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Effect of salicylic acid on date bunch fading disorder

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Abstract

Salicylic acid is a phenolic compound which could have an active role in the process of drought tolerance of plants. This study was conducted to investigate the effects of salicylic acid on date bunch fading disorder of Mazafati date cultivar, in a randomized complete block design with 5 levels of salicylic acid treatments' 0, 25, 50, 75 and 100ppm, at beginning of Khalal stage, in three replicates and two location of Bam region. In this study the percentage of damaged fruits caused by date bunch fading disorder, length of fruit, diameter of fruit, weight of fruit, pulp to seed ratio, yield, total soluble solids, pH, titratable acidity, percentage of total sugar, and percentage of protein were evaluated. Experimental date palm trees treated with 75ppm salicylic acid showed 45% reduction of damaged fruits caused by date bunch fading disorder in compare to other treatments at the 1% level of probability by Duncan's test. Furthermore, the application of 100ppm salicylic acid caused yield increase rate of 12kg per tree in compare to controls at the 1% level of probability by Duncan's test. Although some of salicylic acid treatments were effective in reduction of the damaged fruits caused by date bunch fading disorder and increase in yield of palm trees, but showed no significant effect on the physico-chemical characteristics of fruit. According to the biennium ANOVA table, some characteristics (length of fruit, weight of fruit, pulp to seed ratio, pH, total soluble solids, total sugar, percentage of protein, percentage of fruit moisture, and titratable acidity) did not show any significant differences, but the results of the classification by Duncan's test showed that spraying with salicylic acid solution in all treatments increased length of fruit, diameter of fruit, weight of fruit, pulp to seed ratio, yield, titratable acidity and pH, except for a reduction in the amount of total soluble solids and total sugar in compare to control.

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Introduction

Date is one of Iranian valuable horticultural products on which the income and occupation of the majority of the stares' southern provinces' residences are depended. In the recent years, the date bunch fading disorder has been the most important challenge of date production in Iran. However, none of the main date-producing countries of the world have reported this disorder yet. In Kerman province, Mazafati date cultivar is very susceptible to this disorder (Panahi *et al.,* 2011; Panahi *et al*., 2012). The date bunch fading and wilting disorder may be observed in the fruits in their Kharak-to-Rutab transformation stage. The symptoms of this disorder first appear in form of fruits wilt. Simultaneously with fruits wilting, tip of bunches' axis becomes shriveled, then brown-color spots appear on them and gradually whole axis of bunch and fruits turn shriveled. In some cultivars (Mazafati) a brown-color strip appears on the tail of the main bunch which finally becomes dried and woody (Panahi *et al*., 2011; Panahi *et al*., 2012).

There are several hypotheses about causes of outbreak and development of this disorder in this country, the most important of which include climatic causes, pathogens- especially fungal agents, nutrient deficiencies or disorders and lack of observing optimal cultivation and management principles in the groves (Pezhman, 2004). Darini (2001) reported that no evidence of this disorder was observed in Zehmkan and Dehkahan regions, and claimed that this was due to the lower level of temperature and lack of warm and hot winds in these regions.

Salicylic acid has been reported to be a phenolic compound which is produced in plants naturally. This compound has aromatic ring with hydroxyl group and its derivations (Hayat *et al*., 2010), and is commonly applied in plant's reactions against environmental and physical stresses (Malamy *et al*., 1992; Raskin, 1992). Various studies have approved the role of salicylic acid as an important messenger molecule in plant's responses to various environmental and non-environmental stresses (Larcher, 2003). Khassem *et al.* (2012) found that foliar application of salicylic acid by concentration of 50ppm on Berhi date cultivar results in increased chlorophyll amount and delayed fruit ripening. Salicylic acid delays the ripening process of some fruits through causing disorder in biosynthesis and action of ethylene hormones, abscisic acid and cytokinin. In summer, when temperature exceeds the desired thermal amplitude for Kentucky grass, its quality is reduced. By applying salicylic acid, the thermal resistance of Kentucky grass was increased. In fact, this compound stops the increase of oxygen production (He *et al*., 2005). External application of salicylic acid can contribute to increase of endogenous salicylic acid (Seo *et al*., 1995) which has an active effect on drought resistance process. Endogenous salicylic acid is an induction sign for the plants' special defensive responses (Klessig *et al*., 2000; Shah & Klessig, 1999). Status of nutritional and chemical elements, especially in many stressful factors results in some changes in quality and quantity of the active chemical substances of the plant, which in turn influence on other components of ecosystem (Khassem *et al*., 2012). Several studies illustrate the role of salicylic acid in increasing acidity, non-reducing sugars and chlorophyll content and also on reducing the fruit's TSS, reducing sugars, total sugar and carotene content (Stern *et al*., 2006). Results of the research conducted by Panahi *et al.* (2012) on the chemical effect of salicylic acid on date fruits showed that salicylic acid application results in increased titratable acidity and reduced TSS and total sugar of the fruit.

