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Application of exogenous spermidine treatment for reducing of chilling on fruit quality and quantity of Valencia orange var. olinda

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ABSTRACT: With due attention to importance of citrus as one of the most important horticulture and exports crops of iran and in order to increasing of shelf life and quality improvement of citrus, research in this field is necessary, and the purpose in this study is improvement the Qualitative andquantitativecharacteristics of orange at storage via Polyamins application. This experiment was performed in factorial base on a completely randomized blocks design (CRBD) with three replications during 2010. Treatment included three levels of Spermidine (0, 1 and 1.5 mM) with control that use pure water and temperatures at two levels (2 and 4 °C) and incubation storage period at three levels (one, three and five weeks after harvest). The results indicated that the effect of Spermidine on chilling-injury, weight of single fruit, TA, flavor index (TSS/TA), fruit weight loss and TSS was significant. Effect of temperature on the mentioned characters (exceptional TA) was not significant. The effect of storage period on the percentage of fruit weight loss, vitamin C, TSS, TA, percentage of fruit juice and TSS/TA was significant. The best concentration in this experiment for reduce of chilling injury was spermidine 1.5 mM (Fi 1 & 2).

***Keywords*:** Valencia oranges, Spermidine, chilling-injury

INTRODUCTION

Citrus is a member of world's subtropical fruits and rutaceae family. The Importance of citrus fruits in order of high vitamin C and anti-cancer effects. Oranges are second fruit after apple that people consumed in the world. Valencia oranges is a late- season fruit, high yielding that transferred from spain to the other parts of the world (Fotuhighazvini, 1999; Walheime, 2007). Food and agriculture organization (FAO)reported iran with 4 million tons of citrus fruits production in 2010, It has been ranked between 20th major produced countries in world(FAO, 2011). chilling injury and freezing every few years in iran and many citrus producing countries, inters severe damage to citrus orchards. Many factors affected on freezing hardening that some of the them are genetically and others have physiological aspect (Mirmohammadi&Metbodi, 2002). Freezing in citrus is one of the biggest environmental factors that lead to severe damages to citrus producers (Fotouhi-Ghazvini, 2002). On the other inattention of growers to appropriate harvesting time of fruits and regulation of harvesting time according to market demand caused to chilling injury of citrus fruits on tree (Willis et al., 2008).Losses of agricultural products are agricultural issuesdue to numerous reasons, such as failure to observe the principles of proper harvesting, handling, transportation and storage of agricultural products. Amount of losses in developing countries and in the case of some crops like banana and citrus are reported up to 50% (Fotuhighazvini, 1999). Polyamines are the new group of plant growth regulators that the history of polyamine biochemistry goes back more than 300 years. They are low molecular weight cations and with linear nitrogen groups present in all living organisms. Polyamines and their

biosynthetic enzymes have been implicated in a wide range of metabolic processes in plants and animals, ranging from cell division and organogenesis to protection against stress (Kaur et al., 2013). The common Polyamines are ubiquitous in plant cells, including putrescine, spermidine and spermine. Spermidine increase the more longevity of fruits in storage and decrease damage of storage (Esan ashari and zokaee khosroshahi, 2008). It is believed that Exogenous polyamines causes increase post harvest life and quality improvement of fruits due to maintenance of firmness, reduce ethylene production, water loss, delay changes of color, soluble solids, titrable acidity, and fruit protection against chilling and mechanical injury.one of the most important effects treatment of exogenous polyamine is increase tissue firmness during storage of fruits and vegetables. increase firmness and reducetissue softening were reported in many horticultural crops such as apple, strawberry, tomato, lemon and plum (Walheime, 2007; Zokaee khosroshahi and Esna ashari, 2008). Yoshikawa *et al*(Yoshikawa et al., 2007) stated that effect of low temperatures with polyamines, jasmonates, abscisic acid on antioxidant activities causes reduced low temperature injuries such as fruit cracking in apple fruits. Also its results showed that abscisic acid, jasmonates and polyamines may be associated with resistance of chilling stress in apples fruits. Neda seyf *et al* (Seyf et al., 2008) investigated application of polyamines and benzyladenine on nutritional value of punica during storage period of punica. Results showed that application of spermine and spermidineinduce cold acclimation, which lead to maintenance of membrane fluidity at low temperatures and increase endogenous polyamines, and finally reduced electrolyte leakage and skin browning in treated fruits. the aim of this study is the improvement of qualitative and quantitative traits of orange via Polyamins application at storage.

