase.

Shoot height and leaf area

Shoot height and leaf area significantly declined by decreasing field capacity from 100% to 25% (Table 3). Shoot height and leaf area decreased (47.09% and 27.77%, respectively) at 25% FC compared to 100% FC (Table 3). Ryan (2011) reported that growth can be reduced through impairment of cell division and cell expansion which occurs at a lower water stress threshold rather than photosynthetic inhibition. Fu and Huang (2001) reported that shoot growth of both

Table 3 - Effect of field capacity and photoperiod and their interaction on shoot length, leaf area, shoot fresh and dry weight, root fresh and dry weight and chlorophyll content

Variables	Photo-period	Field capacity (%)				Mean
		100%	75%	50%	25%	
Shoot length (cm)	LDL	22.25 d*	21.27 e	16.67 h	11.05 k	17.81 C
	IDL	23.75 c	22.77 d	18.17 g	12.55 j	19.31 B
	SDL	25.35 a	24.37 b	19.77 f	14.15 i	20.91 A
	Mean	23.78 A	22.80 B	18.20 C	12.58 D	
Leaf area (cm^2)	LDL	1.22 bc	1.22 bc	1.17 c	0.87 e	1.12 B
	IDL	1.23 bc	1.23 bc	1.18 c	0.88 e	1.13 B
	SDL	1.32 a	1.32 a	1.27 ab	0.97 d	1.22 A
	Mean	1.26 A	1.25 A	1.20 B	0.91 C	
Shoot fresh weight (g)	LDL	20.30 a	20.26 a	16.16 b	14.06 c	17.69 A
	IDL	16.32 b	16.26 b	12.81 d	9.13 e	13.63 B
	SDL	13.85 c	12.83 d	9.69 e	5.34 f	10.42 C
	Mean	16.82 A	16.45 A	12.88 B	9.51 C	
Shoot dry weight (g)	LDL	11.30 a	11.26 a	7.16 d	5.06 e	8.69 A
	IDL	10.32 b	10.26 b	6.81 d	3.13 f	7.63 B
	SDL	9.85 b	8.83 c	5.69 e	1.34 g	6.42 C
	Mean	10.49 A	10.11 A	6.55 B	3.17 C	
Root fresh weight (g)	LDL	39.78 a	38.69 b	29.54 g	16.99 j	31.25 A
	IDL	37.80 c	36.71 e	27.56 h	15.01 k	29.27 B
	SDL	36.81 d	35.72 f	26.57 i	14.02 l	28.28 C
	Mean	38.13 A	37.04 B	27.89 C	15.34 D	
Root dry weight (g)	LDL	19.78 a	18.69 b	13.46 d	8.54 f	15.12 A
	IDL	19.69 a	18.60 b	13.37 d	8.45 f	15.03 A
	SDL	18.82 b	17.73 c	12.50 e	7.43 g	14.12 B
	Mean	19.43 A	18.34 B	13.11 C	8.14 D	
Proline content (mol g^{-1} f.w.)	LDL	5.57 i*	7.06 g	14.35 f	23.25 c	12.56 C
	IDL	5.63 i	6.51 h	15.19 e	24.20 b	12.88 B
	SDL	5.66 i	6.89 gh	17.29 d	24.74 a	13.65 A
	Mean	5.62 D	6.82 C	15.61 B	24.07 A	

*In each variable, data followed by the same letters (small letters for interactions and capital letters for means) are not significantly different using LSD at 5% level.
LDL= long day length.
IDL= intermediate day length
SDL= short day length.

Table 1 - Analysis of variance of photoperiod, field capacity and interaction between photoperiod and field capacity measured traits

Source of variability	df	Shoot height (cm)	Leaf area (cm^2)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Sugars of shoot (mg g^{-1} d.w.)
Photoperiod	2	38.45 **	0.04**	212.24**	20.59**	36.59**	4.87**	5.77 NS
Field capacity	3	314.82**	0.33**	141.40**	141.40**	1337.54**	324.86**	79732.82**
Photoperiod * field capacity	6	0.0 NS	0.0 NS	1.25**	1.25**	0.0 NS	0.0 NS	0.0 NS
Error	33	0.17	0.002	0.24	0.24	0.00	0.08	7.01
CV	-	2.18	4.32	3.58	6.57	0.00	2.02	1.96

** and NS significant at the 0.01 level and not significant respectively.

Table 2 - Analysis of variance of photoperiod, field capacity and interaction between photoperiod and field capacity measured traits

Source of variability	df	Superoxide dismutase (Ug^{-1} FW)	Catalase (Ug^{-1} FW)	Peroxidase (Ug^{-1} FW)	Ascorbate peroxidase (Ug^{-1} FW)	Chlorophyll (mg g^{-1} FW)	Proline (µmol g^{-1} FW)	Starch content (mg g^{-1} DW)
Photoperiod	2	4044.08**	97.06**	969.12**	28933.33**	0.59**	4.99**	3.33**
Field capacity	3	55360.44**	234.71**	5248.15**	257973.85**	1.74**	7238.96**	105235.35**
Photoperiod * Field capacity	6	2.52 NS	0.0 NS	0.0 NS	0.0 NS	0.00 NS	2.27**	0.00 NS
Error	33	235,20	3.26	27.65	260.03	0.00	0.12	0.00
CV	-	9.91	5.65	6.67	1.79	0.0	2.68	0.00

** and NS significant at the 0.01 level and not significant respectively.

kentucky bluegrass and tall fescue generally were not affected by surface soil drying but under full drying, shoot growth declined for both species. The reduced leaf area is a modification to avoid evopo-transpiration loss and to increase water use efficiency in grasses which helps to tolerate water stress. Low leaf surface area would reduce transpiration rate also by lowering stomatal activity (Riaz *et al*., 2010). Turf shoot height showed considerable difference in LDL treatments compared to SDL treatments. Reducing photoperiod significantly increased the shoot height and leaf area (Table 3). Shoot height and leaf area increased significantly with shortening day length that it's maximum and minimum decreased (14.82% and 8.19%, respectively) was observed at SLD compared to LDL (Table 3). Similar results have reported on bermudagrass (Tegg and Lane, 2004) and zoysiagrass (Qian and Engelke, 1999).

Shoot fresh weight

Reducing field capacity from 100% FC to 25% FC significantly decreased the shoot fresh weight to 43.46% at 25% FC compared to 100% FC (Table 3). Riaz *et al.* (2010) demonstrated that, water deficit conditions had a significant inhibitory effect on shoot fresh and dry weights of three bermudagrass cultivars. The extended photoperiod (16 h) significantly increased fresh weight compared to shorter photoperiods (12 h and 8 h). Shoot fresh weight increased 41.09% under LDL compared to the SDL condition (Table 3). Sinclair *et al.* (2004) demonstrated that the extended photoperiod increased biomass accumulation of four grasses ('Pensacola' bahiagrass, *Paspalum notatum* Flugge var. *Saurde* Parodi; 'Tifton 85' bermudagrass, *Cynodon* spp. L. Pers.; 'Florakirk' bermudagrass; and 'Florona' stargrass, *Cynodon nlemfuensis* Vanderyst var. *nlemfuensis*) compared to short day condition. Interaction between field capacity and photoperiod resulted in the highest and lowest fresh weight in 100% FC-LDL and 25% FC-SDL treatments (Table 3).

Shoot dry weight

Different percentages of field capacity and photoperiod had significant effects on dry weight (Table 3). Reducing field capacity and photoperiod significantly decreased the dry weight. The shoot dry weight in 100% FC conditions decreased 69.78% compared to 25% FC condition (Table 3). Similar results have been reported on creeping bentgrass (*Agrostis stolonifera* L.), rough bluegrass (*Poa trivialis* L.), and perennial ryegrass (*Lolium perenne* L.) (Pessarakli and Kopec, 2008), bermudagrass (*Cynodon dactylon* L.) (Riaz *et al*., 2010). The highest and lowest dry weight was observed in 100% FC-LDL and 25% FC-SDL treatments, respectively (Table 3). Burton *et al.* (1988) stated that day length was highly correlated with yield of 'Coastal' bermudagrass, with yield reduction occurring in day lengths under 13 h. Therefore, photoperiod influenced dry matter production of forage grasses. Extended photoperiod throughout the cool-season in short-day length conditions substantially decreased forage yield (Sinclair *et al*., 1997, 2001, 2003).

Root fresh weight

Root fresh weight significantly declined by decreasing field capacity from 100% to 25%. Root fresh weight decreased (59.76%) at 25% FC compared to 100% FC (Table 3). The impact of partially closing stomata limits CO_2 availability and reduces photosynthesis, which is vital to produce and translocate carbohydrates to roots to explore deeper moisture (Huang, 2006). Huang and Gao (2000) found that severe leakage of organic solutes from roots in drying soil gives evidence that root death of tall fescue cultivars during drought stress may correlate with root desiccation. There was a significant difference between LDL, IDL and SDL treatments and the highest and lowest root fresh weights were obtained in LDL and SDL treatments, respectively. Root fresh weight decreased 9.50% at SDL compared to LDL (Table 3). This is in agreement with Wang *et al*. (2004) who reported that an increase in root growth is associated with extended light duration and is related to increase in internal cytokinin concentration and its increased activity in root tips.

Root dry weight

As shown in Table 3, reduction in field capacity decreased root dry weight of plants. The highest and lowest root dry weights were observed in 100% FC and 25% FC treatments, respectively and in 25% FC decreased 58.10% compared to 100% FC. Pessarakli and Kopec (2008) demonstrated that, water deficit conditions showed a significant decrease in root dry weight of three turfgrass species. The highest and lowest root dry weight was obtained in LDL and SDL treatments, respectively (Table 3). Root dry weight decreased 6.61% at SDL compared to LDL (Table 3). Beard (1972) reviewed the morphological responses of turfgrasses under shade based on the research conducted before 1995, and found alterations such as: reduced tillering and shoot density, longer internodes with a reduced stem diameter, increased leaf length, decreased leaf width, thinner leaves, more

vertical leaf orientation, and fewer roots (McBee and Holt, 1966; Almodares, 1980; Dudeck and Peacock, 1992). A shift in allocation of dry matter occurs in response to shade, resulting in more dry matter partitioning into shoots rather than roots (Allard *et al*., 1991; Dias-Filho, 2000). In response to lower irradiance, accelerated leaf elongation and decrease in partitioning to root dry matter are adaptive strategies to enhance light capture (Semchenko *et al*., 2012).

Proline content

Reducing field capacity and photoperiod significantly increased proline content in all plants. The highest amount of proline content was obtained in 25% FC and the lowest one was obtained in 100% FC treatment (Table 3). This is in agreement with (Etemadi *et al*., 2005) who demonstrated that the increase in drought increased proline content in bermudagrass (*Cynodon dactylon* L.). During drought stress, plants respond to different stresses with changes they create in their physiological features. Accumulation of soluble material in response to drought is a way to maintain turger. It seems that the accumulation of free proline in plants is the general reaction to the stress. However several other amino acids increase under drought and salinity stress. But the degree of changes is not comparable with proline accumulation (Gzik, 1996). In a comparative study between perennial ryegrass and red fescue for the amount of resistance to the drought, it was seen that the amount of proline in red fescue was more than perennial ryegrass (Bandurska and Jozwiak, 2010). The highest and lowest proline content was obtained in SDL and LDL treatments, respectively (Table 3). This is in agreement to the findings reported on the effects of decreased photoperiod on bermodagrass (Esmaili and Salehi, 2012). Interaction between field capacity and photoperiod resulted in the highest and lowest proline content in 25% FC-SDL and 100% FC-LDL treatments (Table 3).

Chlorophyll content

Field capacity and light durations had significant effects on leaf chlorophyll content. The highest and lowest chlorophyll content, were observed in 100% FC and 25% FC treatments, respectively (Table 4). Induction of drought has caused a reduction of electron carrier in photosynthesis and a reduction in chlorophyll content which has been reported by (Zuily *et al*., 1990; Moran *et al*., 1994). Prolonged drought, heat, and the combined stresses could lead to loss of chlorophyll and lipid peroxidation, resulting in further turf quality decline (Jiang and Huang, 2001). Water is required to facilitate photosynthesis in plants. Low energy electrons are extracted from water and are energized through light energy captured by chlorophyll. These energized electrons enable the production of NADPH and ATP which are then used to reduce CO_2. CO_2 is taken up from the atmosphere through stomata. Stomata are very sensitive to external environmental factors such as light, CO_2, water status, and temperature (Hopkins and Hüner, 2004). The loss of chlorophyll by the plant in an intense stress can be associated with photo oxidation and consequently oxidative stress (Kato and Shimizu, 1985). Kaiser (1987) indicated that an irreversible decrease in plant photosynthetic capacity occurs as RWC declines below 30%, leading to cell death from membrane damage in chloroplasts.

Table 4 - Effect of field capacity and photoperiod and their interaction on proline, sugars and starch contents, activity of Superoxidase dismutase, Catalase, Peroxidase, and Ascorbate peroxidase enzymes

Variables	Photo-period	Field capacity (%)				Mean
		100%	75%	50%	25%	
Chlorophyll	LDL	1.81 a	1.78 b	1.48 e	0.99 j	1.52 A
content	IDL	1.74 c	1.71 d	1.41 h	0.92 k	1.44 B
(mg Chl g^{-1}f.w.)	SDL	1.45 f	1.42 g	1.12 i	0.63 l	1.15 C
	Mean	1.67 A	1.64 B	1.33 C	0.85 D	
Sugars of shoot	LDL	62.63 de	67.19 c	199.39 b	212.15 a	135.34 A
(mg g^{-1}d.w.)	IDL	61.81 e	66.37 cd	198.22 b	211.33 a	134.52 A
	SDL	61.45 e	66.02 cd	198.22 b	210.98 a	134.17 A
	Mean	61.96 D	66.53 C	198.72 B	211.49 A	
Starch content	LDL	225.30 a	224.10 d	91.50 g	41.30 j	145.50 A
(mg g^{-1} d.w.)	IDL	224.90 b	223.70 e	91.10 h	40.90 k	145.10 B
	SDL	224.30 c	223.20 f	90.60 i	40.40 l	144.60 C
	Mean	224.80 A	223.70 B	91.10 C	40.90 D	
Superoxide	LDL	136.00 cd	148.50 c	266.00 a	116.00 def	166.62 A
dismutase	IDL	128.50 cde	143.50 c	261.50 a	109.50 ef	160.75 A
(Ug^{-1} FW)	SDL	106.00 fg	118.50 def	236.00 b	86.00 g	136.62 B
	Mean	123.50 C	136.83 B	254.50 A	103.83 D	
Catalase	LDL	31.48 ef	34.45 cd	40.71 a	31.18 efg	34.45 A
(Ug^{-1} f.w.)	IDL	28.89 fgh	31.86 de	38.12 ab	28.59 ghi	31.86 B
	SDL	29.53 efg	29.53 efg	35.78 bc	26.25 i	29.53 C
	Mean	28.98 C	31.95 B	38.20 A	28.67 C	
Peroxidase	LDL	68.40 fg	70.75 f	94.58 c	112.40 a	86.53 A
(Ug^{-1} f.w.)	IDL	60.80 hi	63.15 hi	86.99 d	104.81 b	78.94 B
	SDL	52.83 j	55.18 ji	79.02 e	96.84 c	70.97 C
	Mean	60.68 C	63.03 C	86.86 B	104.68 A	
Ascorbate	LDL	869.64 de	879.64 d	1160.36 a	854.29 ef	940.98 A
peroxidase	IDL	829.64 gh	839.64 fg	1120.36 b	814.29 hi	900.98 B
(Ug^{-1} f.w.)	SDL	784.64 kj	794.64 ij	1075.36 c	769.29 k	855.98 C
	Mean	827.97 B	837.97 B	1118.69 A	812.61 C	

*In each variable, data followed by the same letters (small letters for interactions and capital letters for means) are not significantly different using LSD at 5% level.
LDL= long day length.
IDL= intermediate day length
SDL= short day length.

Detrimental effects on chloroplast biochemistry or chlorophyll fluorescence occur when RWC drops below 60% in tall fescue (Huang *et al*., 1998). Surface drying had no effects on chlorophyll content in kentucky bluegrass (*Poa pratensis* L.) and tall fescue (*Festuca arundinacea* Schreb.) while under full drying, chlorophyll content decreased in both grasses (Fu and Huang, 2001). Our findings were in agreement are (Fu and Huang, 2001) who reported that amount of chlorophyll in bermudagrass under moderate stress is not reduced, but it will be reduced in the severe drought. Chlorophyll content decreased with decreasing day length and the highest and lowest ones were observed in LDL and SDL treatments, respectively (Table 4). Shorting photoperiod caused decrease in chlorophyll content. In a research, the resistances to low light stress in both bermudagrass and paspalum have been examined and it was concluded that resistance to low light stress in the paspalum is more than bermudagrass (Jiang *et al.,* 2004). Baldwin *et al.* (2008) reported that bermudagrass showed significant decrease in chlorophyll content in response to short day length condition.

Total soluble sugars and starch content

Regardless of photoperiod, decrease in field capacity significantly increased total soluble sugars in the shoot (Table 4). Starch content declined by decreasing field capacity from 100% to 25% (Table 4). Shoot starch content, were highest and lowest in 100% and 25% FC treatments, respectively (Table 4). On the other hand, total soluble sugars during the drought can increase making these compounds non-photosynthetic routes and growth stopping due to the destruction of in soluble sugars and their change to soluble sugars (Hissao, 1973). Although some researchers have suggested that the destruction of starch can also increase monosaccharaides (Düring, 1992). The researchers stated that an increase of amylase in water stress causes starch degradation and the conversion of this large molecule into smaller units (Movahhedi-Dehnavi *et al.*, 2004). Different photoperiod had no significant effects on total soluble sugars (Table 4). Shoot starch content decreased by different light durations and the highest and lowest one was observed in LDL and SDL treatments, respectively (Table 4). Starch content decreased in response to shortening the photoperiod. Some researchers have reported that prolonging photoperiod increases carbohydrates (Hay and Pederson, 1986; Solhoug, 1991; Wang *et al*., 1998). Other researchers reported that the photoperiod had no effect on carbohydrates production (Sicher *et al*., 1982; Logendra and Janes, 1992).