Considering the role of salicylic acid plays in drought resistance (Klessig *et al*., 2000; Shah & Klessig, 1999; and Sayyari *et al*., 2013), thermal resistance (He *et al*., 2005), increasing tissues' tolerance against stress (Bernard *et al*., 2002) including biological and physical stresses (Larcher, 2003; Malamy *et al*., 1992; and Raskin, 1992), reducing ethylene production (Mazaheri Tirani *et al*., 2009) and oxygen production stop (He *et al*., 2005), and also according to the results of previous researches which consider the environmental severe and sudden stresses to be effective on outbreak of this disorder (Panahi, 1999), and confirming the role of temperature and relative humidity in the disorder exacerbation (Darini, 2001; Mohammadi & Moghtaderi, 2005; and Pouzesh Shirazi *et al*., 2004), the hypothesis

of applying salicylic acid for reducing the damage percentage of the disorder seems to be necessary. The effect of salicylic acid on qualitative and quantitative characteristics of "Berhee" date fruits were reported by Khassem *et al.* (2012). However, no more research work has been reported until now by application of salicylic acid on date bunch fading disorder. With considering of high percentage of damages to "Mazafati" date fruits, the current research work aimed to investigate the effect of salicylic acid on date bunch fading disorder; reduction in fruits damage percentage and on qualitative and quantitative characteristics of "Mazafati" date fruits.

Materials and methods

This experiment was carried out in a randomized complete block design by foliar application treatment in five levels of salicylic acid (0, 25, 50, 75 and 100ppm) in three replicates on 15 palm trees in the research station of Azizabad located in Bam area, Kerman province, Iran. Considering the different severity of the disorder in consecutive years, the project was carried out during the years 2010-2011, and any palm was considered as one experimental unit. Foliar application was performed at the beginning of fruits' color change (at the beginning of Kharak stage).

Measurements of studied characteristics

The measurements of studied characteristics included the disorder damage percentage, length, diameter and weight of the fruit, pulp to seed ratio, total sugar percentage, TSS, pH, titratable acidity, protein, moisture and yield.

After note takings, the gathered data were analyzed using MSTATC software, and means comparison test was performed through Duncan's multiple range tests.

Physical analysis

At the harvesting time, 20 full *Rutab* fruits per bunch were randomly collected for *Physical* measurements. Fruit weight, fruit diameter, fruit length, fruit pulp weight, and seed weight were recorded independently in each of the 20 fruits per replicate. An homogenous sample was prepared from each 20 fruits replicate for measurements of *chemical* characteristics (Ranganna, 1979).

Date bunch fading disorder (%)

In order to calculate date bunch fading disorder, 50 fruits were picked randomly from four bunches in four direction of each palm.

pH

The pH of palm sugar concentrate was measured by a pH meter. Calibration was standardized using pH 7.0 and 4.0 buffers (A.O.A.C, 2000). Each sample was measured in three replications.

Total acidity

Total acidity was determined by titration method with 0.1 N sodium hydroxide, which was standardized using potassium hydrogenpthalate (3.2% w/v) prior each titration. A few drops of 1% phenolphthalein were used as an indicator. A sample of palm sugar concentrate (15 ml) was titrated with 0.1 N sodium hydroxide until reached end point and persisted for 15-20 seconds, and the colour was changed to pink colour at pH 8.1. Three titrations were performed for each sample. The percentage of total acidity obtained was equivalent to lactic acid content (A.O.A.C, 2000).

Total soluble solids

Total soluble solids of palm sugar concentrate was measured using Atago hand-held refractometer. All samples were measured in three replications and reported in degree brix (Brix^0) (A.O.A.C, 2000).

Total sugar content:

The determination of total sugars by Lane and Eynon volumetric method was employed. This method consists of the chemical preparation, sample preparation and titration method (A.O.A.C, 2000).

Protein content

Content of nitrogen (N) was measured by the Kjeldahl method (AOAC 1990). The crude protein (CP) was calculated as N x 6.25.