MATERIALS AND METHODS

The experiment was carried out in 2010 at agriculture and natural resources research center of jirooft, kerman province, in iran country. The area of jiroft are about 50 thousands Km2 at east sought of kerman province (56', 17' to 59' 2' longitude and 26' 43' to 29' 35' latitude, 625.6 m ASL, mean of relative humidity 50%, 48˚C maximum of temperature, 0˚C minimum of temperature, 150 mm Average annual precipitation).The experimental design was a factorial study, based on Randomized Complete Block Design (RCBD) with three replications.The tereatments were Spermidine in three levels (0, 1 and 1.5 mM), temperatures at two levels (2 and 4 °C) and storage period at three levels (one, three and five weeks after harvest) that their investigated onValencia orange (Citrus sinensis L. Osbeck var Olinda) with rootstock (Citrus aurantium) at jirooft region.Fruits were harvestedrandomly of the four cardinal points of tree crown without injury to fruit with plastic gloves in early morning and placed in wooden boxes. Fruits were transported to laboratory immediately after harvest from the field. Then healthy and uniform fruits isolated and divided into groups that 10 fruit in each group and it was placed in a plastic bag, that 5 fruits each treatment used for initial experiments and other half for chilling experiment. Fruits were wished with water and then air dried inshade. After preparing of fruits was treated with 1 and 1.5 mM/L concentration of spermidine by dipping for 5 min. following treatments, fruit were placed in two isolated storage with 2 and 4 ˚C after 1 , 3 and 5 weeks.After these times fruits were brought out from cold storage And then transferred into storage with 20˚C, 85-90% relative humidity during 1 week and then evaluated qualitative and quantitative factors and cold damage. Characters were measured like tipchilling index, weight loss, percentage of juice, degree brix of juice (TSS), Vitamin C, total acidity (TA), flavor index (TSS/TA), weight of single fruit. The data was analyzed using SAS version 9 and the means were compared by Duncan's multiple range test.

chilling injury index calculated with formula: chilling injury index = sum (number fruit each group * score of chilling)

Rate of lose weight with formula: $\frac{first\ weight - secondry\ weight}{first\ weight} \times 100$

RESULTS AND DISCUSSION

RESULTS

The result indicated that effect of spermidine on weight of single fruit, TA, TSS/TA were significant at 1% probability and on characters weight loss of fruit and TSS at 5% probability and also effect of spermidine on characters juice and vitamin C was not significant. Temperature was not significant on none of the characters (except TA at 1% level). Storage time was significant on characters percent of weight loss, vitamin C, TSS, TA at 1% probability, but was significant on characters percent of juice and TSS/TA at 5% probability and it was not significant on fruit weight. Interaction Effects of spermidine, temperature and storage time were not significant on mentioned characters. (Amin et al ., Amin and Rahemi, 2007) indicated that effects of three polyamines (spermine,