Antioxidant enzyme activities

APX, POD, CAT and SOD enzymes activities showed significant differences among field capacity and photoperiod treatments. The activities of APX were not significantly different between 100% FC and 75% FC treatments while were significantly increased in 50% FC treatment and minimum APX activity was observed at 25% FC treatment (Table 4). Bian and Jiang (2009) investigated the accumulation of reactive species of oxygen and antioxidants activity and the pattern of gene expression of antioxidant enzymes in the kentuchy bluegrass in the drought condition. They observed that drought stress increased the activity of APX and CAT and decreased SOD and they stated that antioxidant enzymes and their gene expression might be different or occur in the immune system of kentuchy bluegrass roots and leaves. POD enzyme activities increased with decrease in field capacity levels. Differences in leaf POD enzyme activities were not detected between 100% FC and 75% FC treatments. The maximum and minimum POD activity was obtained in 25% FC and 100% FC treatments, respectively (Table 4). In a research on drought tolerance of three cultivars of creeping bentgrass, it was observed that long-term drought stress reduced the activity of antioxidants such as POD and increased lipid peroxidation and the 'Greenwich' showed high resistance to drought (DaCosta and Huang, 2007). CAT and SOD enzymes activities significantly increased with decreasing field capacity from 100% to 50% then, declined in 25% FC treatment (Table 4). Shao *et al.* (2005) reported that in the of drought stress, the production amount of three enzymes, CAT, SOD and POD in resistant bermudagrass varieties have been significantly more than drought-sensitive ones. General declines in antioxidants, including CAT were reported in the response of three species of creeping bentgrass to drought stress. Moreover, they found that the species *Agrostis canina* L. was the most resistant species to drought (DaCosta and Huang, 2007). Liu *et al*. (2008) in a research, physiologically and morphologically investigated the five cultivars of kentuchy bluegrass under drought and heat stress and observed that drought and heat stress simultaneously reduces SOD enzyme in all cultivars and stated that an increase of SOD enzyme activity cannot inhibit stress and would only delay free radicals accumulation. Results of present study indicated that regard-

less of field capacity treatments, APX, POD, CAT and SOD enzymes activities significantly decreased in response to decreasing day length therefore, the maximum and minimum enzymes activity was observed in LDL and SDL treatments (Table 4). Similar findings have been previously reported by (Burritt and Mackenzie, 2003) who stated that when the begonia plant is transferred from low light to bright light, CAT activity increases. Also, they stated that when the (*Picea abies* L.) seedlings are transferred from low light to high light, the activity of CAT enzyme decreases. Xu *et al.* (2010) investigated the effect of nitric oxide and sodium nitroprusside in tall fescue under high light stress and concluded that using sodium nitroprusside reduces enzyme activity of SOD, CAT and APX, but using nitric oxide increases the activity of mentioned enzymes. Jiang *et al.* (2005) demonstrated that, low light conditions showed a significant decrease in activity APX and CAT of bermudagrss and paspalum. Grace and Logan (1996) reported that the CAT enzyme activity varies depending on light intensity. The CAT enzyme activity in Schefflera [*Schefflera arboricola* (Hayata) Merrill] and Vinca (*Vinca major* L.) plants did not change with a change in light intensity, but in Mahonia (*Mahonia repens* (Lindley) Don.), CAT enzyme activity increased with an increase of light intensity. Interaction between field capacity and photoperiod resulted in the highest and lowest SOD enzyme activities in 50% FC-LDL and 25% FC-SDL treatments (Table 4).

4. Conclusions

The results proved that the reduction in photoperiod led to a progressive increase in shoot height and leaf area, however, the increase in irrigation inhibited their progressive growths. Additionally, the reduction in photoperiod caused a decrease in fresh and dry weight of root and shoot. However, the increase in irrigation led to alleviation of these negative effects during the day-time and thus increased the fresh and dry weight of root and shoot. Therefore, it appears as though the increased irrigation might have contributed to the enlargement and flexibility of cells, which, in turn helped increasing the dry and fresh weight of root and shoot. The reduced photoperiod led to a reduction in chlorophyll and starch contents and enzymes activities, and the increased irrigation compensated this reduction to some extent. This phenomenon might be, at least in part, explained by the fact that irrigation reduced ABA production, inhibited ROS production and thus inhibited the closure of stomata. In overall, the increase in irrigation caused the destructive effects of reduced photoperiod to diminish, and vice versa. It seems that the interaction of photoperiod and irrigation treatments has superior effects on alleviating of the symptoms of stressed plants, than their separate. Further studies are needed to clarify more the interaction between irrigation and light treatments at structural and ultrastructural levels, in common bermudagrass.

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Salicylic acid at different plant growth stages affects secondary metabolites and phisico-chemical parameters of greenhouse tomato

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Key words: antioxidant activity, flavonoids, *Solanum lycopersicum*, total phenolics, yield components.

Abstract: Most of the researches on Salicylic acid (SA) have focused on postharvest application or acquiring stress resistance, while studies on its effect on plant growth, secondary metabolites and fruit quality are limited. SA as foliar application (0, 150, 300 and 450 mg/L) at different plant growth stages on fruit yield, secondary metabolites and quality features of tomato (*Solanum lycopersicum* L. cv. Kardelen) under greenhouse conditions were evaluated. The highest fruit yield per plant (about 1.3-fold greater than control) was obtained from 300 mg/L SA when applied three weeks after fruit set. Comparing to control plants, the highest fruit firmness, 10 days prolonged storability, highest total phenolics (22.6 mg gallic acid equivalent per 100 g FW); and highest antioxidant activity (65.11) were observed when 450 mg/L SA applied at fruiting stage and 3 weeks later. An increasing pattern in ascorbic acid content was observed with increasing SA concentration irrespective to application time. The same concentration effect was observed in flavonoid content when plants treated at 3 weeks after fruiting. The highest effect of flavonoids on antioxidant activity was calculated using Pearson correlation (r=0.82). SA concentrations greater than 450 mg/L showed significant adverse effects on all measured traits. The effect of exogenous SA on tomato plant depends on the developmental stage and SA concentrations tested. Improved fruit quality factors may happen in a certain concentration range, while over that may have negative or adverse effect.

1. Introduction

Tomato as one of the most widely produced and consumed 'vegetable' in the world (Heuvelink, 2005) contains high levels of antioxidant active compounds such as vitamin C, polyphenlos and carotenoids (Tommonaro *et al.*, 2012).

Salicylic acid (SA) has been the focus of intensive research due to its role in plant defense mechanisms and response to abiotic stresses (Rivas-San Vicente and Plasencia, 2011). Besides, it is stated that SA plays a crucial role in physiological and biochemical processes during the entire lifespan of the plant (Rivas-San Vicente and Plasencia, 2011). SA as an endogenous plant growth regulator controls a large variety of physiological processes: from regulatory signal in plants mediating defense against pathogens, to ethylene biosynthesis, action and inhibition. It is also involved in plant responses to abiotic stress conditions such as salt and osmotic stresses (Khalil, 2014). Exogenous application of SA also results in many different changes in plant physiological processes and reactions such as prevention of ethylene production (Khan *et al.*, 2003); increases in plant height, number of branches, number of leaves (Saharkhiz *et al.*, 2011) and antioxidant activity (Ananieva *et al.*, 2004).

Most of the researches on SA have focused on mediating local and systemic plant defense and resistance to biotic and abiotic stresses (Atkinson and Urwin, 2012), while studies on its effect on physiological, biochemical and quality features of fruit are limited (Ali *et al.*, 2014). Since plant growth, development and the level of bioactive compounds especially antioxidant active substances depend on the cultivar, and by agronomic and environmental conditions (Tommonaro *et al.*, 2012), the aim of this work was to study the influences of SA on growth, fruit quality attributes including fruit firmness and storability, vitamin C, antioxidant activity, total phenolic, flavonoids and yield of tomato under greenhouse conditions.

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2. Materials and Methods

Plant material and experimental design

The tomato (*Solanum lycopersicum* cv. Kardelen) seeds were obtained from Gento Seeds® Co., Turkey. Six weeks old seedlings having 4-5 leaves were transplanted into a 500 m^2 polycarbonate greenhouse silt-loam soil (Greenhouse Research Center, College of Agriculture, Shiraz University, Shiraz, Iran) at 50 cm double rows and 150 cm between rows spacing (density of 2 plants/m^2). The greenhouse conditions were set to temperature of 25±2°C and relative humidity of 60-70% during the entire study. Soil was already fertilized according to the soil test results and certified lab recommendations for growing greenhouse tomato (Papadopoulos, 1991).

Treatments consisted of SA (Merck Millipore Corporation, Germany) foliar application at four concentrations (0, 150, 300, and 450 mg/L) at different growth stages (transplant establishment, onset of flowering, fruit set, 3 weeks after fruit set) and their all possible combinations (Table 1). The experiment was arranged in a completely randomized design with three replications. Each sample for analyses consisted of three fruits (per plant) of four plants for each replicate per treatment.

Measurement methods

Fruits were harvested at mature red stage based on the "Color Classification Requirement in United States Standards for Grades of Fresh Tomatoes" chart, published by USDA.

Tomato produces fruits on clusters. Fruits of two first clusters were considered as yield and expressed as Kg/plant. Fruit firmness was measured as penetration force on the fruit flesh (over the fruit locules) using Force Gauge, FG-5005 (Lutron Electronic Enterprise Co. Taipei, Taiwan) with a probe diameter of 8 mm. The average values obtained for each fruit was calculated and expressed as Newton. Fruit storability was measured as the number of days from keeping red fruits in a storage at 12±1°C and 80% relative humidity to starting fruit shrinkage or lose their shiny appearance.

Vitamin C quantification was performed according to the method described by the AOAC (1984) and results were expressed as mg ascorbic acid per 100 grams of fruit DW.

Total phenolic content (TPC) was determined with the Folin-Ciocalteau reagent using the method of Spanos and Wrolstad (1990) and the results of three replicates were expressed as mg gallic acid equivalent per 100 grams of fruit DW (mg GAE/100g DW).

Table 1 - Effect of salicylic acid foliar application at different tomato plant phonological stages on some fruit characteristics

Measured trait	Salicylic acid (mg/l)	Application time										
		Establishment	Flowering	Fruiting	3 weeks after fruiting	Establishment + Flowering	Establishment + Fruiting	Establishment + 3 weeks after fruiting	Flowering + Fruiting	Flowering + 3 weeks after fruiting	Fruiting + 3 weeks after fruiting	All 4 times
Fruit firmness (N)	150	9.97±0.47 no	10.76±0.59 mn	12.59±0.13 kl	12.20±0.21 lm	12.33±0.14 lm	12.62±0.24 kl	12.27±0.05 lm	12.67±0.36 kl	10.89±0.64 mn	13.49±0.99 j-l	13.94±0.20 i-k
	300	13.96±0.35 i-k	14.69±0.68 g-j	16.14±0.82 e-g	14.51±0.39 g-i	15.07±0.24 f-j	15.56±0.12 e-h	14±0.50 h-k	15.33±0.28 f-i	14.38±0.07 g-i	16.11±0.52 e-g	16.10±0.65 e-g
	450	16.19±0.83 e-g	16.28±0.87 e-g	19.92±0.78 a	16.95±0.21 c-e	16.23±0.40 e-g	18.28±0.31 bc	16.45±0.37 ef	18.86±0.12 ab	16.60±0.32 d-f	18.89±1.17 ab	18.10±0.54 b-d
	Control	9.07±0.51 o										
Shelf life (days at 12°C)	150	21.00±0.57 d	21.66±0.33 b-d	21.66±0.33 b-d	22.00±1.0 b-d	21.66±0.23 b-d	21.66±0.66 b-d	21.66±0.31 b-d	21.33±0.33 cd	21.66±0.41 b-d	22±0.57 b-d	22±0 b-d
	300	21.66±0.33 b-d	22±0.57 b-d	22±0.51 b-d	22.33±0.33 b-d	22.00±0.54 b-d	22.33±0.33 b-d	22.33±0.13 b-d	22.33±0.34 b-d	22±0.02 b-d	22.33±0.33 b-d	22.33±0.33 b-d
	450	22.33±0.66 b-d	22±0.57 b-d	22.33±0.88 b-d	24.66±1.33 a	21.83±0.44 b-d	22.33±0.33 b-d	22.66±0.88 bc	22.66±0.23 bc	23±0.57 b	23±0.57 b	22.33±0.33 b-d
	Control	17.00±0.00 e										
Vitamin C (mg/100 g dw)	150	13.88±0.35 pq	13.99±0.35 pq	14 39±0.42 o-q	18.28±0.26 l-o	15.42±0.81 n-q	16.92±0.62 m-p	19.12±0.51 l-n	14.74±0.39 o-q	20.37±0.92 k-m	21.03±1.24 j-l	21.87±1.90 i-l
	300	23.56±0.22 h-k	24.91±1.63 h-j	27.15±1.25 e-h	28.47±1.63 b-g	25.10±0.68 h-g	25.13±0.13 h-g	26.01±0.48 f-h	27.30±0.51 d-h	27.52±1.53 d-h	27.81±1.06 c-g	29.35±2.28 b-f
	450	31.11±0.79 a-e	31.62±0.89 a-c	32.47±0.12 ab	32.50±0.09 ab	31.18±0.73 a-e	31.26±0.72 a-d	31.95±0.92 ab	31.73±0.92 a-c	32.25±0.38 ab	34.34±2.07 a	32.50±0.84 ab
	Control	11.82±0.24 q										
Total phenolics (mg GAE/100 g dw)	150	14.19±0.09 l	14.51±0.16 k	15.60±0.25 i	16.14±0.14 gh	15.12±0.10 j	15.18±0.10 j	15.71±0.05 i	14.59±0.23 k	15.70±0.20 i	15.84±0.11 hi	16.26±0.22 g
	300	19.66±0.17 f	19.73±0.14 f	20.13±0.12 e	20.24±0.06 e	20.04±0.06 ef	20.12±0.065 e	20.13±0.06 e	19.99±0.12 ef	20.22±0.03 e	20.26±0.05 e	21.45±0.06 d
	450	21.47±0.03 d	21.71±0.29 b-d	22.64±0.09 a	22.67±0.12 a	21.54±0.07 cd	21.51±0.09 cd	21.59±0.11 cd	21.49±0.02 cd	21.88±0.18 bc	21.73±0.19 b-d	22.01±0.06 b
	Control	10.84±0.10 m										
Flavonoids (mg GAE/100 g dw)	150	1.05±0.08 n	1.06±0.08 n	1.06±0.01 n	1.09±0.03 kl	1.09±0.08 kl	1.07±0.03 mn	1.09±0.08 lm	1.08±0.03 lm	1.08±0.01 lm	1.08±0.09 lm	1.09±0.06 j-l
	300	1.09±0.06 j-l	1.13±0.04 i-k	1.11±0.07 h-j	1.14±0.01 b-e	1.09±0.06 j-l	1.09±0.09 j-l	1.12±0.06 g-i	1.10±0.05 j-l	1.12±0.03 f-h	1.14±0.09 d-g	1.09±0.10 j-l
	450	1.14±0.06 d-g	1.14±0.06 c-f	1.13±0.01 e-g	1.18±0.05 a	1.14±0.07 c-f	1.14±0.05 c-f	1.16±0.06 bc	1.14±0.01 d-g	1.15±0.07 b-d	1.16±0.08 ab	1.14±0.08 c-f
	Control	0.99±0.07 o										

Averages±SD for each measured trait with the same letters showing no significant differences using LSD test at p<0.05.
Control plants did not receive any SA at any time.

Total antioxidant activity (TAA) was measured using DPPH (2,2-diphenil-1-picrylhydrazyl) (Merck Millipore Corporation, Germany) assay as described by Patras *et al.* (2009) at the absorbance of 517 nm using micro plate reader (Epoch, Germany). TAA was calculated according to the following equation:

$$TAA = [1- (A_{sample\ at\ 517nm} / A_{control\ at\ 517nm})]\times 100$$

Total flavonoid content (TFC) was determined by the aluminum chloride colorimetric assay based on the formation of a complex flavonoid-aluminum, having a maximum absorbance at 510 nm (Toor and Savage, 2005) and results expressed as mg gallic acid equivalent per 100 gram of fruit DW (mg GAE/100 g DW).

Statistics

Data were analyzed using SAS 9.1 statistical software (SAS Institute Inc., Cary, NC, USA). Means were compared using LSD test at $p \leq 0.05$. Pearson correlation statistical method was used to determine the correlation between secondary metabolites and antioxidant activity.

3. Results and Discussion

Our preliminary tests on different concentrations of SA application showed significant adverse effects on all measured traits when concentration was higher than 450 mg/l (data not shown). This could be due to the nature of SA that acts as a plant growth regulator. It is stated that the responses to SA are highly concentration dependent, so that moderate doses of SA improve features such as antioxidant status and induce stress resistance, while higher concentrations trigger a hypersensitive cell death pathway (Tounekti *et al.*, 2013).

Fruit yield

Fruits of the first two clusters were evaluated as yield. The greatest yield was obtained from 300 mg/l SA applied at three weeks after fruit set (Fig. 1). This was 1.26 fold greater than control. The stimulatory effect of SA on flowering regulation which has been well known for a long time (Raskin, 1992; Rivas-San Vicente and Plasencia, 2011) would eventually affect total number of fruits (Ondrašek *et al.*, 2007) and enhance efficiency in fruit production. Previously, increased yield of strawberry (Aghaeifard *et al.*, 2015) and tomato (Javaheri *et al.*, 2012) has been related to promoted cell division and cell enlargement due to SA (Hayat *et al.*, 2010) through its influence on other plant hormones such as auxin,

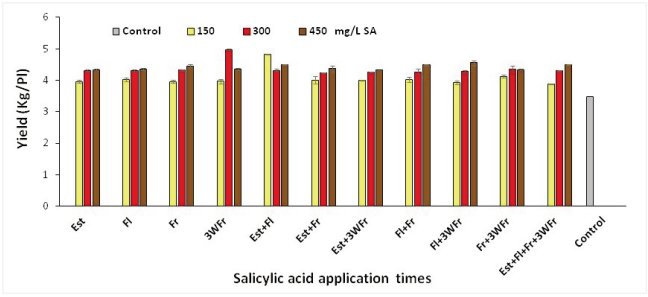

Fig. 1 - Effect of salicylic acid (SA) foliar application at different tomato plant phenological stages on yield (two first clusters) at greenhouse condition. Transplant establishment (Est), flowering (Fl), fruiting (Fr), three weeks after fruiting (3WFr).

cytokinin and ABA balances (Shakirova, 2007) and enhanced net photosynthetic rate, internal CO_2 concentration and water use efficiency (Fariduddin *et al.*, 2003). It is reported that in non-thermogenic plants such as tobacco, SA levels increase 5- and 2-fold in their leaves at the initiation of or during transition to flowering, respectively (Abreu and Munné-Bosch, 2009).

Fruit firmness

Firmness is an important physical parameter for postharvest storage, transportation and monitoring the fruit ripening process. SA and its derivatives are widely in use to enhance fruits postharvest life by enhancing fruit firmness during storage (Wang *et al.*, 2006). In our experiment, SA significantly affected fruit firmness. The firmness became 2.9 times higher than the control group when 450 mg/L SA was applied at fruiting stage. Plants treated with 150 mg/L SA at establishment stage showed the lowest fruit firmness as equal to control plants (Table 1). A 2-fold greater tomato fruit skin thickness due to 0.01M SA application comparing to control has been previously reported (Javaheri *et al.*, 2012). Metabolic reactions respiration, ethylene production (Aktas *et al.*, 2012) breakdown of cell wall and activation of enzymes involved therein are key events in fruit ripening and softening (Prasanna *et al.*, 2007). These metabolic activities can be harmful to maintain fruit quality. There are reports that SA could prevent the activity of such enzymes, while affect the swelling of cells in a manner that results in firmer fruit (Zhang *et al.*, 2003; Prasanna *et al.*, 2007). SA prevents fruit softening. Shafiee *et al.* (2010) have cited several reports indicating that rapid softening of fruits during ripening was simultaneous with rapid decrease in endogenous SA of fruits. Increased firmness in climacteric fruits due to pre-harvest SA spray has been attributed to the role of SA in preventing cell wall and membrane degrading enzymes (polygalacturonase,

lipoxygenase, cellulase, pectinemethylesterase), ethylene production (Zhang *et al.*, 2003) and reduced hydrolysis of soluble starch and therefore higher firmness (Tareen *et al.*, 2012 b), while in non-climacteric fruits such as "Flame seedless" grape it has been attributed to the role of SA in preventing decay (Khalil, 2014).