Results and discussion

Tables 1 and 2 show the results of combined variance analysis, and average of the characteristics under study during two experimental years of 2010 and 2011 in regard with the treatments' effects have been presented in tables 3 and 4.

Table 1. Analysis of variance of the effect of the treatments on the measured traits during 2010 – 2011.

Mean Squares (MS)						Df	Source of variations
Date bunch fading disorder	yield	pulp to seed ratio	weight of fruit	diameter of fruit	length of fruit		
*	n.s	n.s	n.s	n.s	n.s	1	Year (A)
n.s	n.s	**	n.s	**	n.s	1	Location (B)
**	n.s	n.s	n.s	n.s	n.s	1	(A) ×(B)
n.s	*	n.s	*	*	n.s	8	Replication (A) ×(B)
**	**	n.s	n.s	n.s	n.s	4	Treatment C)
n.s	n.s	n.s	n.s	n.s	n.s	4	(A) ×(C)
n.s	*	n.s	n.s	*	n.s	4	(B) ×(C)
n.s	n.s	n.s	n.s	n.s	n.s	4	(A) ×(B) ×(C)
78.95	51.04	3.69	607.52	2.71	12.84	32	Error
19.11	32.9	17.88	16.83	5.02	6.95		CV (%)

N.s, non significant; *, significant at P≤0.05; **, significant at P≤0.01.

Table 2. Analysis of variance of the effect of the treatments on the measured traits during 2010 – 2011.

Mean Squares (MS)					Df	Source of variations
Acidity	Protein	pH	TSS	Total sugar		
**	n.s	n.s	**	**	1	Year (A)
**	**	**	**	**	1	Location (B)
**	n.s	n.s	**	**	1	(A) ×(B)
n.s	n.s	*	**	n.s	8	Replication (A) ×(B)
n.s	n.s	n.s	n.s	n.s	4	Treatment C)
n.s	n.s	n.s	n.s	n.s	4	(A) ×(C)
n.s	n.s	n.s	n.s	n.s	4	(B) ×(C)
n.s	n.s	n.s	n.s	n.s	4	(A) ×(B) ×(C)
0.000	0.23	0.008	17.74	24.83	32	Error
11	16.15	1.3	6.78	6.78		CV (%)

N.s, non significant; *, significant at P≤0.05; **, significant at P≤0.01.

Discussion

Date bunch fading disorder

As the results indicate, the trees treated by foliar application of salicylic acid showed lower damage percentage in compare with the control. Among the different levels of salicylic acid concentrations, concentration of 75ppm of salicylic acid showed to have better effect on reducing the disorder damage in compare to other treatments, and, by average damage percentage of 22 percent, showed 45 percent reduction in compare to the control (Table1).Reduction of the disorder damage percentage can be attributed to the role of salicylic acid as an important messenger molecule in plant's responses to various biological and non-biological stresses (Larcher, 2003). Some researchers (Panahi, 1999) consider the disorder outbreak related to the time during which the plant is under severe and sudden environmental stresses. The present project tries to increase the endogenous salicylic acid production by external application of salicylic acid (Seo *et al.*, 1995) so that the plant may get resistant against factors such as reduced relative humidity and increased temperature (Mohammadi & Moghtaderi, 2005) which play role in the disorder exacerbation. Salicylic acid concentration of 75ppm was more effective than 100ppm concentration in reducing the disorder damage. In rapeseed plants treated by salicylic acid in concentrations of 0.5 and 1 mm, amount of produced ethylene was reduced. However, in treatment with concentration of 1.5mm, amount of ethylene produced in the plant was increased (Mazaheri Tirani *et al.*, 2009). Generally, results of this research confirm this hypothesis that salicylic acid is effective in plant's reactions to the environmental stresses (Malamy *et al.*,

1992; Raskin, 1992).

Length, diameter, weight and pulp to seed ratio of the fruit

According to the ANOVA table (Table 1), salicylic acid did not affect length, diameter, weight and pulp to seed ratio of the fruit of Mazafati date cultivar, significantly. However, according to the classification table, the highest effects of salicylic acid treatment are related to concentration of 75ppm on fruit length, diameter and pulp to seed ratio, and also related to concentration of 100ppm on the fruit weight.In the study conducted by Khassem *et al.* (2012) on Berhi cultivar date, salicylic acid did not affect length, diameter and weight of fruit pulp, significantly.

Table 3. Biennium mean comparison of different levels of salicylic acid treatments on the measured traits (2010-2011).