spermidine and putrescine) with concentration (0, 0.5, 1 and 1.5 mM) in 3 and 10˚ C on reducing of chilling on sweet lemon (*Citrus limetta*)caused that untreated fruits showed symptoms of chillingas brown spotsand skin necrosis In 3˚C after 2 weeks, whereas Treated fruits with polyamines showed no symptoms of chilling in 3˚C. and reducing of chilling with increasing of spermidine and spermine content have positive correlation.Effect of polyamines in increasing of tissue firmness related to linkage those to carboxyl Group (-Coo) of pectic compounds in cell walls. This linkage lead towall stability that is detectable immediately after treatment. It'spreventfrom activity of cell wall degrading enzyme such as pectin methylesterase , pectinesterase and polygalacturonase and lead to reduce of fruit softening during storage period. decrease of fruit tissue softening during storage period in controlled atmosphere storage have closely correlation with increase levels of endogenous polyamines (Esan ashari and zokaee khosroshahi, 2008). Yuko *et al* (Yuko et al., 1993) investigated effects of storage temperature on polyamines content in four varieties of vegetables and results indicated that reduced level of chlorophyll and polyamines in 20 and 32˚C ,respectively. Whereas chlorophyll and spermidine tend to remain in 2˚C and but increased level of putrescine. Bibi *et al* (Bibi et al., 2010) reported that application of endogenous putrescine on improvement effects of high temperature in flower and fruit development of cotton caused increase level of putrescine in cotton flowers and this action despite of negative effects of increase in temperature caused increase of seed set. Valero *et al* (Valero et al., 2002) reported that application of free polyamines (spermine, spermidine and putrescine) and abscisic acid in two stage of fruit ripening of lemon when bicolor and complete yellow fruit caused increase tissue firmness compared to control fruits in first stage of ripening. Treated fruit with putrescine indicated high level of firmness and lowest of weight loss compared to treated fruits with calcium and control fruits during storage period.

Chilling injury index

Results of this experiment indicated that effect of espermidine on chilling index was significant (table1).So that most of chilling injury percentrelated to control treatment with mean of score 1.72 and least obtained in spermidine 1.5 mMwith mean 10.). According these results can say that with increasing concentration of spermidine reduced percent of chilling injury, and best concentration was spermidine 1.5 mM for reducing chilling injury(Fig 1 & 2). (Ggo*et al.,* 2008) were reported that relation of polyamines in control of chilling sensitivity of corn genotype indicated that cold injury in roots related with spermidineconcentration .obtained results in this study corresponded with Ggo*et al*results.

Weight of fruit single

Effect of exogenous spermidine on weight of fruit single was significant at 1% probability. The highest weight was control with mean of weight 287.38 g and it was lowest in treatment spermidine 1 mM per liter with mean of weight 236.79 g (table 2). (Ramezanian et al., Ramezanian et al., 2010) stated that application of spermidine on pomegranate fruit caused reduced weight loss and increased amount of juice than control fruits and caused improvement of low temperature on quality of pomegranate fruits during cold storage.

Percentage of weight loss

Weight loss percent of orange affected by spermidine treatment significantly ($p<0.05$). the comparison means indicated that spermidine 1.5 mM per liter has highest of weight loss with 4.78% and control has least of weight loss with 3.85% (table2). Zokaee Khosroshahi & Esna ashari (Zokaee khosroshahi et al., 2007) stated that storage life of strawberry fruits was significantly increased by the use of putrescine and it caused also preventd the softening of fruit flesh weight loss during the storage.

TSS

Effect of spermidine on TSS was significant at 5% probability. Highest rate of TSS was related to the 1 mM per liter spermidine with 9.42% and lowest rate was related to control with 8.67% (table 2). Zokaee khosroshahi & esna ashari (Zokaee khosroshahi and Esna ashari, 2008) stated that application of putrescine on post harvest life of

strawberry, apricot, peach and sweet cherry caused ethylene production and juice losses were decreased significantly and use of putrescine prevented the softening of fruit flesh and also application of putrescine increased TSS rate in sweet cherry.

TA

TA rate affected by spermidine significantly ($p<0.01$). Highest rate of acidity was related to control with 1.106 and lowest rate occurred in 1 mM per liter spermidine with 0.95 (table 2). Mirdehghan & Rahemi(Mirdehghan and Rahemi, 2002)stated that application of polyamines on storage life of pomegranate prevented browning of skin and weight loss of fruit and it also delayed in acidification process, soluble solids and transpiration rate.