Fruit storability

The greatest fruit storability period was observed in 450 mg/l SA when applied at 3 weeks after fruiting (Table 1). This was over 10 days more than control plants. Aghdam *et al.* (2014) attributed longer storability and higher chilling resistance of detached tomato fruits treated with SA to increased endogenous proline content. Lowered ethylene biosynthesis has been also considered to be the main cause of prolonged storability due to regulatory potential of exogenous SA on fruit ripening of green mature tomato fruits (Kant *et al.*, 2013). Parallel to this, SA can activate the alternative respiration pathway in many plant tissues which results in a lower respiratory rate and delay in the climacteric peak (Raskin, 1992). Srivastava and Dwivedi (2000) stated that the concentration of SA determines the extent to which these effects actualize. Reduced the quality loss during storage due to SA were previously reported for tomato (Ding *et al.*, 2001) and sweet peppers (Fung *et al.*, 2004).

Vitamin C

Human diet consists of about 91% of ascorbic acid coming from fruits and vegetables (Tareen *et al.*, 2012 a). An increasing pattern of ascorbic acid content was observed with increasing SA concentration irrespective to application time (Table 1). The greatest ascorbic acid content was obtained from 450 mg/L SA. In most cases, this increase was about 3 times than control plants. Some researches indicated that the treatment of tomatoes (Javaheri *et al.*, 2012; Kalarani *et al.*, 2002) and strawberry (Aghaeifard *et al.*, 2015) with SA caused them to acquire higher levels of ascorbic acid comparing to control plants. SA can activate ascorbate peroxidase, which is the precursor to ascorbic acid in fruits and prevents vitamin C from being destroyed in cells and therefore causes the accumulation of ascorbic acid in the fruit (Wiśniewska and Chełkowski, 1999).

Total Phenolics

Phenolic compounds as secondary plant metabolites are synthesized by all plants and responsible for the flavor and color of fruit products (Jeong *et al.*, 2008). The highest amount of phenolic compounds was observed in plants treated with 450 mg/L SA during the fruiting stage and three weeks after the fruiting (Table 1). In those treatments, a 2.9 times higher phenolic compounds content than control plants was observed. Although Aghdam *et al.* (2012) reported no significant effect of SA application on total phenolics content of mature green tomato fruits, our results were similar to reports on sweet cherry (Valero *et al.*, 2011) and grapes (Ranjbaran *et al.*, 2011; Khalil, 2014), which all concluded SA application induced greater total phenolics and other secondary metabolites with antioxidant properties (Ranjbaran *et al.*, 2011). Previously, the application time of SA on sweet cherry at three fruit developmental stages (pit hardening, initial color changes and onset of ripening) increased fruit weight and led to higher concentration of total phenolics and total anthocyanins, as well as higher antioxidant activity (Giménez *et al.*, 2014).

Antioxidant activity

It is well known that the positive effect on health associated with tomato consumption is exerted by the pool of antioxidants, with noticeable synergistic effects (Tommonaro *et al.*, 2012). The highest antioxidant activity was observed in 450 mg/L SA treatment when applied on fruiting stage plus three weeks after fruiting. This was 1.84 times greater than the control, which had the least antioxidant activity. An increasing pattern in antioxidant activity was observed with increasing SA concentration, irrespective to application time (Fig. 2). The same pattern was previously found in orange (Huang *et al.*, 2008 b) and pears (Cao *et al.*, 2006). Increased total antioxidant activity of strawberry due to SA has been previously reported (Aghaeifard *et al.*, 2015). Regular applications of salicylic acid at different stages of plant growth and fruit development can increase the antioxidant activity

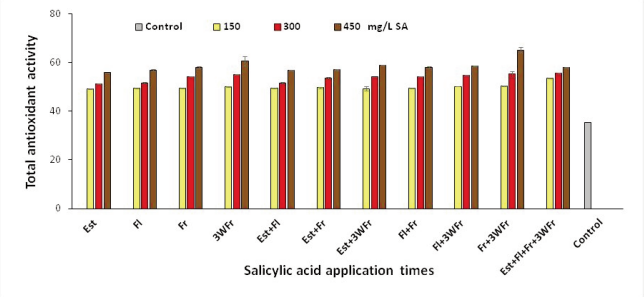

Fig. 2 - Effect of salicylic acid (SA) foliar application at different tomato plant phenological stages on fruit total antioxidant activity at greenhouse condition. Transplant establishment (Est), flowering (Fl), fruiting (Fr), three weeks after fruiting (3WFr).

(Shakirova, 2007). It is frequently hypothesized that SA has direct physiological effects on the activity of antioxidant enzymes which promote the synthesis of metabolites existing in fruits and vegetables, especially those with nutritional value in the product (Huang *et al.*, 2008 a).

Flavonoids

Flavonoids comprise a diverse group of natural compounds and are among the best-known natural phenols, exhibiting an array of chemical and biological pathways such as radical scavenging and antimicrobial activities. An increasing pattern in the amount of flavonoids was found by increasing SA concentration irrespective to application time. The highest amount of flavonoids was observed in 450 mg/L SA treatment when plants were treated either in three weeks after fruiting or fruiting plus three weeks after fruiting stages with about 1.18 times greater than control treatment (Table 1). Reports have been cited stating that exogenous SA applications boost the accumulation of flavonoids in several plant species (Tounekti *et al.*, 2013). On the other hand, research has proved that flavonoids possess antibiotic activities (Al-Matani *et al.*, 2015). This can generate debate as to whether higher amounts of flavonoids can contribute to a longer shelf life against the rot of perishable fruits like tomato. When vegetables are heated for special purposes in food industries, excess heat can cause degradations in flavonoids and thus can reduce its overall content (Sharma *et al.*, 2015).

Correlation between secondary metabolites and antioxidant activity

A Pearson correlation analysis was performed to determine relationships between the individual parameters phenolic compounds, flavonoids and ascorbic acid which contribute in antioxidant activity. Significant correlations were found for all measured traits with the highest effect of flavonoids on antioxidant activity; however, it was not a simple sum of their contribution (Table 2). This has been related to synergistic effect among all antioxidants and their interactions with other constituents of the fraction (Jimenez *et al.*, 2002; Lenucci *et al.*, 2006). Similar to our results, Ilahy *et al.* (2011) found a good significant correlation between antioxidant activity and main antioxidants (vitamin C, flavonoids and total phenols). Given the key role of SA in increasing ascorbic acid (Dat *et al.*, 1998), total phenolics and other secondary metabolites with antioxidant properties (Ranjbaran *et al.*, 2011), the rise in antioxidant activity can thus be explained.

Table 2 - Pearson correlation analysis between secondary metabolites and antioxidant activity

	Phenolic compounds	Flavonoid	Vitamin C
Antioxidant activity	0.621 (*)	0.823 (*)	0.639 (*)

(*) Significant differences at p<0.05.

4. Conclusions

Treatments with SA could be a promising tool to improve tomato yield, fruit quality attributes and health beneficial compounds (including phenolic compounds, vitamin C and flavonoids having antioxidant activity) because of its diverse regulatory roles in plant metabolism. The effect of exogenous SA on plant depends on the plant species, developmental stage, and the SA concentrations tested. Fruit setting stage and 3 weeks later are the best two important stages for SA application. A concentration of 300 mg/L SA for increased yield and 450 mg/L SA for improved fruit quality attributes are recommended. It is possible that exogenous application of SA, out of recommended rates, have negative or adverse effect on desired characteristics.

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Pre-storage putrescine treatment maintains quality and prolongs postharvest life of *Musa acuminata* L.

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Key words: firmness, polyphenol oxidase, postharvest, skin color, weight loss.

Abstract: The study was carried out to determine the effect of putrescine on quality and postharvest life of *Musa acuminata* L. during storage. The fruits were dipped at different concentrations of putrescine (0.5, 1 and 2 mM for 30 min) and distilled water as 'control'. Changes in fruit quality attributes such as weight loss, firmness, skin color (L*, hue angle), total soluble solids (TSS), titratable acidity (TA), pH, ascorbic acid, polyphenol oxidase (PPO) and polygalacturonase (PG) enzymatic activity were calculated at harvest and after 5, 10, 15 and 20 days of storage at 0±1°C, 80-85% relative humidity. Weight loss, fruit softening, skin color changes, TSS, pH, the activity of PPO and PG increased during fruit ripening but the rate of changes was significantly slowed in putrescine treated fruits. Moreover, putrescine application maintained higher levels of TA, ascorbic acid and reduced the loss of sensory acceptability and decay incidence compared to control. In conclusion, the postharvest dip treatment of putrescine could be an effective means for extending the storage life of *Musa acuminata* L.

1. Introduction

Banana (*Musa* spp.) is a climacteric fruit; therefore, ripening process is induced by ethylene production via ACC (1-aminocyclopropane 1-carboxylic acid) biosynthesis. The rate of respiration is followed by reaching a threshold level of ethylene within the cells of fruit then rises rapidly to a peak and subsequently falls as ripening progress. During fruit softening, starch is turned to sugars, the peel color changes to yellow and fruit flavor develop by losing its astringency (Pathak *et al*., 2003).

Polyamines as natural compounds suppress ethylene synthesis by inhibition of ethylene biosynthesis enzymes activities (Lee *et al.*, 1997). They are present ubiquitously in plant organs. The main polyamines are putrescine (1, 4-diaminobutane), spermidine (*N*-3-aminopropyl-1, 4-diaminobutane), and spermine [bis (*N*-3-aminopropyl)-1, 4-diaminobutane] which are essential in plant growth, differentiation and stress responses (Valero and Serrano, 2010). They are known to improve the storage life of fruits by inhibiting ethylene production and delaying the ripening process, respectively.

Polyamines and ethylene have opposite impacts on fruit ripening and senescence. Thus, a balance between them is crucial to enhance and retard the fruit ripening process. In general, polyamines level declines throughout fruit senescence along with accelerating ethylene synthesis (Valero *et al*., 2002).

Much researches have indicated the positive effects of pre and postharvest polyamines application on retarding fruit softening in mango (Malik *et al*., 2003) and pear (Franco-Mora *et al*., 2005), reducing weight loss in apricot (Martinez-Romero *et al*., 2002), inhibition of ethylene production in peach (Zokaee Khosroshahi and Esna-Ashari, 2008), delaying ripening process in nectarine (Torrigiani *et al*., 2004) and peach (Bregoli *et al.*, 2002), and maintaining TA at higher levels, diminishing the increase in TSS, and declining color change in plum (Khan *et al*., 2008).

Thus, the present study was carried out to evaluate the application of putrescine for extending quality and storage life of *Musa acuminata* L.

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2. Materials and Methods

Mature green bananas (*Musa acuminata* L.) were harvested from a commercial orchard in Minab, Iran, and then transported to the laboratory for experiments. Fruits uniform in size and color, without any noticeable defects, were selected and dipped in putrescine solution at different concentrations (0.5, 1 and 2 mM for 30 min) and distilled water as 'control'.

Then all of treated and untreated fruits were stored at 13°C and 80-85% relative humidity. Then, some physico-chemical attributes were measured at harvest and after 5, 10, 15 and 20 days of cold storage.

Quality parameters evaluation

Fruit weight was recorded just after harvest and after the different sampling dates and then expressed as percentage of weight loss relative to the initial weight (Soto-Zamora *et al*., 2005).

Fruit firmness was measured using a FG-5020 penetrometer (Lutron Electronic Enterprise Co.) of 5 mm in diameter at 2 equatorial points and was expressed as newton (N).

Color was determined at opposite sides of each fruit from each replicate with a Minolta Chromameter CR400; the following parameters were considered: L* (0= black; 100= white), a* (green to red) and b* (blue to yellow) then expressed as L* and hue angle (h°)= arctan (b* a^{*-1}) (Ozdemir, 2016).

Total Soluble solids (TSS) content was assessed by a digital refractometer (Atago N1, Japan) at 20˚C and expressed as a percent. Titratable Acidity (TA) was estimated by titrating 5 ml of diluted juice against 0.1 N NaOH using phenolphthalein as an indicator and was expressed as percent malic acid (%). The pH of fruit juice was measured using a MTT65 (Japan) pH meter calibrated by pH 4 and 7 buffer solutions.

Ascorbic acid assessment

Ascorbic acid content was estimated using the methods of Marisa and Wall (2006).

Polyphenol oxidase (PPO) and Polygalactronase (PG) activities measurement

PPO and PG were assessed using the procedure of Marquez Cardozo *et al.* (2015) and Zhu *et al.* (2015) respectively.

Decay incidence and sensory acceptability determination

Fruit deteriorations were measured on individual fruit by visual observations. From each fruit 5 slices were obtained and fruits decay was recorded using the following formula: A/B ×100 in which A is the number of decayed fruit slices and B the initial number of all fruit slices.

The fruits were rated by a panel of 10 judges on the basis of color, texture, taste and flavor and overall acceptability (as 1-2 unusable, 3-4 unsalable, 5-6 salable, 7-8 good, 9-10 very good).

Statistical analysis

To estimate storability of fruit, a factorial design completely randomized was carried out in three replications. All data were analyzed using SAS software package 9.4 for windows and mean comparisons were conducted using Duncan's multiple range tests.

3. Results and Discussion

Weight loss and firmness

Weight loss percentage increased in all the treatments along the storage. However, all putrescine concentrations demonstrated significantly lower weight loss than control. Fruits treated with 2 mM putrescine exhibited the lowest weight loss amongst the putrescine concentrations during storage while highest weight loss was registered by control (Fig. 1 A). The effect of putrescine on reducing weight loss

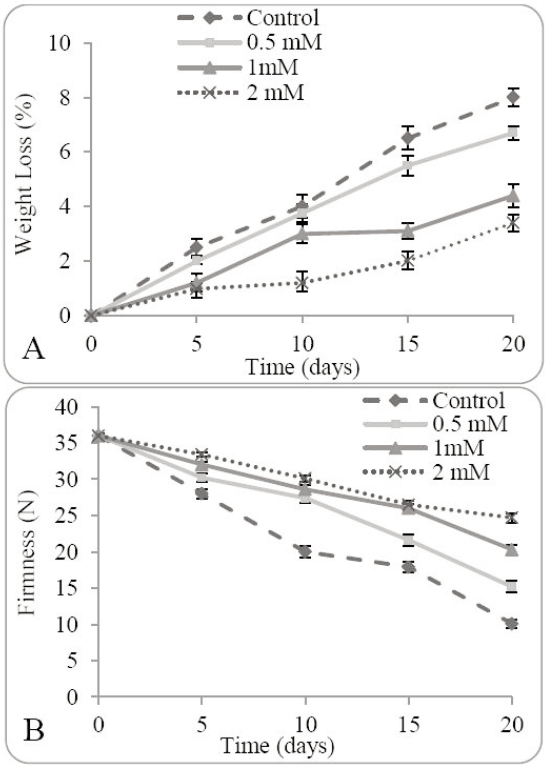

Fig. 1 - The effect of putrescine at different concentrations (0.5, 1 and 2 mM) on weight loss (A) and firmness (B) of *Musa acuminata* L. during storage.

may be ascribed to conjugation of polyamines to the cell membrane phospholipids that result in cell membrane integrity (Mirdehghan and Rahimi, 2016). Similar results have been reported in apricot (Enas *et al.*, 2010).

As shown in figure 1B irrespective of treatments, fruit firmness decreased significantly over storage but putrescine treated fruits were observed firmer, and especially 2 mM putrescine treatments was more effective than others in keeping the firmness. It is suggested that polyamines maintain fruit firmness by their cross-linkage to the pectin substances carboxyl groups in the cell wall and lead to rigidification of cell wall; consequently cell wall degrading enzymes activities of pectin methyl esterase (PME), pectin esterase (PE) and polygalactouronase (PG) are decreased (Valero *et al*., 2002). The results are in line with peach (Bregoli *et al*., 2002).

Color changes

Skin color alteration from green to yellow is a predominant index used for evaluating the stage of ripening in banana (Gomes *et al.*, 2013). As the storage time progressed, fruit color changed as a result of chlorophyll degradation along with carotenoid synthesis. However, putrescine treated fruits showed higher L* and hue angle than control (Fig. 2 A and B).

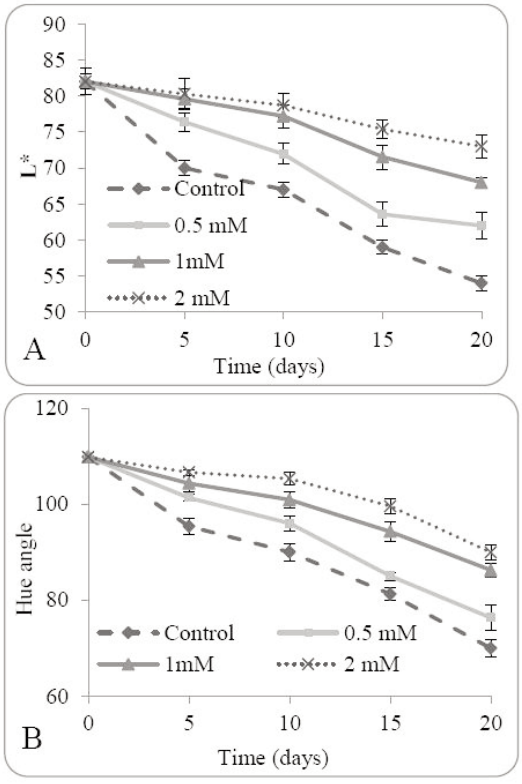

Fig. 2 - The effect of putrescine at different concentrations (0.5, 1 and 2 mM) on L* (A) and hue angle (B) of *Musa acuminata* L. during storage.

Delayed color changes can be associated to the effect of putrescine as anti-senescence by reducing ethylene production and subsequently delaying fruit ripening as well as senescence (Drake and Chen, 2000). Similar results have been observed in apricot (Martinez-Romero *et al*., 2002).

Total soluble solids (TSS), titratable acidity (TA) and pH

TSS content and TA increased along the storage period while pH demonstrated reverse trend in all treated and untreated fruits (Fig. 3 A, B and C). Lower

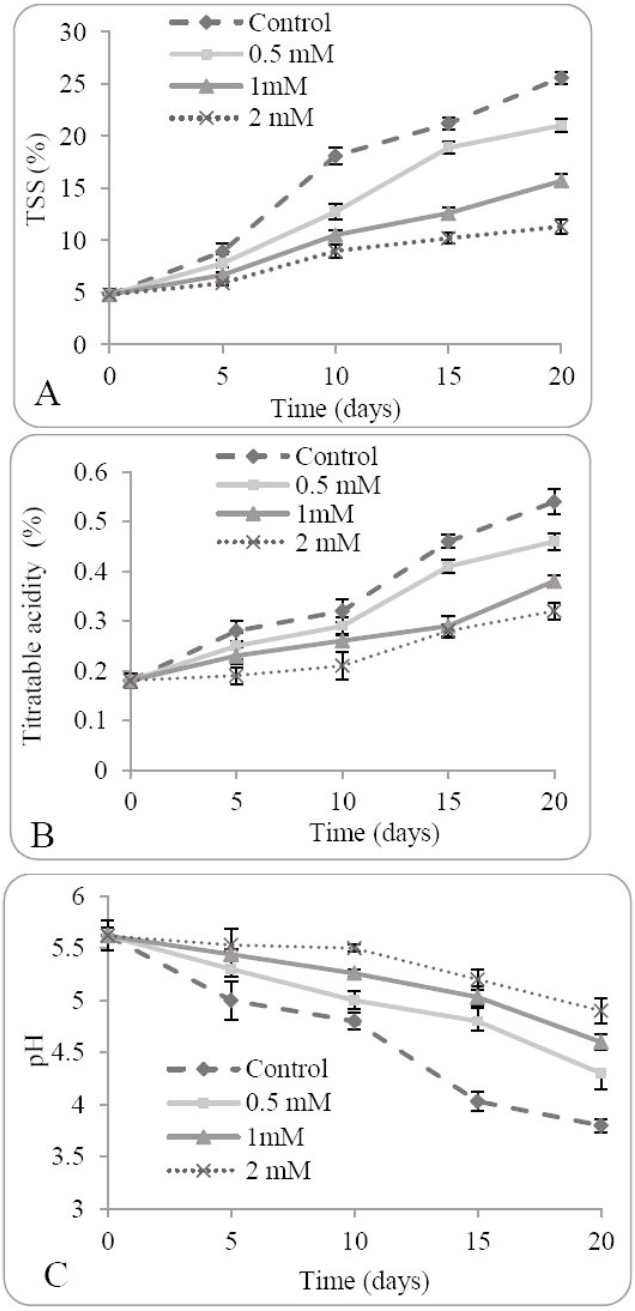

Fig. 3 - The effect of putrescine at different concentrations (0.5, 1 and 2 mM) on TSS (A), TA (B) and pH (C) of *Musa acuminata* L. during storage.

values of TSS, TA and higher value of pH content were observed in putrescine treated fruits compared to control (Fig. 3). That is ascribed to the role of putrescine on delaying fruit ripening process by reducing ethylene production and respiration rate in fruit (Valero *et al*., 2002). The results are in agreement with those observed in mango (Malik and Singh, 2006).