Salicylic acid	length of fruit (Cm)	diameter of fruit (Cm)	weight fruit(g)	of pulp to seed ratio	Yield (Kg)	Date bunch fading disorder (%)
Control	51.43a	32.57ab	143.1a	10.21a	17.0b	67.5d
25ppm	51.48a	32.93ab	142.3a	10.12a	23.3a	56.67d
50ppm	51.72a	32.5ab	140.5a	10.76a	15.4b	44.17c
75ppm	52.88a	33.9a	150.1a	11.54a	23.3a	22.5a
100ppm	50.17a	32.23b	156.1a	11.1a	29.1a	41.67c

Values in each column (Mean) with the same alphabet are not significantly different at 5% level whereas values with different alphabets are significant at 5% level.

Yield

The results inserted in table 1 show that salicylic acid application in level 1% was effective on the yield of Mazafati date cultivar in Bam region, as application by concentration of 100ppm resulted in increased yield. This result conforms to reports of salicylic acid effect on yield increase in some plant such as soya (Kumar, 1999), peas (Kumar *et al.*, 1997) and cowpeas (Singh, 1980).

Table 4. Biennium mean comparison of different levels of salicylic acid treatments on the measured traits (2010-2011).

Salicylic acid	Total sugar (%)	TSS	pH	Protein (%)	Acidity (%)
Control	62.34a	63.5a	6.94a	2.966a	0.08a
25ppm	60.24a	62.3a	6.93a	3.11a	0.09a
50ppm	59.11a	61.4a	6.97a	2.924a	0.09a
75ppm	61.41a	61.8a	6.97a	2.964a	0.08a
100ppm	60.59a	61.3a	6.94a	3.123a	0.09a

Values in each column (Mean) with the same alphabet are not significantly different at 5% level whereas values with different alphabets are significant at 5% level.

Reducing total sugar content and TSS of the fruits

Results inserted in table 2 indicate that salicylic acid application on the experimental trees, in spite of reducing total sugar content and TSS of the fruits, does not have significant effect in compare with the control. Khassem *et al.* (2012) investigated salicylic acid treatment of fruit trees on Berhi cultivar and also Stern *et al.* (2006) on apple fruit reported reduced total sugar and TSS of the fruit.

pH

Results of the experiment showed that salicylic acid application did not affect on pH changes of the fruit (Table 2). In investigating the effectiveness of salicylic

acid (SA) at different concentrations (0, 0.5, 1.0, 1.5 or 2.0 mmol L–1) on postharvest life of peach fruit it was found that salicylic acid treatment with The higher concentration of SA (2.0 mmol) resulted in increased fruit weight, firmness and decreased juice pH (Tareen *et al.*, 2012).

Protein

Results of the experiment showed that salicylic acid application did not affect the protein content of the fruit of Mazafati date cultivar significantly (Table 2). In investigating the biochemical effect of salicylic acid application on rapeseed plant, results of measuring protein amount showed that 0.5mm concentration of salicylic acid had no effect on proteins' amount, but 1 and 1.5 mm concentrations increased and reduced proteins' amount respectively (Mazaheri Tirani *et al.*, 2009).

Titratable acidity

Titratable acidity includes acidity of total fruit water content which is measured based on the predominant organic acid of the fruit. In date fruit, the predominant organic acid is malic acid. The results presented in table 2 indicate that salicylic acid application on the experimental trees, in spite of increasing the amount of titratable acidity of the fruit of Mazafati date cultivar, had no significant effect in compare to control trees. In studying the salicylic acid treatments on fruit trees, Khassem *et al.* (2012) reported that salicylic acid application resulted in increased acidity of the fruit of Berhi date cultivar.

Conclusion

According to the above-mentioned results, we can observe that foliar application of salicylic acid on trees of Mazafati date cultivar at the beginning of Kharak stage at Bam region contributed to reduction of damage percentage of date bunch fading disorder (45% reduction). In addition, it had a positive and significant effect on increase of the trees' yield (12kg). As it was explained in the discussion section as well, salicylic acid treatments, in spite of being effective on reduction of the disorder damage percentage and yield of palms, but had no significant effect on physicochemical characteristics of date fruits. Lack of significant effect of salicylic acid application on physicochemical characteristics of the fruits of Mazafati date cultivar may before to the foliar application time, which was the beginning of treatment that is the beginning of Kharak stage. Answering this question whether treatment before this stage, or other concentrations of salicylic acid, can be effective on the physiochemical characteristics of the fruits requires complementary experiments; however, it shall be emphasized again that these treatments contributed to reducing the damage percentage of date bunch fading disorder and increasing the yield of Mazafati trees yield in Bam region.

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