TSS/TA

Effect of spermidine on TSS/TA was significant at 1% probability. Lowest rate of TSS/TA was related in control with 7.76 and highest rate eas related in 1 mM per liter spermidine with 9.66 (table 2). Serrano *et al* (Serrano et al., 2003) stated that effectof putrescine on post harvest life of four plum cultivars increased shelf life,delayed ripening process and higher quality attributed when compared with controls was significantly for all vultivars.

DISCUSSION

According to obtained results treatment of exogenous spermidinehave desired effectsin order to reduce the effect of chilling on quality and quantity of Valencia orange fruits var. olinda. The best concentration in this experiment for reduce of chilling injury was spermidine 1.5 mM (Fi 1 & 2). Polyamines with linkage to cell membrane caused membrane stability and preservation waxes of cuticle layer and so they have important role in reduced water exchanges via fruit skin. Pretreatments that reduced symptoms of chilling injury, caused increasing of polyamine levels.alsoIn many cropssuch as lemon, tangerine, grape fruit, pumpkin, cucumber, eggplant and pepper associated with increasing polyamine levels. So it is believed that polyamines with preservation stability and health of cell membrane can protected plant organs against cold injury(Esnaashari and Aokaekhosroshahi, 2008). Polyamines of Putrescine, spermidine and spermineact as disinfectant, because their capacity have changed in linkage with ionized compounds in cell membraneand absorb free radicals, So their caused stabilization lipids and prevention of membrane degradation (Serrano et al., 1996). According to study results recommended that results in this research matched in large scale and performed with cooperation and consultation ofcitrus expert. And so looks higher concentration this plant growth regulator to be effective in freezing temperature that its require to more research.alsoit seems necessary that using of hormone as exogenous form before chilling occurrence in region with foliage application on citrus trees.

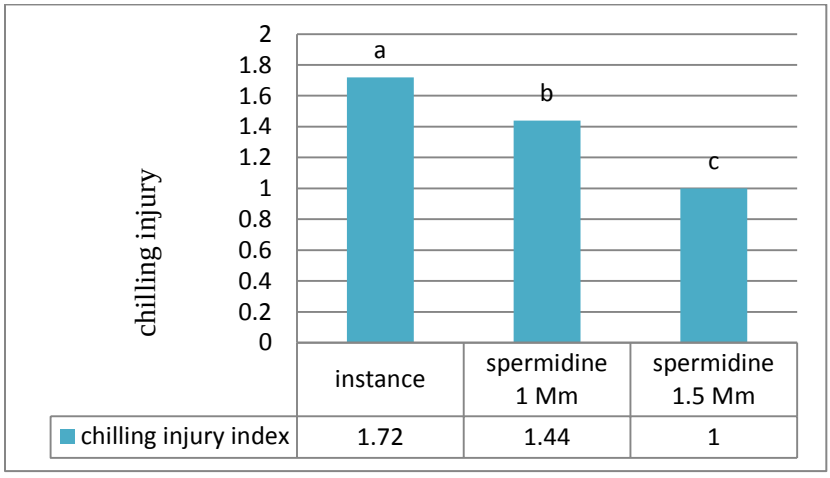

Figure 1. Comparing the mean of various concentrations effect of spermidine on the chilling injury index