Ascorbic acid

The content of ascorbic acid was significantly influenced by putrescine. The value of ascorbic acid was higher in treated fruits than control throughout the storage (Fig. 4). It is possible that putrescine inhibits ascorbic acid oxidation by decreasing ascorbate oxidase activity and consequently maintaining ascorbic acid (Ishaq *et al*., 2009). This result is in line with the finding of Davarynejad *et al.* (2013).

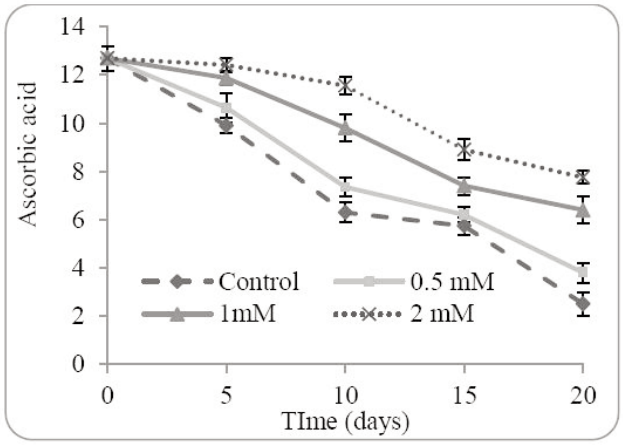

Fig. 4 - The effect of putrescine at different concentrations (0.5, 1 and 2 mM) on ascorbic acid of *Musa acuminata* L. during storage.

Enzymatic activity of polyphenol oxidase (PPO)

Irrespective of treatments, the activity of PPO increased during ripening process and it was significantly higher in control than treated fruits (Figure 5 A). This trend may be attributed to the role of putrescine on reducing polyphenol oxidase activity (Koushesh saba *et al*., 2012). Previously, it has been observed in kiwifruit (Jhalegari *et al*., 2012).

Polygalacturonase (PG) activity

PG is known as an important enzyme on fruit softening, whereas, a reduction in PG activity results in a delay in fruit softening and consequently an increase in the storage life (Jhalegari *et al*., 2012). In this study, the activity of PG increased during the storage. As shown in figure 5 B, untreated fruits demonstrated the highest values of PG activity (1.74 mmol kg^{-1} s^{-1} on the 20th day of storage). Fruits treated with 2 mM exhibited the lowest PG activity, followed by putrescine at 1 and 0.5 mM respectively. This trend is associated to declining fruit firmness and increasing fruit softening by the loss of membrane integrity (Sitrit and Bennett, 1998).

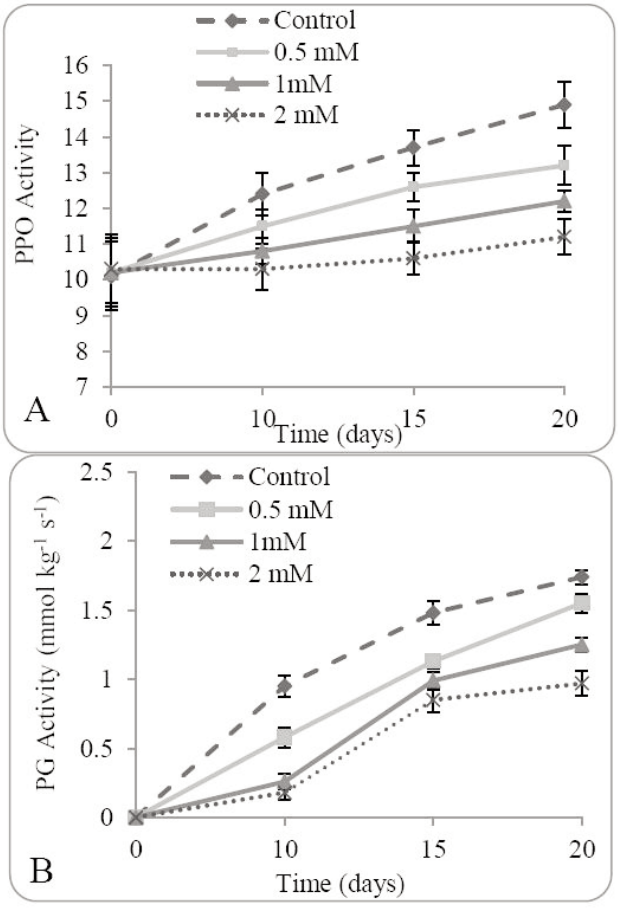

Fig. 5 - The effect of putrescine at different concentrations (0.5, 1 and 2 mM) PPO (A) and PG (B) activities of *Musa acuminata* L. during storage.

Decay incidence and sensory acceptability

The highest rate of fruit decay percent was observed in control while all three concentrations of putrescine reduced the decay development significantly during storage; in particular, the fruits dipped in 2 mM putrescine showed the lowest decay incidence in comparison to others (Fig. 6 A). While time passed, sensor acceptability declined. However, fruit treated by putrescine exhibited higher scores of sensor acceptability compared to control at the end storage (Fig. 6 B).

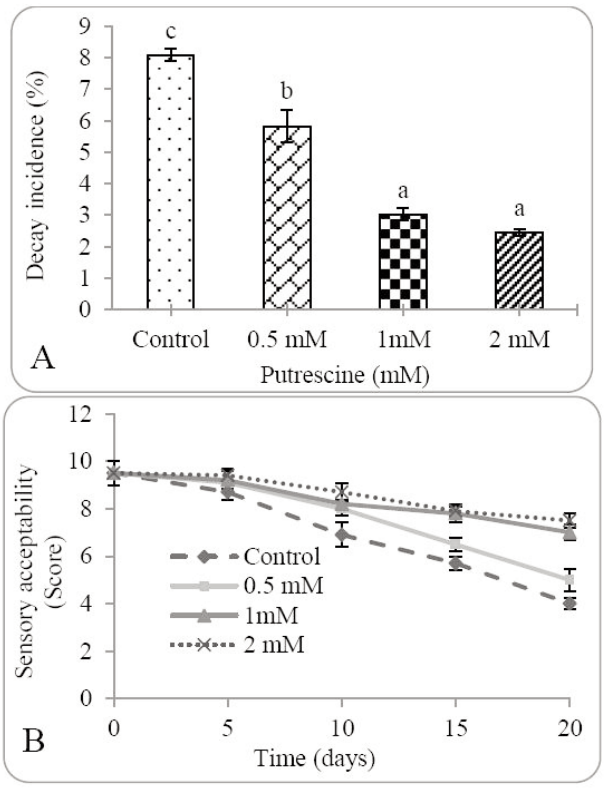

Fig. 6 - The effect of putrescine at different concentrations (0.5, 1 and 2 mM) on decay incidence (A) and sensory acceptability (B) of *Musa acuminata* L. during storage.

4. Conclusions

The effect of putrescine treatment at different concentrations (0.5, 1 and 2 mM for 30 min) was investigated to improve and extend storage life of banana (*Musa acuminata* L.). Application of putrescine maintained fruit quality attributes such as firmness, color, TSS, TA, pH and sensory acceptability. In addition, the reduction of weight loss, PPO, PG, and decay incidence were observed in putrescin treated fruits compared to control. Thus, the postharvest dip treatment of putrescine may be an effective tool for prolonging the storage life of *Musa acuminata* L.

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Bioactivity of selected essential oils from medicinal plants found in Fiji against the Spiralling whiteflies (*Aleurodicus dispersus* Russell)

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Key words: *Aleurodicus dispersus* Russell, essential oils, fumigant and repellent toxicity, GC-MS.

Abstract: The concentration of essential oil solutions [0.25%, 0.5% and 5% (v/v)] of five medicinal plants found in the South Pacific (Fiji) were subjected to the whitefly (*Aleurodicus dispersus* Russell) in order to assess eventual control activities, by both fumigant and repellent tests. The essential oil of *Ocimum tenuiflorum* L. exhibited the strongest fumigant activity against the Spiralling whiteflies with an LC_{50} value of 0.003% followed by the essential oil of *Cymbopogon citratus* (DC.) Stapf. (LC_{50} = 0.004%), *Cananga odorata* (Lam.) Hook F. and Thoms (LC_{50} = 0.050%), *Murraya koenigii* (L.) Spreng. (LC_{50} = 0.113%), and *Euodia hortensis* forma *hortensis* (LC_{50} = 0.114%). The essential oil of *M. koenigii* (RI=52%) and *C. citratus* (RI=52%) at 5% (v/v) concentration were found to have a higher repellent toxicity against the Spiralling whiteflies. The chemical composition of the selected essential oils was also determined using GC-MS. The trend in the chemical constituent of essential oils revealed that the phenolic and alcoholic compounds were the major groups of contributors to the tested activities. Thus, these data suggested that essential oils from the selected medicinal plants found in the South Pacific (Fiji) have the potential to be employed in the pesticidal activities.

1. Introduction

The whitefly, *Aleurodicus dispersus* Russell is commonly known as Spiralling whitefly, a native to the Caribbean region and Central America. The Spiralling whiteflies are thought to be widely spread in the Pacific Islands, America (North and South), Asia and Africa (Waterhouse and Norris, 1989). The Spiralling whiteflies were first discovered in Suva, Fiji Islands in April 1986 and since then was regarded as a serious pest (Kumar *et al.*, 1987; Waterhouse and Norris, 1989). These Spiralling whiteflies pose extreme threats to the agricultural and horticultural crops in glasshouses and fields worldwide (Oliveira *et al.*, 2001; Mani and Krishnamoorthy, 2002; Stansly and Natwick, 2010). Some specific plants that are usually attacked include cassava, pepper, papaya, mango, eggplant, citrus, guava, banana, coconut, breadfruit, tropical almond, sea grape, paper bark and rose (Russell, 1965; Kessing *et al.*, 1993; Neuenschwander, 1994; Reddy, 2015).

There are many synthetic chemicals i.e. pyriproxyfen, imidacloprid, buprofezin and pyridaben which are used by farmers to control the different species of whiteflies (Bi *et al.*, 2002; Toscano and Bi, 2007; Reddy, 2015). The use of synthetic chemicals has led to the development of resistance in the insects (Palumbo *et al.*, 2001; Horowitz *et al.*, 2007; Carabalí *et al.*, 2010; Li *et al.*, 2014). The use of synthetic chemicals also arouse major concern to the environment and human health through the bioaccumulation of chemical compounds in the food chains, resulting in severe physiological disorders and diseases (Oliva *et al.*, 2001; Baldi *et al.*, 2003; Briggs, 2003; Saiyed *et al.*, 2003; Lemaire *et al.*, 2004). As a result, an alternative search for chemical pesticides has led to the global effort to test the efficacy of various natural product for the pest control and crop protection.

Natural pesticides such as plant essential oil can represent an alternative in the crop protection (Coats, 1994; Isman, 2000; Koul *et al.*, 2008). The

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diverse use of essential oil can represent a good alternative due to its novel, safe and eco-friendly substitute for its effective insecticidal properties (Li *et al.*, 2014; Palanisami *et al.*, 2014). Several essential oils from medicinal plants have been screened for the repellence and toxicity against grain storage insects, fleas, ticks and lice (Leal and Uchida, 1998; Gandhi *et al.*, 2010; Olivero-Verbel *et al.*, 2010; Caballero-Gallardo *et al.*, 2011; Cheng *et al.*, 2012; Seo *et al.*, 2012; Vera *et al.*, 2014). However, very little information on the fumigant and repellent toxicity of essential oils from medicinal plants found in the South Pacific (Fiji) was available against the Spiralling whiteflies. The aim of this study was to assess if essential oils of determined medicinal plants could serve as bio-pesticides for the control of the whitefly pest. The chemical profile of selected essential oils was also studied in order to provide justification for the presence of active compounds in the tested activities.

2. Materials and Methods

Essential oils extraction and analysis

The plant materials from *Cananga odorata* (Lam.) Hook F. and Thoms (Makosoi flowers), *Cymbopogon citratus* (DC.) Stapf. (Lemongrass leaves), *Murraya koenigii* (L.) Spreng. (Curry leaves), *Ocimum tenuiflorum* L. (Tulsi leaves) and *Eudioa hortensis* forma *hortensis* (Uci leaves) were collected from Fiji islands in April to November, 2015. The selected plant materials were verified with the voucher specimens placed at University of the South Pacific Herbarium and Koronivia Research Station, Suva, Fiji Islands. The plant materials from the medicinal plants were hydro-distilled using Clevenger apparatus for 5-7 hours. A meniscus layer (essential oils) was formed in the collecting tube which was then collected in a vial. The samples were dried over anhydrous sodium sulphate (Na_2SO_4) and stored at 4°C.

The analysis of essential oils using Gas Chromatography equipped with Mass spectrometry (Agilent Technologies 6890) was performed using an HP-5MS non polar fused silica capillary column (0.25 mm, 30 m, 0.25 µm film thickness; Model Number: 19091S-433) with the following conditions: The oven temperature was programmed from 50°C to 325°C over 5 min, at equilibration time of 0.50 min. The transfer source and quadrupole temperatures were 150°C, 200°C, 230°C and 250°C respectively, operating at 71 eV ionization energy. For the front inlet the mode used was split with an initial temperature of 250°C at 42.5 kPa at a split ratio of 50:1 and split flow of 43.8 mL/min. Helium was used as a carrier gas at a constant linear velocity of 35 cm/sec, flow rate of 0.9 mL/min; the injected sample volume was 1.0 µL which was diluted in hexane (1000 µL). The analysis was carried at the Southern Cross University, Australia. The constituents of essential oils were identified based on mass spectra comparison of retention indices (RI) with standard compounds. For the reference purpose, the database search was done using Essoils and Adams library. For the purpose of semi-quantification, the normalized peak areas of reported compounds were used without any correction factors for establishing abundance. Retention Indices (RI) and abundance were calculated using the mean values of 3 injections (El Bouzidi *et al.*, 2011).

Breeding of Spiralling whiteflies

The adult Spiralling whiteflies were brought from a nearby farm (Rewa Province) without any insecticidal exposure. The collected Spiralling whiteflies were brought to the green house where they were introduced to the cassava plants [*Manihot esculenta* (Crantz)] in order for them to grow and multiply. The plants were maintained in the greenhouse for appropriately 6-7 months without any pesticide contact before carrying out the actual experiment. The adult Spiralling whiteflies were collected in petri dish using a small paintbrush. The conditions that were set in the laboratory were similar to the environment that they were found, that is, under the condition of 28±2°C, 75±5% RH and light regime of 14:10 h (L:D). The Spiralling whiteflies (*Aleurodicus dispersus* Russell) bred in the greenhouse were brought into the laboratory when required to carry out the fumigant and repellent test.

Fumigant toxicity assessment

The leaves of the cassava pot plants were enclosed with a clear pocket plastic bag (16 cm in length) with 50 whiteflies in each bag irrespective of their sex. The treatments [0.25%, 0.5% and 5% (v/v)] were introduced into each plastic bag using a filter paper (~2 cm in diameter) based on the randomisation. The filter paper discs (~2 cm in diameter) were impregnated on the side of the plastic bag. The control filter disc had Tween 20 (5%) (Purchased from Sigma-Aldrich, Australia) mixed with the distilled water. The mortality count results after 3, 6, 9, 12

and 24 hours were calculated.

Repellent toxicity assessment

A T-shaped olfactometer set was constructed in order to test the repellency on the adult Spiralling whiteflies. The setup consisted of a long glass tube (diameter of 50 cm). The external light source was placed between site 1 and site 2. Site 1 had the control leaf disc (2 cm in diameter) dipped in tween 20 (5%) solution, while site 2 had the leaf disc with selected concentration of the essential oil. The essential oil concentration for all the five plants tested were 0.25%, 0.5% and 5% (v/v). The test was performed on 50 adult whiteflies with 4 replicates for each concentration. After 6-8 hours the number of whiteflies were counted using a hand lens for each site (chamber). The Repellency Index (RI %) was calculated using the formula (Abdellaoui *et al.*, 2009):

$$RI\ \% = (C\text{-}T/\ C+T) \times 100$$

where [C= whitefly counts on the control side of the olfactometer] and [T = whitefly counts on the treatment side of the olfactometer].

If the Repellency Index calculated (RI %) is positive, it means that the whiteflies were repelled with the tested concentration of essential oils and vice versa if the Repellency Index (RI %) calculated is negative.

Statistical analysis

Fumigant test assessment. A Factorial ANOVA (5x4x5 split plot design) using Tukey's HSD test was performed. Prior to performing ANOVA (significant at p=0.05), the percentage mortalities were transformed by the arcsine of the square root. The total mortalities were converted to percentage mortality. The Lethal concentration (LC_{50}) values for the mortality after 24 hours were assessed using Probit in XLSTAT software (version 2015.1) (Kabir *et al.*, 2007; Postelnicu, 2011). The morality was corrected using Abbott's formula for those that exceed 10% by natural mortality (Abbott, 1925).

Repellent test assessment. To evaluate the statistical difference at 5% level of significance between each essential oil with its respective control, an independent sample t-test was performed. The Probit analysis in XLSTAT software (version 2015.1) was also used to calculate the EC_{50} for the repelling effect of each essential oil (Padhy and Panigrahi, 2016, Olufayo and Alade, 2012).

3. Result and Discussion

Chemical analysis of the essential oils

A total of 88 compounds were detected in the selected essential oils from the medicinal plants, accounting for 92.76-97.88% of total composition as summarised in Table 1 and 2. The main chemical compounds identified in the essential oil of *C. odorata* were *trans*, *trans*-farnesol (29.71%), benzyl benzoate (21.69%), linalool (16.65%) and *trans*, *trans*-farnesyl acetate (6.93%). While for *M. koenigii* the major compounds identified were sabinene (43.80%), β-caryophyllene (16.52%), terpinen-4-ol (7.20%) and α-pinene (5.67%). In case of *E. hortensis,* menthofuran (55.17%) and evodone (25.91%) were the main compounds. The essential oil from *O. tenuiflorum* revealed the presence of eugenol (58.20%), germacrene D (11.68%) and *cis*-β-ocimene (10.79%) as the major compounds. The major compounds identified in the *C. citratus* essential oil were citronellal (45.09%), citronellol (19.11%), geraniol (13.57%) and elemol (6.15%).

Table 1 - Composition of essential oils (%) from *C. odorata* (Makasoi), *M. koenigi*i (Curry leaves), *E. hortenis* (Uci), *O. tenuiflorum* (Tulsi) and *C. citratus* (Lemon grass)

Chemical compounds	*Ocimum tenuiflorum* (%)	*Cymbopogon citratus* (%)	*Cananga odorata* (%)	*Euodia hortensis* forma *hortensis* (%)	*Murraya koenigii* (%)
α-thujene	0.61	-	0.31#	-	1.79
linalool	0.21	0.27	16.65	0.10#	-
myrcene	0.38	-	0.11	0.37	1.84
sabinene	0.43	-	0.58#	-	43.80
iso-pulegol	-	1:17	-	-	-
α-pinene	-	-	0.32	-	5.67
limonene	-	-	-	4.64	-
1-octen-3-ol	0.19#	-	-	-	-
citronellal	-	45.09	-	0.20	-
iso iso-pulegol	-	0.46#	-	-	-
β-pinene	-	-	-	-	1.55
α-terpinene	0.23#	-	-	-	2.64
decanal	-	0.14#	-	-	-
methyl benzoate	-	-	1.64	-	-
menthofuran	-	-	-	55.17	-
p-cymene	0:23	-	-	-	0.67
citronellol	-	19:11	-	0.13#	-
cis-β-ocimene	10.79	-	-	-	0.11
neral	-	0:55	-	-	-
ethyl benzoate	-	-	0.14	-	-
limonene-10-ol	-	-	-	0.60	-
trans-β-ocimene	0.43#	-	-	-	0.39
geraniol	-	13:57	0.74	-	-
terpinen-4-ol	1:01	-	0.15	-	7.20
evodone	-	-	-	25.97	-
β-phellandrene	-	-	-	-	0.69
ϒ-terpinene	0:37	-	-	-	4.82
geranial	-	0.74	-	-	-
methyl salicylate	-	-	3.15	-	-
α-copaene	1.98#	-	-	0.79	-
citronellic acid	-	0.37#	-	-	-

to be continued

Table 1 (continued)

Chemical compounds [z]	*Ocimum tenuiflorum* (%)	*Cymbopogon citratus* (%)	*Cananga odorata* (%)	*Euodia hortensis* forma *hortensis* (%)	*Murraya koenigii* (%)
methyl chavicol	-	-	0.45#	-	-
β-cubebene	-	-	-	0.26	-
allo-ocimene	0.17#	-	-	-	-
citronellyl acetate	-	1.05#	-	-	-
limonene-10-yl acetate	-	-	-	0.60	-
geranyl acetate	-	0.44	-	-	-
trans-anethole	-	-	0.27#	-	-
α-(2) gurjunene	-	-	-	0.59#	-
trans-sabinene hydrate	-	-	-	-	0.59
α-cubebene	0.18#	-	-	-	-
β-elemene	-	0.59#	-	-	-
δ-elemene	-	-	0.24#	-	-
β- caryophyllene	-	-	-	0.54	-
isoterpinolene	-	-	-	-	0.95#
eugenol	58.20	-	1.38	-	0.33#
germacrene D	11.68	0.79#	2.74	0.27#	0.14
trans-α-bergamotene	-	-	-	0.18#	-
trans-p-menth-2-en-1-ol	-	-	-	-	0.47#
δ-cadinene	1.44#	0.88	-	-	-
methyl eugenol	-	-	1.77	-	-
trans-β-farnesene	-	-	-	0.20#	-
β-bourbonene	0.93	-	-	-	-
elemol	-	6.15	-	-	-
β-caryophyllene	4.31	-	0.49	-	16.52
β-funebrene	-	-	-	0.23#	-
α-terpineol	-	-	-	-	0.28
4-α-hydroxyl germacral (10), 5-diene	-	1.15#	-	-	-
humulene	0.33#	-	-	0.29#	-
cis-piperitol	-	-	-	-	0.12#
β-copaene	0.35	-	-	-	-
ϒ-eudesmol	-	0.72#	-	-	-
β-selinene	-	-	0.31#	-	0.40#
trans-piperitol	-	-	-	-	0.17#
δ-cardinol	-	0.27#	-	-	-
α-germacrene	-	-	0.35#	-	0.18#
AR-curcumene	-	-	-	0.60	-
ϒ-muurolene	0.40#	-	-	-	-
α-cardinol	-	3.70	-	-	-
β- elemene	-	-	-	-	1.50
trans, trans-farnesol	-	-	29.71	-	-
cis, trans-farnesol	-	0.46#	-	-	-
bicyclogermacrene	-	-	-	0.41#	-
trans, trans-farnesal	-	-	0.43#	-	-
benzyl benzoate	-	0.21#	21.69	-	-
β-curcumene	-	-	-	0.56#	-
α-cardinene	0.55#	-	-	-	-
ϒ-cardinene	0.22#	-	-	-	-
trans, trans-farnesyl acetate	-	-	6.93	-	-
δ-cardinene	-	-	-	0.46#	-
α-selinene	-	-	-	-	0.78#
benzyl salicyate	-	-	2.21	-	-
caryophyllene oxide	0.24	-	-	-	0.75#
epi-1-cubenol	0.13#	-	-	-	-
trans-nerolidol	-	-	-	-	0.24#
α-cadinol	0.87#	-	-	-	-
intermedeol	-	-	-	-	0.27#
ϒ-curcumene	-	-	-	3.79#	-

(z) Compounds listed in order of elution from a HP-5MS non polar fused silica capillary column.

Indicate that the compounds were detected for the first time as compared to the literature.

Variability in the essential oils

The results obtained also showed variability in terms of the quality, quantity and composition of essential oils in all the selected plants when compared to the available literature, that is, *O. tenuiflorum* (Pino *et al.*, 1998; Naquvi *et al.*, 2012), *C. citratus* (Negrelle and Gomes, 2007; Olivero-Verbel *et al.*, 2010; Matasyoh *et al.*, 2011; Tyagi *et al.*, 2014), *C. odorata* (Katague and Kirch, 1963; Gaydou *et al.*, 1986; Murbach Teles Andrade *et al.*, 2013), *E. hortensis* (Brophy *et al.*, 1985) and *M. koenigii* (Raina *et al.*, 2002; Chowdhury *et al.*, 2008) (Table 1). The variability in the composition of essential oil is mainly due to the genetic variations, climatic, ecological locations, soil composition, plant organs, age and vegetative cycle stages of the plant (Pietschmann *et al.*, 1998; Masotti *et al.*, 2003; Stewart, 2005; Tchoumbougnang *et al.*, 2005; Angioni *et al.*, 2006; Koba *et al.*, 2007; Nascimento *et al.*, 2008; Katoch *et al.*, 2013; Erbil *et al.*, 2015; Ríos, 2016).

Table 2 - The major chemical groups present in the essential oils of *C. odorata*, *M. koenigii*, *E. hortensis*, *O. tenuiflorum*, and *C. citratus*

Chemical groups	*Cananga odorata* (%)	*Murraya koenigii* (%)	*Euodia hortensis* forma *hortensis* (%)	*Ocimum tenuiflorum* (%)	*Cymbopogon citratus* (%)
Monoterpenes	1.32	65.51	60.18	13.64	-
Ester	35.76	-	0.60	-	1.70
Alcohol and phenol	50.85	9.08	0.83	60.61	45.88
Sesquiterpenes	4.13	20:27	9.17	22.61	3.41
Aldehyde	0:43	-	0.20	-	46.52
ketones	-	-	25.97	-	-
Acid	-	-	-	-	0.37
Miscellaneous	0.27	-	-	-	-
Total (%)	92.76	94.86	96.95	96.86	97.88

Fumigant toxicity of selected essential oils

Among the five tested essential oils (Fig. 1 a-c), *O. tenuiflorum* essential oil showed the most robust fumigant effect against the Spiralling whiteflies with LC_{50} value of 0.003% followed by the essential oils of *C. citratus* (LC_{50} = 0.004%), *C. odorata* (LC_{50} = 0.050%), *M. koenigii* (LC_{50} = 0.113%), and *E. hortensis* (LC_{50} = 0.114%) (Table 3). Statistically, the fumigant activity of *O. tenuiflorum* and *C. citratus* essential oils at 0.5% and 5% (v/v) concentrations were significantly higher than the other species (p=0.00). The significant threshold was set at p<0.05. The mortality count of the Spiralling whiteflies were also higher at 5% (v/v) concentration for *C. odorata*, *E. hortensis* and *M. koenigii* essential oils as compared to 0.25% and 0.5% (v/v) concentrations. Generally the increasing concentrations of the tested essential oils led to increased mortality of whiteflies.

The robust effect of *O. tenuiflorum* essential oil

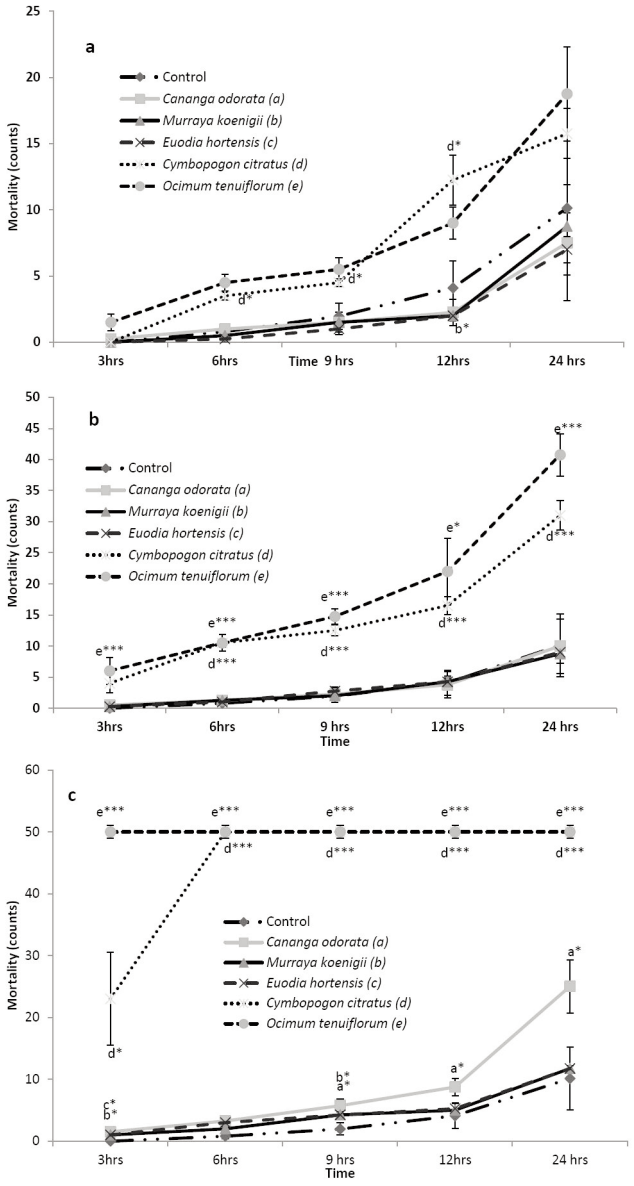

Fig. 1 - Fumigant effect (Mean±SE) of selected essential oils on the Spiralling whiteflies over different time intervals using different solution concentrations: (a) 0.25%; (b) 0.5%; (c) 5% (v/v). The alphabetical letters represent the respective essential oils and the asterisks indicate results statistically different from the control at p<0.05 (*), p<0.01 (**), p<0.001 (***) using Tukey's test.

could be attributed to the chemical constituents present in the oil. In this study, *O. tenuiflorum* essential oil had 60.61% of alcoholic and phenolic compounds as compared to *C. citratus* (45.88%), *C. odorata* (50.85%), *E. hortensis* (0.83%) and *M. koenigii* (9.08%). According to Isman (2000), eugenol compounds were found to be 7-9 times more toxic than terpenes and terpinene-4-ol. This confirms that *O. tenuiflorum* essential oils from the present study showed a strong fumigant effect due to eugenol (58.20%) compound. In previous studies, eugenols were also reported as a major cause of toxicity against the adult beetle (*Callosobruchus maculatus*) (Ajayi *et al.*, 2014), bean weevil (*Acanthoscelides obrectus*) (Regnault-Roger and Hamraoui, 1995), yellow fever mosquito (*Aedes aegypti*) (Sosan *et al.*, 2001) and rice weevil (*Sitophilus oryzae*) (Lee *et al.*, 2003).

Cymbopogon citratus essential oil also showed strong fumigant effect (Fig. 1 a-c). Such effect can be attributed to the major chemical compounds, alcohols and phenols (45.88%), especially citronellol (19.11%) and geraniol (13.57%). These major chemical compounds have showed toxicity and repellent effects on different pests (Fradin and Day, 2002; Ansari *et al.*, 2005; Choochote *et al.*, 2007; Paluch *et al.*, 2009; Sakulku *et al.*, 2009; Maia and Moore, 2011).

Similarly, the interaction of different chemical compounds could have played a major role in the repression of the fumigant effect. According to Chang *et al.* (2009), when linalool compound from the Basil oil (*Ocimum* family) was mixed with cuelure compounds, the level of toxicity on the tested insect (Melon fly, *Bactrocera cucurbitae*) decreased. The above scenario could explain why *C. odorata* essential oil had the second highest percentage of alcohol and phenol compounds (50.85%) while it was not able to produce a greater fumigant effect as compared to *C. citratus* (45.88%) essential oil.

Table 3 - Dose-effect analysis of the fumigant properties of essential oils on the Spiralling whiteflies after 24 hours at 0.25%, 0.5% and 5% (v/v) concentrations

Essential oils	Time (hours)	Equation	R^2	LC_{50}/EC_{50} (%)	$\chi^2_{statistic}$	P-value	df
Cananga odorata	24	y = 4.998 + 4.086x	0.750	0.050	118.149	<0.0001	1
Murraya koenigii	24	y = 3.408+ 3.933x	0.316	0.113	76.080	<0.0001	1
Euodia hortensis forma *hortensis*	24	y = 3.349+3.887x	0.586	0.114	78.574	<0.0001	1
Cymbopogon citratus	24	y = 8.725+3.764x	0.902	0.004	279.950	<0.0001	1
Ocimum tenuiflorum	24	y = 12.286+5.020x	0.651	0.003	253.512	<0.0001	1

The χ^2 probability ≤0.0001, indicated that the significant difference was brought by the log (concentration) variable and the repellency. Each test represents the mean of four replicates of 50 whiteflies.

Repellent toxicity of selected essential oils

The Table 4 revealed that none of the essential oils showed a very strong repelling effect on the Spiralling whiteflies. In order, based on the Repellency index (RI %) of selected essential oils at the highest concentration [5%(v/v)], we found *C. citratus* (52%), *M. koenigii* (52%), *O. tenuiflorum* (12%), *E. hortensis* (10%) and *C. odorata* (9%). A direct relationship was seen between the repellent effect and the concentration (Table 4). Statistically, it was found that only *C. citratus* had a strong significant difference (R^2 =0.611, p=0.00) at tested concentrations. The EC_{50} values in ascending order of the repellent effect of selected essential oils were 3.05% (*C. odorata*), 2.73% (*O. tenuiflorum*), 0.96% (*E. hortensis*), 0.43% (*C. citratus*) and 0.41% (*M. koenigii*).

The chemical analysis in this study revealed the presence of α-pinene (5.67%), β-pinene (1.55%) and myrcene (1.84%) only in the essential oil of *M. koenigii*. The other active compounds that might have contributed towards the repellent effect can be terpinene-4-ol (7.20%) and eugenol (0.33%). In previous report, these compounds were found to repel yellow fever mosquito (*Aedes aegypti*) (Coats *et al.*, 1991; Debboun *et al.*, 2014), bean weevil (*Callosobruchus chinensis*) (Haidri *et al.*, 2014) and two-spotted spider mites (Lee *et al.*, 1997).

The repellent activity of *C. citratus* and *M. koenigii* essential oils at the highest concentration [5% (v/v)] were similar. In agreement with Nerio *et al.* (2010), essential oil from *C. citratus* family were found to have promising repellent properties. The active compounds from previous studies such as α-pinene, limonene, citronellol, citronellal, camphor and thymol have shown higher repellent activity against ticks (*Amblyomma americanum*) and yellow fever mosquito *(Aedes aegypti*) (Nerio *et al.*, 2010; Debboun *et al.*, 2014). This study also reported the presence of citronellal (45.09%), citronellol (19.11%) and geraniol (13.57%) that may have caused the repellent effect (Table 1).

Interestingly, the essential oil activity of *O. tenuiflorum* showed a weak repellency against the Spiralling whiteflies, despite the fact that the mode of action of essential oils against the Spiralling whiteflies in both fumigant and repellent test are known to be similar. In fact, the mode of action of essential oils against the Spiralling whiteflies was via neurotoxicity and respiratory toxicity (Tanada and Kaya, 1993; Isman and Machial, 2006; Satar *et al.*, 2008; Li *et al.*, 2014; Tehri and Singh, 2015). The weak repellent activity of *O. tenuiflorum* essential oil could be due the eugenol content (58.20%) which could have attracted the Spiralling whiteflies rather than repelling. In previous study, eugenol caused attractancy to the Japanese beetle (*Popillia japonica*) (Isman and Machial, 2006). The other chemical compounds from literature that were found to attract the insects were cinnamyl alcohol, 4-methoxy-cinnamaldehyde, cinnamaldehyde, geranylacetone and α-terpineol (Hammack, 1996; Petroski and Hammack, 1998). The overall trend of repellent effect of selected essential oils on the Spiralling whiteflies can be ranked as *C. citratus* and *M. koenigii* followed by *E. hortensis, O. tenuiflorum* and *C. odorata.*

4. Conclusions

All the five essential oils from medicinal plants

Table 4 - Summary of repellent effect (6-8 hours) on the adult whiteflies at different concentrations (Using Probit analysis)

Essential oils	Conc (v/v) (%)	RI (%)	Equation	R^2	EC_{50} (%)	$\chi^2_{statistic}$	P-value	Df
Cananga odorata	0.25	-29	y = -0.140+0.290x	0.0795	3.046	5.93	0.015	1
	0.05	-17						
	5	9						
Murraya koenigii	0.25	-13	y = 0.260+0.663x	0.3232	0.406	38.214	< 0.0001	1
	0.05	8						
	5	52						
Euodia hortensis forma hortensis	0.25	-10	y=0.003+0.188x	0.028	0.964	3.277	0.070	1
	0.05	-3						
	5	10						
Cymbopogon citratus	0.25	-9	y = -0.285+0.953x	0.6111	0.434	27.474	< 0.0001	1
	0.05	3						
	5	52						
Ocimum tenuiflorum	0.25	-18	y = -0.163+0.374x	0.1582	2.728	13.928	0.000	1
	0.05	-11						
	5	12						

The χ^2 probability ≤0.0001, indicated that the significant difference was brought by the log (concentration) variable and the repellency. Each test represents the mean of four replicates of 50 whiteflies.

tested against the Spiralling whiteflies showed fumigant and repellent effects. The strongest fumigant effect was shown by *O. tenuiflorum* essential oils, while for repellent test none of the essential oils showed strong effect. However the *M. koenigii* and *C. citratus* showed higher repellency when compared with other tested essential oils. In addition, the results presented in this study are the first given information on the chemical composition of essential oils from the South Pacific on the selected plant species. So far only *E. hortensis* essential oil composition data from Fiji is reported (Brophy *et al.*, 1985). The selected essential oils from medicinal plants showed potential for the development of possible natural form of controlling the whitefly but needs to be further evaluated to enhance their activity and safety to the humans.