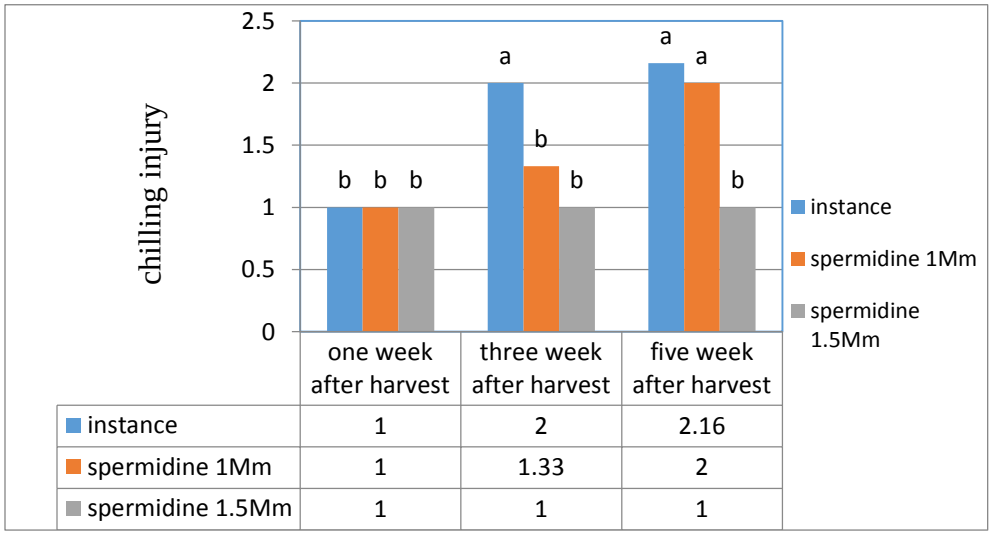

Figure 2. Comparing the mean of interaction effect of spermidine and different storage time on the chilling injury index

Table 1. Variance analysis of the characteristic studied

S.O.V	D.F	Mean squares							
		Chilling injury index	Percent fruit juice	Percentage of fruit weight	Fruit weight	TSS	TA	Vitamin C	TSS/TA
replication	2	0.055	3.283	0.503	487.351	0.297	0.002	336.789	0.174
Spermidine (A)	2	2.389**	9.509^{ns}	4.374*	11520/6**	2.689*	0.127**	49.023^{ns}	17.961**
Temperature (B)	1	0.018^{ns}	0.153^{ns}	0.300^{ns}	426.726^{ns}	1.356^{ns}	0.020^{ns}	244.907^{ns}	0.098^{ns}
Storage time (C)	2	2.389**	15.313*	19.919**	573.490^{ns}	3.923**	0.059**	584.827**	6.153*
Interaction A*B	2	0.018^{ns}	3.989^{ns}	0.083^{ns}	769.650^{ns}	0.042^{ns}	0.024^{ns}	1.297^{ns}	1.234^{ns}
Interaction A*C	4	0.78**	4.153^{ns}	1.374^{ns}	382.757^{ns}	1.161^{ns}	0.002^{ns}	52/682^{ns}	1.116^{ns}
Interaction B*C	2	0.351*	0.259^{ns}	0.688^{ns}	1419.75^{ns}	0.945^{ns}	0.002^{ns}	6.314^{ns}	1.297^{ns}
Interaction A*B*C	4	0.185^{ns}	0.949^{ns}	0.894^{ns}	526.144^{ns}	0.781^{ns}	0.014^{ns}	56.073^{ns}	1.297^{ns}
error	34	0.075	5.416	1.033	865.638	0.716	0.012	74.239	1.882
C.V		19.73	5.468	23.064	11.218	10.047	10.917	10.18	15.752

ns: non-significant, **,* : Significant at 1% and 5% of probability levels, respectively

Table 2. mean comparison of the traits in the Valencia orange fruits var Olinda

Treatment	Percent fruit juice	Percentage of fruit weight	Fruit weight	TSS	TA	Vitamin C	TSS/TA
instance	34.4^{a}	3.85^{b}	278.389^{a}	8.67^{b}	1.106^{a}	85.028^{a}	7.67^{b}
Spermidine 1mM	42.09^{a}	4.5^{a}	236.794^{c}	9.42^{a}	0.95^{b}	86.044^{a}	9.66^{a}
Spermidine 1.5mM	49.19^{a}	4.78^{a}	262.606^{b}	0.97^{b}	0.97^{b}	82.817^{a}	8.78^{a}

Mean in each column with similar letters not significatly different at 5% of probability level using dancan's multiple

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