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Comparative characterization of fruit quality, phenols and antioxidant activity of de-pigmented "Ghiaccio" and white flesh peaches

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Key words: antioxidant capacity, depigmented peaches, phenolic compounds, phytochemicals, *Prunus persica.*

Abstract: Quality traits and nutraceutical potential of new de-pigmented peaches ("Ghiaccio") were investigated and compared with those of four white flesh peaches. Total soluble solids (TSS) were measured by digital refractometer, and titratable acidity (TA) by volumetric titration. Total phenolic (TPC) and anthocyanin content (TAC) were analysed spectrophotometrically and the antioxidant capacity (AC) evaluated by DPPH• assay. A strong influence of genotype on quality traits and phytochemical profile of "Ghiaccio" peaches was observed. "Ghiaccio" series showed, on average, a higher TPC content than that of white flesh peaches, both in flesh and peels (+129% and +14%, respectively). The peels of all peaches analysed were significantly richer in TPC than the flesh. TAC was not detectable in de-pigmented genotypes; on the contrary, in the white flesh peaches, it was higher in the peels than in the flesh. AC correlated well with TPC. Data confirm, for all peaches analysed, the influence of genotype and fruit tissue on the nutraceutical properties. Among genotypes, the best candidates for "Ghiaccio" peaches with enriched nutraceutical properties are the advanced selections GØ and GX. Observed differences in the nutraceutical potential among "Ghiaccio" series may open new opportunities for breeding de-pigmented peach varieties with a higher nutritional value.

1. Introduction

Peach (*Prunus persica*, L.) is the second most important temperate fruit crop after apple. The top producer of peaches is China, followed by the EU with Italy, Greece and Spain being the biggest European producers (Faostat, 2015). At present, the demand from the market to develop and introduce new varieties with different characteristics, which could make possible to expand cultivation areas and production calendars and improve technology with regards to production and post-harvest handling of these delicate fruits, is increasing. However, breeders have traditionally selected new cultivars mainly for external fruit traits (i.e. size and appearance), with organoleptic and nutritional characteristics being a secondary goal. In spite of this, fruit quality is fundamental for the acceptance of different cultivars by consumers, due to the high competition in the markets with the presence of numerous new varieties, other fruits and other foods (Iglesias and Echeverria, 2009).

Abbot (1999) indicates that food quality is a concept, which includes sensory, mechanical and functional properties as well as chemical composition and nutritional values. The latter is a key point as fruit has long been promoted for its health benefits in preventing various cancer and age-related diseases (Bazzano *et al*., 2002; Liu, 2003; Casacchia and Sofo, 2013). This is due to the presence of high added value bioactive compounds, named phytochemicals (Iriti and Faoro, 2006). These compounds have strong antioxidant properties that enable them to scavenge free radicals, donate hydrogen, chelate metals, break radical chain reactions, and quench singlet oxygen *in vitro* and *in vivo* (Dai and Mumper, 2010). All these properties enable them to act in the prevention of oxidative stress-related diseases (Pandey and Rizvi, 2009). Among phytochemicals, the most abundant class present in fruit is that of polyphenols (Manach *et al*., 2004). Phenolic rings have the capacity to scavenge free radicals, first of all hydroxyl ones, by virtue

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of the aromatic hydroxylation at the ortho-position (Xia *et al*., 2010).

These compounds are distributed in every part of the fruit in different ratios, focusing more in the peels (rich in anthocyanins, hydroxycinnamic acids and flavans) and in the seeds (rich in proanthocyanidins and flavans) (Lachman *et al.*, 2009). Peaches, even though having a lower antiradical capacity than other fruits, are ones of the most important commodities consumed worldwide, both as fresh and processed product (i.e. fruit juice, jam or canned) (Cantin *et al*., 2009). As a consequence, breeding programs aimed to increase peach nutraceutical value is desirable. "Ghiaccio" peach series is a new type of peach variety, resulting from a breeding program conducted in the past years at the Fruit Tree Research Centre of Rome with the aim to obtain varieties with enhanced postharvest fruit characteristics and an improved resistance to disease and pests (Nicotra *et al*., 2001). Their progenitor is a stony hard-type peach cultivar, the Korean "Yumyeong" (Kim *et al*., 1978), from which "Ghiaccio" selections have been obtained by self-pollination (Nicotra *et al*., 2001). Peaches of "Ghiaccio" series have different ripening times but similar pomological traits (Nicotra *et al*., 2001). To the best of our knowledge, there is no phytochemical and nutritional characterisation of "Ghiaccio" peaches in literature, making this study quite relevant, providing breeders and consumers with experimental data on this emerging varieties.

Hence, the aim of the present study was to characterise the quality and nutraceutical properties of these new genotypes by measuring their total polyphenolic and total anthocyanin content, and their relative antioxidant capacity. A comparison with four commercial white flesh peach cultivars was also performed. The ultimate goal was to select "Ghiaccio" peach genotypes with enhanced nutraceutical traits, to provide breeders with new varieties having more healthful properties, making them competitive with other fruits known for their healthy properties.

2. Materials and Methods

Chemicals

All used reagents were of analytical spectrophotometric grade (Carlo Erba, Rome, Italy). Cyanidin chloride was purchased from Extrasyntese (Genay Cedex, France). Folin-Ciocalteu reagent, malic acid, chlorogenic acid, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich (Milan, Italy).

Plant materials

Peach fruits from five early-to-late ripening "Ghiaccio" genotypes (Table 1) and four white flesh peach cultivars were collected in the experimental orchards of the Fruit Tree Research Centre of Rome (CREA-FRU, Italy), at fully ripening phase. All plants (six years old) were grafted on the same rootstock (GF677), spaced at 4.5x2.5 m and standard pruning, drip irrigation and cultural practices were performed. For each genotype, 15 undamaged and disease-free fruits were collected in order to have three replications of five fruits each for the analysis.

Table 1 - Pomological and phenological traits of "Ghiaccio" series [z]

Traits	Cultivar/Selection				
	Ghiaccio Ø (G Ø)	Ghiaccio 1® (G1)	Ghiaccio X (GX)	Ghiaccio 2® (G2)	Ghiaccio 3® (G3)
Size (g)	large (168)	very large (178)	very large (217)	very large (205)	very large (200)
Shape	oblate	oblate	oblate	oblate	oblate
Shape of pistil end	weakly depressed	weakly depressed	weakly depressed	weakly depressed	weakly depressed
Symmetry	symmetric	symmetric	asymmetric	symmetric	symmetric
Prominence of suture	weak	weak	weak	weak	weak
Ground colour	cream white	cream white	cream white	cream white	cream white
Over colour	present	absent	absent	present	absent
Hue of over colour	pink			pink	
Density of pubescence	medium	sparse	medium	very sparse	very sparse
Firmness of flesh	very firm	very firm	very firm	very firm	very firm
Ground colour of flesh	cream white	cream white	cream white	cream white	cream white
Sweetness	high	high	high	high	high
Acidity	low	low	low	low	low
Ripening time [y]	-20	-8	-5	11	25

[z] Data were detected as Community of Plant Varity Office (CPVO) descriptors.
[y] The reference cv. Rome Star ripens in Center Italy between 25th-30th of July.

Fruit quality attributes

After harvesting, fruits were washed, stoned and homogenized, and the homogenate samples were analysed for total soluble solid (TSS) content using a digital refractometer (Refracto 30 PX, Mettler Toledo, Milan, Italy); data are given as °Brix. The method for analysis of titratable acidity (TA) was based on titration of the acids present in the fruit juice with sodium hydroxide (0.1 N). Data are given as g malic acid L^{-1}, since this is the dominant organic acid in peach (David *et al*., 1956). The pH value was measured using a digital pH-meter (785 DMP, Methrom, Milan, Italy). Every analysis was replicated three times.

Extraction of bioactive compounds

Fruits from the different genotypes were carefully separated in two different tissues: peel and flesh. The peel fraction was removed from the whole fruit with a sharp knife and immediately frozen with liquid nitrogen, placed in a plastic freezer bag, and stored at -80°C until evaluation. The flesh fraction consisted of a peeled wedge, which was chopped into small pieces, frozen in liquid nitrogen, and also stored at -80°C. Samples (5 g) of the different fractions were extracted with a 25 mL hydro alcoholic solution (methanol:water= 70:30, v/v) acidified with HCl (0.005 N) and homogenized with an Ultra-Turrax blender (Ultra Turrax T25, IKA, Milan, Italy) at 9000 rpm. Then, the homogenates were allowed to stand for 2 hours at 37°C under magnetic stirring to rich a complete solvent extraction. Extracts were centrifuged at 8400 rpm for 15 min at 5°C and then the obtained supernatants were analysed as follows. Extractions were repeated on three independent samples of the initial homogenate to give triplicate readings.

Determination of total polyphenol content (TPC)

TPC of both flesh and peel samples was determined using the Folin-Ciocalteu (F-C) method (Waterhouse, 2002). TPC was calculated from a calibration curve, using chlorogenic acid as a standard. Results were expressed as milligrams of chlorogenic acid equivalents (CAE) *per* 100 g fresh weight (FW). Every analysis was replicated three times.

Determination of total anthocyanin content (TAC)

TAC of both flesh and peel fractions was estimated according to the method of Mondello *et al.* (2000). TAC was calculated from a calibration curve, using cyanidin chloride (CC) as a standard. Results were expressed on a fresh weight basis as milligrams of CC equivalents (CCE) x 100 g FW. Every analysis was replicated three times.

Measurement of antioxidant capacity (AC)

AC was assessed by measuring the effect of the bioactive extracts on the content of 2,2-diphenyl-1, picrylhydrazyl radical ($DPPH^{\bullet}$) according to Brand-Williams *et al.* (1995). All the measurements were made in triplicate. AC was expressed as micrograms of Trolox equivalents (TE) x mg FW.

Statistical analysis

Data analysis was performed with SPSS 17.0 software (SPSS, Inc., Chicago, Illinois). All measurements were performed at least in triplicate and data were reported, where not specified differently, as means ± standard error of the mean (SE). An exploratory data analysis was made to check the data normal distribution (Shapiro-Wilkinson test) and the equality of variances (Levene's test). When these conditions were met (TSS, TA, AC), data were subjected to one-way analysis of variance (ANOVA) and comparisons between means were determined according to Tukey's HSD test. Significant differences were accepted at $p<0.05$ and represented by different letters.

When ANOVA assumptions were violated (TPC), even after mathematical transformation of data, a non-parametric data analysis was carried out (Kruskal-Wallis non parametric test) and significant mean differences were established using the Mann-Whitney test for independent and non-parametric procedures and a Bonferroni's correction to set the critical value for significance for each test. Box-plots were used to display the range, median and distribution density of phytochemicals and AC in the peel and flesh of genotypes analysed. Spearman's correlation coefficient (ρ) was used to determine the correlation among variables in the non-parametric analysis ($p<0.01$).

3. Results and Discussion

Fruit quality attributes

Table 1 shows the main pomological and phenological traits of "Ghiaccio" series analysed.

Ghiaccio means ice in Italian to remind consumers that the colour of the fruit is white or pale cream. They are very different from the common peach type grown in Europe and the U.S. These peaches are characterized by totally white cream skin and flesh, very firm flesh, high sugar content (up to 17°Brix), 20-25 days longevity on the tree, a great productivity and a noteworthy shelf life. Moreover, this variety

shows a great resistance against diseases, rottenness and pathogenic agents making it particularly suitable for organic practice (Nicotra *et al*., 2001). The "Ghiaccio" series includes "Ghiaccio 1" (G1), "Ghiaccio 2" (G2) and "Ghiaccio 3" (G3) genotypes, which have been already licensed, while two advanced selections, "Ghiaccio Ø" (GØ) and "Ghiaccio X" (GX) are still under evaluation. All genotypes analysed were harvested between early July and late August and, generally, significant differences in quality traits were detected (Table 2).

In "Ghiaccio" series, the highest TSS level (15.2 °Brix) was observed in G1 samples, while the lowest one in GØ ones (9.1°Brix). All samples analysed showed a TSS content greater than 8 °Brix, which represents the minimum TSS content established by the EU to market peaches and nectarines [Commission Regulation (EC) No. 1861/2004, 10/28/2004]. Moreover, "Ghiaccio" genotypes showed TSS similar to the white flesh peaches analysed. TA values ranged from 3.18 (G3) to 4.23 (G1) g malic acid L^{-1}, with significant differences among selections (Table 2). On average, these values are lower than that of the progenitor and "Ghiaccio" series can be collocated in sub acidic peach varieties (Crisosto *et al*., 2001). These results are relevant since the acceptance of new cultivars by consumers, which is the ultimate goal of breeders, is linked to the fruit quality. In fact, acid levels, expressed in terms of pH and TA, and sugar concentrations, reported as TSS, affect flavour perception of the fruit influencing peach sensory profile and consumer acceptance of peach fruits (Crisosto *et al*., 2001; Crisosto and Crisosto, 2005).

In the present study, we also reported the TSS/TA ratio as the relationship between these parameters has an important role in fruit consumer acceptance (Diaz-Mula *et al*., 2009). Among "Ghiaccio" peaches, TSS/TA values ranged from 3.62 for G3 to 3.16 for G2, being the latter significant different from the other genotypes of the series. In the fresh market, consumers desire large shaped and flavourful fruit with a high sugar content and low to moderate acidity and these new genotypes appear to respond adequately to such requests. In this regard, "Ghiaccio" series showed, on average, a TA content lower than that of the white flesh cultivars analysed, which is reflected in a higher value of the TSS/TA ratio.

Bioactive compounds distribution within the peach genotypes and relative antioxidant capacity

Fruit antioxidant potential varies in relation to the phytochemical moieties present, and variations can occur among genotypes within a single species (Van der Sluis *et al*., 2001; Cantin *et al.*, 2009). Moreover, it is well known that the content of phytochemicals can vary within different tissues (Carbone *et al*., 2011). In figure 1, box-plots showed the distribution of polyphenols, and the antiradical activity between the peel and the flesh of "Ghiaccio" peaches analysed, independently from the genotype. TPC was significantly higher in the peach peel extracts than in the flesh ones (+103%). These data are consistent with those reported in the literature about the influence of the type of fruit tissue on the accumulation of nutraceutical substances (Carbone *et al*., 2011;

Table 2 - Fruit quality attributes of genotype analysed (mean±SE)

Genotype	Harvest date	TSS (°Brix)	TA (g L^{-1} malic acid)	TSS/TA ratio
Ghiaccio 1	July, 22nd	15.20±0.03 e	4.23±0.06 b	3.58±0.04 d
Ghiaccio 2	August, 11th	13.00±0.03 d	4.13±0.01 b	3.16±0.02 c
Ghiaccio 3	August, 25th	11.5±0.1 b	3.18±0.02 a	3.62±0.05 d
Ghiaccio Ø	July, 7th	9.10±0.07 a	ND	ND
Ghiaccio X	July, 21st	12.10±0.07 c	3.43±0.04 a	3.54±0.06 d
Crizia	June, 28th	9.6±0.4 a	7.96±0.03 c	1.21±0.05 a
Maria Anna	August, 4th	13.60±0.03 d	12.0±0.2 e	1.13±0.02 a
Redhaven Bianca	July, 21st	12.2±0.1 c	8.74±0.01 d	1.39±0.01 b
Silver Late	September, 8th	13.20±0.06 d	12.61±0.02 f	1.05±0.00 a
"Ghiaccio" Series		12.2±0.5 a	3.7±0.1a	3.47±0.06 b
White Flesh cvs.		12.1±0.5 a	10.3±0.6 b	1.19±0.04 a

TSS= Total soluble solids.
TA= Titratable acidity.
ND = Not determined.
Significant differences were accepted at $p<0.05$ and represented by different letters on the column, within the cultivars or within the groups ("Ghiaccio" series and white flesh cultivars).

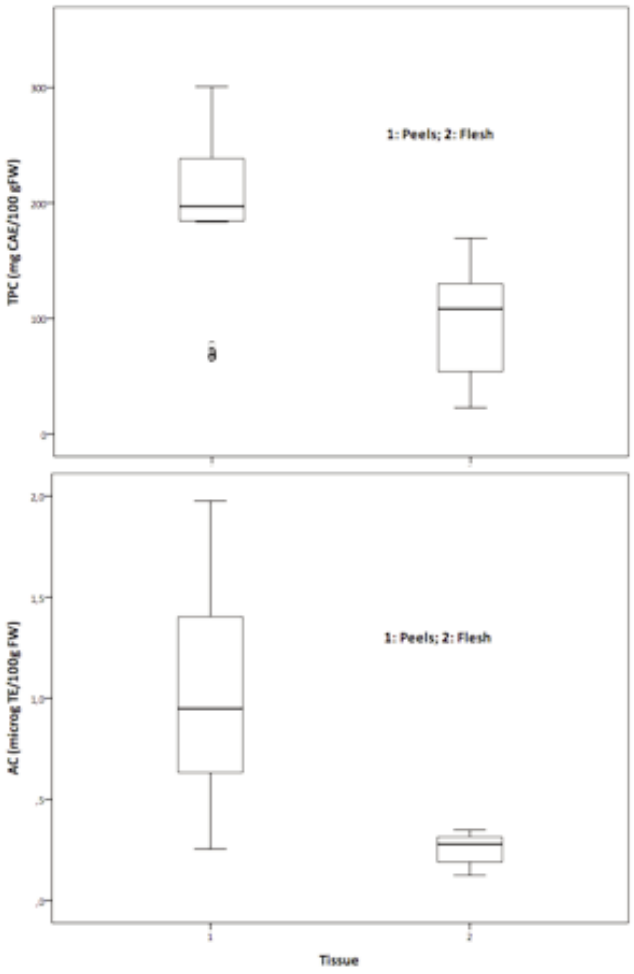

Fig. 1 - Box-plots for the phytochemicals and antioxidant capacity of different peach tissues. The line in the box indicates the median value of the data; the right and the left edges of the box respectively indicates the 75th and the 25th percentiles of the data set, the ends of the horizontal lines indicate the minimum and maximun data values; the point outside the box are outliers or suspected outliers. A) TPC= Total polyphenol content (mg CAE 100 g^{-1} FW). B) AC = Antioxidant capacity (µg TE mg^{-1} FW). Outlier: value more than 1.5 and less than 3 box-lengths from end of box.

Tomás-Barberán *et al*., 2001).

Tables 3 and 4 show the flesh and peel phytochemical content and AC of different cultivars analysed. Among "Ghiaccio" genotypes, GØ showed the highest TPC both in the peel and flesh (260 and 134 mg CAE 100 g^{-1} FW, respectively), while G2 the lowest one (70 and 28 mg CAE 100 g^{-1} FW, respectively) (Tables 3 and 4). These findings highlight that TPC of "Ghiaccio" series is related to the genotype, in agreement with those reported in literature, not only for other peaches but also for other fruit species (Tomás-Barberán *et al*., 2001; Ceccarelli *et al*., 2016). Interestingly, the average peel polyphenol content of "Ghiaccio" series (175 mg CAE 100 g^{-1} FW) was not significantly different from that of the white flesh cultivars analysed (Table 3), while the average TPC of the flesh was significantly higher (Table 4). Obtained results emphasize the healthy properties of the flesh of these new de-pigmented peaches.

Table 3 - Phytochemicals and antioxidant capacity of the peel of genotypes analyzed (mean±SE)

Genotype	AC	TPC	TAC
Ghiaccio 1	1.0±0.1 bc	232±6 e	ND
Ghiaccio 2	0.53±0.08 a	70±2 a	ND
Ghiaccio 3	0.7±0.2 ab	197±4 d	ND
Ghiaccio Ø	1.3 ±0.1 cd	260±4 f	ND
Ghiaccio X	1.5±0.1 d	144±4 c	ND
Crizia	1.34±0.03 cd	102±3 b	26.53±0.03 c
Maria Anna	1.42±0.06 cd	260.9±0.7 f	34.55±0.06 d
Redhaven Bianca	1.13±0.04 cd	100±3 b	19.70±0.05 a
Silver Late	1.50±0.08 d	151±2 c	23.40±0.03 b
"Ghiaccio" series	1.00±0.08 a	175±14 a	ND
White flesh cvs.	1.35±0.04 b	154±14 a	26±2

AC= Antioxidant capacity.
TPC= Total polyphenol content.
TAC= Total anthocyanin content.
ND = Not determined.
Significant differences were accepted at p<0.05 and represented by different letters on the column, within the cultivars or within the groups ("Ghiaccio" series and white flesh cultivars).

Table 4 - Phytochemicals and antioxidant capacity of the flesh of genotypes analyzed (mean±SE)

Genotype	AC	TPC	TAC
Ghiaccio 1	0.286±0.007 cd	59±8 c	ND
Ghiaccio 2	0.16±0.01 a	28±2 ab	ND
Ghiaccio 3	0.325±0.007 d	126±2 d	ND
Ghiaccio Ø	0.308±0.007 d	134±4 d	ND
Ghiaccio X	0.20±0.02 b	71±3 c	ND
Crizia	0.256±0.005 c	23±2 a	7.54±0.01 c
Maria Anna	0.157±0.003 a	38±2 b	ND
Redhaven Bianca	0.263±0.004 c	28±2 ab	1.67±0.01 a
Silver Late	0.412±0.005 e	62±2 c	6.45±0.01 b
"Ghiaccio" series	0.25±0.01 a	86±8 b	ND
White flesh cvs.	0.27±0.02 a	38±3 a	4.1±0.9

AC= Antioxidant capacity.
TPC= Total polyphenol content.
TAC= Total anthocyanin content.
ND = Not determined.
Significant differences were accepted at p<0.05 and represented by different letters on the column, within the cultivars or within the groups ("Ghiaccio" series and white flesh cultivars).

As regards total anthocyanins, their content was not detectable in "Ghiaccio" peaches both in the peel and the flesh. Besides, white flesh peaches revealed the presence of these compounds in all tissue analysed.

In agreement with the phytochemical data, genotype also influenced the AC of peaches analysed. Among "Ghiaccio" genotypes, the highest value of AC was found in GØ (1.3 µg TE mg^{-1} FW) and GX (1.5 µg

TE mg^{-1} FW) for what concern the peel and in GØ (0.308 µg TE mg^{-1} FW) and G3 (0.325 µg TE mg^{-1} FW) for the flesh. On average, the antioxidant capacity of the peel was lower in "Ghiaccio" series than in white flesh cultivars (Table 3), probably due to the absence of anthocyanins in the peel of "Ghiaccio" series. Moreover, correlation analysis pointed out that the scavenging capacity against $DPPH^{\bullet}$ of peach extracts and TPC were significantly and positively correlated ($\rho = 0.796$; $p<0.01$). This result confirms previous reports on commercial white and yellow flesh cultivars, showing that phenolic compounds can be considered the main phytochemicals contributing to AC in peaches (Vizzotto *et al*., 2007).

4. Conclusions

Peach breeding has supplied a large number of improved cultivars each year to satisfy different market demands. Nevertheless, there are some critical issues not yet fully addressed through breeding such as the increasing competition between peach and a broad range of other fruits, as well as the consumer eating habits, which are changed in the last years, with a growing emphasis on nutrition and health properties of food. To meet these challenges, it is necessary to explore new germplasm for the production of new cultivars with improved quality and nutritional characteristics as well as to provide fruits to the market for long periods of time. In this context, the "Ghiaccio" series could be a right answer for breeders. The present study describes for the first time the quality traits, phytochemical composition and AC of five genotypes (three cultivars and two advanced selections) belonging to the "Ghiaccio" peaches. Data point out the key role played by the genotype also within the "Ghiaccio" series, underling the importance of the varietal selection. Among the genotypes analysed, the best candidates for a "Ghiaccio" peach with enriched nutraceutical properties are the advanced selections GØ and GX. In addition, data pointed out a higher nutraceutical potential of "Ghiaccio" series than that of the commercial white flesh peaches analysed. Finally, taking into account that the fruit characteristics (i.e. shape, colour and size) are very similar to each other, but with different ripening periods, "Ghiaccio" genotypes, if grown together, would allow the producers to supply the markets with the same type of fruit for a long period of time (i.e. 75 days).

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Effect of pre- and postharvest salicylic acid treatment on quality characteristics of tomato during cold storage

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Key words: ascorbate peroxidase, decay, *Lycopersicon esculentum*, weight loss.

Abstract: Nowadays, there is a considerable tendency to replace dangerous chemicals with natural compounds, compatible with plant, human, and nature. This study was aimed to assess the effect of salicylic acid on quality and storage life of tomato (*Lycopersicon esculentum* cv. Baraka). The salicylic acid application was including plant foliar application three weeks before harvest at concentration 4 mM, followed by the postharvest dipping fruits in salicylic acid at different concentrations (1, 2, 4 mM), then storing at 10°C for 40 days to investigate quantitative and qualitative characteristics. The chilling injury symptoms, electrolyte leakage, decay and a* (redness) value significantly decreased and activity ascorbate peroxidase increased. Ascorbic acid content, total soluble solid, titratable acidity, firmness, and L* (lightness) retained by salicylic acid treatments. The salicylic acid application had no significant influence on weight loss and b*. Application of salicylic acid in all concentrations, especially a combination of treatments preharvest to concentrations 4 mM as well as postharvest 4 mM, had the highest influence on qualitative and quantitative characteristics and increased the postharvest life of the tomato fruit.

1. Introduction

Tomatoes are widely used, and those are a rich source of fiber, phenolics, vitamins A, C, and small amounts of vitamin E and lycopene. Lycopene prevents the harmful effects of free radicals and different types of cancers as well as cardiovascular disease (Pila *et al.*, 2010; Orabi *et al.*, 2015). Major problem of postharvest tomato is softening and ripening during storage, distribution, and marketing because of their susceptibility to damage (Batu, 2004; Agamy *et al.*, 2013). Fruit firmness and color are as effective factors of tomato quality, which are used as fruit quality indicator (Batu, 2004; Agamy *et al.*, 2013). Tomato is climacteric so its ripening continues after harvesting and it can become overripe quickly. Hence, its quality decreases and its shelf life limits (Batu, 2004). Pila *et al.* (2010) reported that owing to lack of information on appropriate postharvest treatments, packaging, temperature, etc., the fruits not only lose their quality but also encounter a substantial postharvest loss. Cold storage is one of the most efficient and most practical postharvest procedures that maintains quality of products from the harvest to consumption time (Bourne, 2006), and extending the storage life of fresh horticultural products, tomatoes can be stored successfully for weeks (Hatami *et al.*, 2013), but the main problem is the postharvest handling, because the tropical and subtropical products are sensitive to chilling injury (CI) (Soleimani Aghdam *et al.*, 2012). Hatami *et al.* (2013) reported that improper temperature management is the primary cause of many postharvest diseases and disorders. Elhadi and Jeffrey (2012) reported that mature green tomatoes are the most sensitive to low temperatures among the commercial fruit, and there is a risk to develop chilling injury if they are held below 13°C or 12.5°C (Rugkong, 2009) and 12°C (Galvez *et al.*, 2010; Zhang *et al.*, 2010). Sevillano *et al.* (2009) reported that chilling injury reduced tomato fruit quality. Commonly visible CI comprise several symptoms such as surface pitting (Soleimani Aghdam *et al.*, 2012) and alteration of ripening process as indicated by delayed or even total failure offruit color development and softening (Rugkong, 2009),

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increased susceptibility to Alternaria rot and decay (Ding *et al.*, 2002), decrease mealy texture when ripened (Alternaria and Cladosporium rots are usually associated with chilling injury) (Elhadi and Jeffrey, 2012). Salicylic acid (SA) is a phenolic compound and plant growth regulator (Zavala *et al.*, 2004) and defenses against biotic and abiotic environmental stresses. Plants produce reactive oxygen species (ROS) when exposed to biotic and abiotic environmental stresses conditions (Agamy *et al.*, 2013). ROS includes superoxide, hydrogen peroxide and hydroxyl ions (Dat *et al.*, 2000). Thus, ROS cause to damage in cellular structures. There is a mixture of non-enzymatic antioxidants (carotenoids, ascorbate) and enzymatic antioxidants in plants, such as catalase (CAT) and ascorbate peroxidase (APX) which inhibit harmful effects of these ROSs. The enzymatic action of APX reduces H_2O_2 using ascorbate as an electron donor (Orabi *et al.*, 2015). Salicylic acid is an antioxidant defense system and regulates different physiological and biochemical processes in plants including: plant growth (Khan *et al.*, 2003), stomatal conductivity (Hayat *et al.*, 2010), photosynthesis (Fariduddin *et al.*, 2003), seed germination (Babalar *et al.*, 2007), disease resistance (Janda *et al.*, 2007), heavy metal stress, low temperature, high temperature and salinity (Hayat *et al.*, 2008). Salicylic acid treatment could be used to enhance the chilling resistance of maize, cucumber and rice (Kang and Saltveit, 2002), pomegranate (Sayyari *et al.*, 2009) and tomato (Ding *et al.*, 2001, 2002). Salicylic acid delays the ripening of banana and kiwifruit during storage (Srivastava and Dwivedi, 2000; Zhang *et al.*, 2003). Babalar *et al.* (2007) reported that pre and postharvest SA treatments caused fruit quality maintenance in strawberry. Fattahi *et al.* (2010) reported that losses in fruit quality are mostly due to its relatively high metabolic activity during storage. Salicylic acid is known as a signal molecule in the induction of defense mechanisms in plants. Due to the risk of inappropriate use of substance chemicals in postharvest technology, it is essential to study the application of safe postharvest treatments along with cold storage. Since the time between tomato fruit harvest and consumption may take long weeks, and during this period many changes could happen that affect the postharvest behavior of fruits. Therefore, the aim of this article was to appraise the effects of pre and postharvest SA application to maintain the qualitative characteristics of tomato fruits at cold storage and increase the postharvest life of the tomato fruit.

2. Materials and Methods

Plant material and salicylic acid treatment

Fruits of tomato (Lycopersicon esculentum cv. Baraka) produced in the greenhouse at University of Hormozgan (Iran) were used. Baraka cultivar is a hybrid seed appropriates for the tropical region. Fruits were harvested at mature green stage in April 2014 and transferred to laboratory where they were selected for health and size, weight, and color uniformity. Fruits were divided randomly. Then, they were washed and dried in the air. Each treatment consisted of 60 fruits, and each treatment was composed of three replicates (20 fruit per replicate): three SA treatments were compared (4+1, 4+2, and 4+4 mM) consisting of a 4 mM SA plant foliar application three weeks before harvest followed by the postharvest fruit dipping for five minutes in SA solutions at different concentrations (1, 2, and 4 mM). Fruits harvested from non-treated plants were used as control. Tomatoes were stored at 10°C temperature and 85-90% relative humidity (RH) for 10, 20, 30 and 40 days (Hatami *et al.*, 2013). Samples were taken at every 10 days intervals during storage for quality evaluation.

Firmness and weight loss

Fruit firmness was measured using a penetrometer equipped with a 6 mm diameter flat probe exerting maximum force on fruit. Units were expressed as kg cm^{-2} (Shafiee *et al.*, 2010). Fruit weight loss was measured immediately after harvest and storage time. The results were calculated as percentage of weight loss at the start of the experiment and at different intervals during storage by this formula: %WL = [(W1-W2)/W1] × (100), where %WL= percentage weight loss, W1= initial fruit weight in (g), W2= final fruit weight in (g) (Zhang *et al.*, 2002).

Superficial color

Superficial color of tomato was measured using a Minolta chronometer model CR 400 and average readings at three points against each other in the fruits were recorded. Color indices inclusive (L*, a*, and b* values) were measured. Superficial color of the fruit was expressed as L* (the ratio of white to black color), a* (the ratio of red to green color) and b* (the ratio of yellow to blue color) (Shafiee *et al.*, 2007).

Chilling injury index (scores) and electrolyte leakage

Chilling injury index of fruits was evaluated at 10°C after 10, 20, 30 and 40 days in cold storage. Symptoms were manifested as surface pitting and

dehydration according to the method of Sayyari *et al.* (2009). The severity of the symptoms was assessed with scores according to the following 3 stage scale: 0 (no symptom), 1 (1-25% of damaged area), 2 (26-50% of damaged area) and 3 (>51% of the damaged area). The average extent of chilling-injury damage was expressed as a chilling-injury (CI) index, which was calculated using the following formula:

CI = [(value of hedonic scale) × (number of fruit with the corresponding scale number)] / (4 × total number of fruit in the sample).

The rate of electrolyte leakage (EL) was measured according to the method of Mirdehghan *et al.* (2007), using 6 discs (10 mm diameter) of peel tissue, cut with a cork borer. Conductivity was measured after 4 h of incubation in 25 mL of 0.4 M mannitol under constant shaking. The conductivity of the solution (L1) was measured with a conductivity meter (Ttracon WTW 325). After readings had been taken, the vials were autoclaved at 121°C for 15 min, and then cooled to 20°C. The conductivity of tissues (L2) was measured. Ion leakage was calculated as the ratio of L1 to L2.

Fruit decay index (scores)

Decay incidence of each fruit was determined by scores. According to the amount of the decay on fruit surface scales from 1 to 5 were given to the each treatment where; 1= normal (no decay on fruit surface), 2= trace (up to 5% of fruit surface were decayed), 3= slight (5-20% of fruit surface were decayed), 4= moderate (20-50% of fruit surface were decayed), and 5= severe (>50% of fruit surface were decayed). Results were expressed as fungal decay index (Babalar *et al.*, 2007).

Vitamin C (ascorbic acid)

Fruit vitamin C content was measured by using titrimetric method with the titration of filtrate against 2,6-dichlorophenol indophenol and the results of vitamin C content were expressed as mg/100 g (Pila *et al.*, 2010).

Titratable acidity and total soluble solids

Five ml of extracted fruit juice was diluted to 45 ml with distilled water. Then, extract fruit juice was titrated with 0.1 N sodium hydroxide to a pH of 8.1. Titratable acidity (TA) was determined as percentage of citric acid by this formula:

$$TA\ (\%) = [(V \times N \times meq)/Y] \times 100$$

where V= volume of sodium hydroxide used ml, N = sodium hydroxide normality, and meq = 0.064, Y = volume of bulk fruit juice ml (Saltveit, 2005). Total soluble solid (TSS) in the extracted fruits juice was measured with a portable refractometer (Model DBR95), and the results were expressed as Brix.

Ascorbate peroxidase

Ascorbate peroxidase (APX) was assayed by recording the decrease in optical density due to ascorbic acid at 290 nm for 1 min in a UV-vis spectrophotometer (model unicuv- 2100). Samples from pulp of 0.5 g fresh tissue homogenized and the homogenized samples were centrifuged at 14000 rpm for 15 min. The supernatant was used as crude enzyme extract for APX enzyme analyses. The 3 ml reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM EDTA, 0.1 mM H_2O_2, and 0.1 ml enzyme. The reaction started with adding of 0.1 mM hydrogen peroxide. The enzyme activity was calculated using the extinction coefficient 2.8 Mm^{-1} cm^{-1} for ascorbate (Nakano and Asada, 1981).

Statistical analysis

Statistical analysis was performed by SAS software (SAS Institute Inc., 1990) according to a split plot in time design on the basis of completely randomized design (CRD), with 3 SA concentration and 3 replicates. Data were analyzed by GLM and differences among means of data were compared by with Least Significant Difference (LSD) Test at a significance level of 0.05.

3. Results

Firmness and weight loss

The results showed a rapid decrease in firmness in the control set compared to the fruits treated with SA during storage, and all treated fruits were firmer than the control set ($P< 0.01$). Maximum fruit flesh firmness (3.2 kg cm^{-2}) was recorded in pre and postharvest SA treatments 4+4 mM and control set had the softest fruits (0.6 kg cm^{-2}) at the end of the experiment but, the difference was not statistically significant compared to the other SA concentrations (Table 1). No significant changes were observed in weight loss during storage for any treatments, with the exception of weight loss in 30 days SA-treated fruit, which was lower than the control set (Table 1).

Color assessment

The results of this investigation showed that pre and postharvest SA treatments had an effect on fruit lightness (L*) and redness (a*) value in comparison

Table 1 - Effect of pre and postharvest treatments salicylic acid in firmness, weight loss, L*, a*, b* of tomato fruit cv. Baraka and stored at 10°C for up to 40 days

Time storage (days)	Salicylic acid treatments	Firmness (kg cm^{-2})	weight loss (%)	Color parameter		
				L*	a*	b*
0	0	4.82±1.02 a	0.00±0.00 f	34.98±2.69 a	-6.13±0.63 g	15.47±1.17 e
10	0	3.38±0.18 cdef	1.62±0.82 e	29.37±1.23 fg	3.83±0.35 d	15.76±1.51 e
	4+1	4.11±0.07 abc	1.32±0.03 e	33.02±1.97 abc	-5.73±0.17 g	13.42±0.59 e
	4+2	4.18±0.03 abc	1.28±0.03 e	33.31±0.89 ab	-6.03±0.82 g	11.75±0.42 e
	4+4	4.29±0.021 ab	1.22±0.02 e	33.84±1.59 ab	-6.86±0.36 g	16.27±2.87 e
20	0	2.88±0.78 f	2.38±0.54 cd	26.62±0.80 hi	5.86±1.58 cd	24.59±2.97 d
	4+1	3.97±0.11 bcd	2.12±0.01 d	31.89±0.96 b-e	-4.94±0.59 fg	24.34±6.31 d
	4+2	4.08±0.08 abc	2.10±0.02 d	32.24±0.84 bcd	-2.45±0.37 ef	34.49±0.08 ab
	4+4	4.15±0.14 abc	2.09±0.01 d	32.46±1.00 bcd	-5.73±0.45 g	24.72±7.10 d
30	0	1.70±0.67 g	3.11±0.14 b	24.41±0.86 ij	11.84±2.14 b	32.06±1.73 bc
	4+1	3.64±0.07 b-f	2.69b±0.02 c	29.73±0.47 efg	-1.99±0.71 ef	31.89±0.99 bc
	4+2	3.75±0.07 b-e	2.66±0.03 c	30.16±0.95 d-g	2.94±0.11 d	28.14±4.10 cd
	4+4	3.78±0.09 b-e	2.65±0.03 c	30.67±0.52 c-f	-4.75±0.55 efg	27.53±9.01cd
40	0	0.60±0.10 h	3.92±0.62 a	22.60±1.07 j	19.87±1.85 a	31.34±3.77 bc
	4+1	3.02±0.08 ef	3.67±0.02 a	27.95±0.96 gh	5.64±0.33 cd	36.85±2.79 ab
	4+2	3.08±0.08 ef	3.64±0.04 a	28.21±0.16 fgh	5.54±0.38 cd	32.40±0.32 bc
	4+4	3.20±0.04 def	3.63±0.02 a	28.87±0.71 fgh	-1.70±0.70 e	39.76±0.70 a

Means within each column with different superscript letters are significantly different (p = 0.05) for each sampling.

with control set (P<0.01). No significant (b*) value were observed in treated fruits, except 20th days in 4+2 mM (34.49) and 40th days in 4+4 mM (39.76) SA-treated fruit, which were higher level than control set (31.34). During storage, the L* value decreased. The control set had the lowest L* value (22.60), and the highest value was recorded in pre and postharvest SA treatments 4+2-4+4 mM (28.21 and 28.87, respectively) at the end of the experiment, and their difference was not statistically significant compared to the other SA concentrations. Our results indicate that SA treatments delayed the loss of L* value in tomato during storage. In general, a* value increased during storage as well as ripening. In the other hand, the color development rate of tomatoes increased with the increase in maturation. The most value of color index a* with a negative value (-6.13) was recorded in the green fruits of the control set. The negative values were observed in immature fruits and treated fruits showed negative/lower a* values than control fruits. The highest value of color index a* (19.87) was recorded in completely ripened tomatoes of the control set at 40th days. The index a* had a sharp increase control set at 10th days with a* value changing from negative (green color) to positive (red color) (Table 1). Storing mature-green fruits, treated with salicylic acid at 10°C increased the postharvest life up to 40 days.

Chilling injury and electrolyte leakage

Our results showed that CI increased during storage, but applying different concentrations of SA could significantly (P<0.01) affect chilling injury index in tomato fruit. Salicylic acid treatments lowered the levels of chilling injury compared to that of the fruits of control set, and the highest chilling injury was observed in control set. No chilling injury symptoms were observed in tomato fruits with pre and postharvest treatments 4+4 mM (Table 2). The results obtained from the present study showed that electrolyte leakage of control set (70.27%) was significantly higher than that of SA-treated fruits end of the storage period, and there was no significant difference between concentrations used for treatment (P<0.01) (Table 2).

Fruit decay index

Decay increases during storage and the results of our evaluation showed that SA, at different concentrations, significantly affected fruits decay (P<0.01). Fruits with SA treatments showed lower levels of decay as compared to that of the fruits of control set. Highest decay was observed in our control set. In pre and postharvest treatments 4+4 mM, there were not any decay symptoms in tomato fruit after 40 days. We did not witness any significant differences between concentrations (4+1 and 4+2 mM) (Table 2).

Table 2 - Effect of pre and postharvest treatments of salicylic acid in chilling injury, electrolyte leakage, decay, ascorbic acid of tomato fruit cv. Baraka and stored at 10°C for up to 40 days

Time storage (days)	Salicylic acid treatments	Chilling injury	Electrolyte leakage (%)	Decay index	Ascorbic acid (mg/100 g)
0	0	0.00± 0.00 d	40.63±10.32 d	0.00±0.00 d	66±6.00 a
	0	0.00±0.00 d	56.59±4.05 c	0.00±0.00 d	32±1.05 f
10	4+1	0.00±0.00 d	26.21±1.81 gh	0.00±0.00 d	65±1.73 a
	4+2	0.00±0.00 d	24.39±0.74 h	0.00±0.00 d	66±3.00 a
	4+4	0.00±0.00 d	23.98±2.00 d	0.00±0.00 d	68±3.46 a
20	0	0.00±0.00 d	61.80±1.30 bc	0.00±0.00 d	18±3.00 g
	4+1	0.00±0.00 d	28.27±0.90 fgh	0.00±0.00 d	49±4.58 bcd
	4+2	0.00±0.00 d	25.63±0.65 gh	0.00±0.00 d	52±4.58 bc
	4+4	0.00±0.00 d	23.68±0.94 h	0.00±0.00 d	55±1.73 ab
30	0	0.60±0.20 b	65.11±5.47 ab	0.46±0.31 b	12±3.00 gh
	4+1	0.20±0.00 c	33.25±2.71 defg	0.20±0.00 c	44±4.58 de
	4+2	0.20±0.00 c	31.81±1.70 efgh	0.20±0.00 c	48±3.00 cd
	4+4	0.00±0.00 d	30.59±0.79 efgh	0.00±0.00 d	50±1.73 bcd
40	0	1.06±0.23 a	70.27±0.92 a	1.07±0.23 a	9±3.00 h
	4+1	0.33±0.12 c	37.53±1.21 de	0.40±0.20 b	39±3.00 e
	4+2	0.27±0.12 c	36.65±2.95 de	0.33±0.23 bc	41±3.46 e
	4+4	0.00±0.00 d	36.51±2.65 def	0.00±d	45±3.00 de

Means within each column with different superscript letters are significantly different (p = 0.05) for each sampling.

Vitamin C (ascorbic acid)

Tomato fruits vitamin C content was decreased during storage, and it was found to be maintained with pre and postharvest treatments of SA, and this result was statistically significant (P<0.01). Tomato fruits treated with SA showed comparatively higher levels of ascorbic acid than the fruits of control set (9 mg/100 g), and the highest ascorbic acid content in pre and postharvest treatments 4+4 mM (45 mg/100 g) was observed, but there was no significant difference between two concentrations of our treatment (Table 2).

Titratable acidity (TA) and total soluble solids (TSS)

Tomato fruits TA content was maintained with pre and postharvest treatments of SA, and it significantly resulted in firmer fruits comparing to the controls (P<0.01). Titratable acidity (TA) content in treated fruits was higher than the control set (0.86%), and our results showed that TA maintained with pre and postharvest treatments of SA and we observed the highest TA content in concentrations of 4+2-4+4 mM (1.02, 1.03%, respectively) at 40th days (Table 3). Also, TSS increased during storage. Highest and lowest TSS were observed in control set and treated fruits, respectively, and this difference was significant (P<0.01). SA application in this experiment had a significant effect on soluble solids. Therefore, soluble solids of control fruits (4.36 °Brix) were more than of treated fruits (2.70 °Brix in 4+4 mM) after 40th days storage (Table 3).

Ascorbate peroxidase (APX)

The results showed APX decreased in control fruits, it increased and then decreased again in treat-

Table 3 - Effect of pre and postharvest treatments SA in TA, TSS, APX of tomato fruit cv. Baraka and stored at 10°C for up to 40 days

Time storage (days)	SA treatments	TA (%)	TSS (°Brix)	APX (mg/g fw)
0	0	0.94±0.19 abcd	3.40±0.69 c	37.14±1.24 e
	0	0.92±0.01 bcd	3.43±0.15 bc	20.90±19.03 f
10	4+1	1.03±0.01 ab	2.23±0.15 d	54.78±1.12 ab
	4+2	1.04±0.01 ab	2.16±0.15 d	55.66±1.29 a
	4+4	1.04±0.02 ab	2.46±0.46 d	56.12±2.49 a
20	0	0.90±0.01 bcd	4.00±0.10 ab	18.80±11.17 f
	4+1	1.01±0.01 abc	2.66±0.12 d	52.31±2.06 abc
	4+2	1.04±0.01 ab	2.60±0.20 d	53.83±4.36 ab
	4+4	1.06±0.01 a	2.53±0.06 d	55.70±4.77 a
30	0	0.89±0.03 cd	4.26±0.21 a	7.38±2.89 g
	4+1	1.01±0.01 abc	2.66±0.06 d	40.18±2.47de
	4+2	1.02±0.01 abc	2.63±0.12 d	43.78±4.81 cde
	4+4	1.03±0.01 ab	2.66±0.06 d	46.42±3.11bcd
40	0	0.86±0.03 d	4.36±0.40 a	6.95±1.38 g
	4+1	1.00±0.01 abcd	2.63±0.12 d	38.06±1.09 de
	4+2	1.02±0.02 abc	2.66±0.0 6d	38.61±1.31 de
	4+4	1.03±0.02 ab	2.70±0.10 c	38.18±2.51 de

Means within each column with different superscript letters are significantly different (p=0.05) for each sampling.

ed fruits. The activity of APX in treated fruits was higher than the controls, and there was no significant difference between the three concentrations at the end of the experiment (P<0.01) (Table 3).

4. Discussion and Conclusions

The results of this study indicate that pre and postharvest treatment with SA produced the firmest fruits. Softening of fruits is one of the most common physical parameters to assess the progress of ripening (Srivastava and Dwivedi, 2000; Brummell, 2006) and softening is a major problem of tomato that limits the quality. Key factors associating with fruit softening are the depolymerisation and degradation of cell wall components (Brummell, 2006). Srivastava and Dwivedi (2000) reported polygalacturonase is primarily responsible for ripening associated pectin degradation and fruit softening. Level of polygalacturonase activity has been positively correlated with fruit ripening and softening in banana and tomato fruits. Application of salicylic acid is useful in inhibiting tissue softening in fruits by reducing cell wall hydrolases activities and maintaining cell membrane consistency (Supapvanich, 2015). Wei *et al.* (2011) reported that exogenous application of SA enhances defense mechanisms and production of antioxidants in fruits during storage that leads to a decrease in lipid peroxidation of the cell membrane and results in maintained cell membrane structure. This result was in agreement with the reports of Babalar *et al.* (2007) and Shafiee *et al*. (2010) that suggested pre and postharvest application of SA on strawberry could decrease the softening and keep them firm during storage. Zhang *et al.* (2003) showed that SA effectively prevented kiwifruit softening during storage and rate of fruit ripening related to internal SA concentration as well as Srivastava and Dwivedi (2000) reported that salicylic acid treatment inhibited the process of banana fruit softening during ripening. Srivastava and Dwivedi (2000), Zhang *et al.* (2003) and Wang *et al.* (2006) reported that rapid softening of fruits during ripening was simultaneous with rapid decrease in endogenous SA of fruits. Tomato fruit weight loss did not show changes in response to SA treatments (except in 30th days lower than the control set). The results of this study did not accord with the ones of Babalar *et al.* (2007) and Shafiee *et al.* (2010).

The L* value decreased during storage. Babalar *et al*. (2007) reported higher lightness in pre and postharvest SA treated strawberry fruits than control. Shafiee *et al.* (2010) showed SA treatments were not effective on fruit lightness in comparison with the control set. Fattahi *et al.* (2010) suggested that the decrease in L* value represented the formation of dark color in the pulp due to oxidative browning reactions or increasing in brown pigment concentrations. Value a* increased during fruit ripening. The same results were obtained from pre and postharvest SA application on strawberry (Babalar *et al.*, 2007), but Shafiee *et al.* (2010) reported that SA treatments were not effective on a* value in comparison with control. The a* value is a useful index of maturation and the degree of ripening in tomato (Artes *et al.*, 1999) and the external color is a key factor indicating the quality of tomato (Supapvanich, 2015). Changes in a* result increase the respiration rate during storage. The salicylic acid treatment causes a decrease in respiration and a delay in the appearance of the climacteric peak, which is concentration-dependent (Srivastava and Dwivedi, 2000). Shafiee *et al.* (2010) reported that the effect of SA treatments might be due to the reduction of respiration, and it prevents from an increase in a* value, so it could have an advantage in delaying the senescence. The SA application did not affect b* value except 20th days in 4+2 mM and 40th days in 4+4 mM SA-treated fruit, which showed higher level than control set. There were not literature about the effect of pre and postharvest application of SA on b*changes.

Salicylic acid treatments lowered the levels of chilling injury compared to that of the fruits of control set, and the highest chilling injury was observed in control set. Ding *et al.* (2001, 2002) reported that chilling injury was manifested in tomato fruit by some symptoms. Severely injured fruit developed sunken areas (blemishes) an increased susceptibility to Alternaria rot and decay. Initially, CI affects the cell membrane with changes in the fatty acid of phospholipids. Secondary damages are on the cell membrane that leads to disruption of the cell structure (Soleimani Aghdam *et al.*, 2012). Asghari and Soleimani Aghdam (2010) suggested that treatment with SA prior to low-temperature storage induce heat shock proteins (HSPs) biosynthesis and, at the same time, CI tolerance in tomatoes and peaches. Accumulation of the heat shock proteins (HSPs) in chilling-sensitive horticultural products with SA treatments would allow their storage at low temperatures without CI development. This membrane damage can be measured by the electrolyte leakage, which the results obtained from the present study showed that electrolyte leakage of control set was significantly

higher than that of SA-treated fruits. Therefore, these results indicate that SA can maintain membrane consistency through enhancing the antioxidant potential of the plant. Reduction of electrolyte leakage and prevention of oxidative damage to cells under stress conditions has been mentioned as primary mechanisms of stress tolerance. The same results were obtained from postharvest treatment with SA to prevent chilling injury (Sayyari *et al.*, 2009; Soleimani Aghdam *et al.*, 2012) and electrolyte leakage (Sayyari *et al.*, 2009; Soleimani Aghdam *et al.*, 2012; Orabi *et al.*, 2015).

Fruits with SA treatments showed lower levels of decay as compared to that of the fruits of control set and in pre and postharvest treatments 4+4 mM, there were not any decay symptoms in tomato fruit after 40th days. Salicylates are major components of the signal transduction pathways of plants playing an important role in disease resistance (Asghari and Soleimani Aghdam, 2010). Different researches show that SA had no direct effect on the decrease of decay in pear fruits (fruits were sprayed with SA), but it might reduce fungus development (Shafiee *et al.*, 2010). Babalar *et al.* (2007) reported SA in a concentration dependent manner from 1 to 2 mM effectively reduced fungal decay in Selva strawberry fruit. Salicylic acid applied to either plant's vegetative stage, fruit development stages or postharvest stage could completely control decay and increased fruit shelf life. Yao and Tian (2005) showed preharvest and postharvest treatments of sweet cherry fruit with SA showed significantly lower disease percentages in storage at 25°C than the control. At 0°C, the inhibitory effects of preharvest SA treatments on postharvest disease were better than those of the postharvest treatment. Shafiee *et al.* (2010) also obtained similar results for pre and postharvest SA treatments on strawberry fruit.

Tomato fruits vitamin C content was decreased during storage and fruits treated with SA showed comparatively higher levels of ascorbic acid than the fruits of control set. Shahkoomahally and Ramezanian (2014) reported that the utilization of ascorbic acid during later storage periods may be the reason for its decreased amounts. Generally, when fruits become overripe, vitamin C content declines concurrently with the degradation of fruit tissues. The results obtained from this study indicate that the SA treatments were beneficial in delaying degradation of ascorbic acid content during storage. Therefore, The SA treated fruits exhibited higher maintenance of ascorbic acid as compared to that of control set. SA prevents vitamin C destruction by increasing the antioxidant ability and resistant of plants and fruits (Wang *et al.*, 2006; Shafiee *et al.*, 2010). Also, exogenous SA could be effective in reducing the rate of respiration and ethylene production (Renhua *et al.*, 2008). Thus, the results of this study confirm previous reports of postharvest treatment with SA to preserve vitamin C content in tomato (Pila *et al.*, 2010), orange (Huang *et al.*, 2008), rambutan fruit (Supapvanich, 2015) and pineapple fruit (Lu *et al.*, 2011).

Titratable acidity content in treated fruits was higher than the control set. Titratable acid depends directly on the concentration of organic acids in the fruit as an important factor in maintaining the quality of fruits (Kazemi *et al.*, 2011). Therefore, any treatment that slows the metabolism and aging of the product can slow down the changes during storage to reduce titratable acid (Zokaee Khosroshahi *et al.*, 2007). A correlation between enhanced respiration and a decrease in TA has been suggested by Shahkoomahally and Ramezanian (2014) to be due to the use of organic acids as respiratory substrates in the respiratory cycle in fruits. Organic acids have a higher ratio of oxygen to carbon compared to carbohydrates or fatty acids; therefore, those are easier to consume as an energy source in the process of respiration. Salicylic acid reduces respiration and ethylene production, leading to the reduction in consumption of organic acids as respiratory substrates (Serrano *et al.*, 2003). Salicylic acid application in this experiment had a significant effect on soluble solids. Therefore, soluble solids of control fruits were higher than of treated fruits. Bal and Celik (2010) revealed that after harvest and during storage and ripening of the fruits was increased the TSS content. Asghari and Soleimani Aghdam (2010) and Bal and Celik (2010) reported that cell walls contain large amounts of polysaccharides, mainly pectins and cellulose, and are digested due to the activity of the cell wall degrading enzymes leading to a significant increase in TSS content. Salicylic acid effectively protects cell walls by decreasing the expression of degrading enzymes and as a consequence prevents from dramatic increase in TSS content of the cells, and caused slow down of ripening. Similar observation was reported with SA treated banana. Salicylic acid treatments inhibited ethylene biosynthesis and delayed the senescence. This is because in the control fruits, due to the aging process (ripening), cell wall was digested and increased soluble solids. On the other hand, SA treatment reduces cellular metabolic activities, such as

respiration and ethylene production, and thus maintains the membranes and cell walls, and prevents from an abnormal increase in the soluble solids (Valero *et al.*, 2006). An increase in TSS content of fruits during storage due to the conversion of starch to be soluble sugars is one of the ripening indexes (Fisk, 2006). This result was in agreement with Babalar *et al.* (2007) who reported that the use of salicylic acid decrease TSS of strawberry fruits and consequently, effectively delays fruit senescence process. Treatment of kiwifruits maintained a lower TSS content than the control fruits at the end of cold storage (Soleimani Aghdam *et al.*, 2009). However, it is in disagreement with Lu *et al.* (2011) results on pineapple fruit and Shafiee *et al.* (2010), report on strawberry, who suggested that SA did not affect soluble solids content and titratable acidity.

The activity of APX in treated fruits was higher than the controls. The study indicated the beneficial effects of SA by pre and postharvest treatments on tomato fruit quality. Wang *et al.* (2006) and Soleimani Aghdam *et al.* (2012) reported that SA might mitigate postharvest CI in fruits and vegetables via different mechanisms. These mechanisms include: a) enhanced alternative oxidase (AOX) gene expression as an efficient ROS avoidance gene, b) increased ascorbate peroxidase (APX) and glutathione reductase (GR) activity, c) enhanced reduced-to-oxidized ascorbate (AsA/DHAsA) and reduced-to-oxidized glutathione ratios (GSH/GSSG) and d) improved heat shock proteins (HSPs) gene expression in peach fruits. Orabi *et al.* (2015) suggested that there is an important link between plant antioxidant ability and the applied doses of the SA. The observed variation (increase) activity of APX is due to SA activates the resistance system and increases the cell antioxidant capacity. Asadi *et al.* (2013) showed that exogenous application of salicylic acid lightened the toxic actions induced by stress and decreased lipid peroxidation rates with increasing antioxidant activity. There is not any report on the effects of pre and postharvest treatments with SA on fruits APX activity.

As a whole, this study showed that pre and postharvest treatments of SA are an effective method of extending storability and postharvest life of tomato fruits at 10°C. The most effective treatment in reducing losses of fruit quality was found to be SA 4+4mM treatments during the storage period of tomato fruit. It was determined that under these conditions Baraka tomato could be stored for 40 days without losing much of its quality.

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