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Foliar application of potassium nitrate and 2,4-dichlorophenoxyacetic acid affect some fruit splitting related characteristics and biochemical traits of mandarin cv. 'page'

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Abstract
Fruit splitting and quality are the most values in mandarin cv. 'Page' since they are playing the main role in the production and exporting potential. Therefore, this study was carried out to evaluated the foliar application of KNO_3 (0, 1 and 2%) and 2,4-dichlorophenoxyacetic acid (2,4-D, 0, 50 and 100 mg/L) at three stages (full bloom, end of petal fall, and end of June drop) during two growing seasons (2016 and 2017) on some fruit splitting related characteristics and biochemical traits of mandarin cv. 'Page'. Foliar application of KNO_3 and 2,4-D treatment significantly reduced fruit splitting severity, peel firmness and SSC/TA ratio, but significantly enhanced fruit size, β-galactosidase and PG activity, SSC, TA, vitamin C, TPC, and TAC as compared with control. A negative correlation was revealed between PG and β-galactosidase activity and fruit splitting severity. Moreover, a synergistic effect of KNO_3 and 2,4-D treatments was found in treated fruits as the highest content of obtained in KNO_3 2% + 2,4-D 100 mg/L treatment. Overall, it is proposed that the foliar application of KNO_3 2% + 2,4-D 100 mg/L in mandarin cv. 'Page' trees could be more suitable to reduce fruit splitting and improve fruit quality.

Keywords: antioxidant capacity, β-galactosidase, KNO_3, peel firmness, polygalacturonase

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INTRODUCTION

Fruit splitting and/or cracking is one of the major pre-harvest physiological disorders which almost occur on various citrus species as well as several mandarin hybrids. This disorder is recognized to be created by pressure resulting from the expanding and enlargement pulp of a unique fruit on the rind, finally creating a rupture at the stylar- or navel-end, and resulting in the splitting of the fruit. Split fruit finally falls from the tree. Not only does fruit splitting negatively influence fruit yield, but it also attracts insects and pathogens which leads to decay and needs serious effort to orchards irrigation. Finally, it is causing a decrease of annual yield up to 60% thus causing severe economic losses (Stander 2013, Cronjé et al. 2014, Juan and Jiezhong 2017).

Many factors might influence citrus fruit splitting such as cultivar characteristics, weather conditions, rootstock, fruit size, peel thickness, peel hardness, growth regulators, and cultural practices such as pruning, thinning, irrigation and plant nutrition (Cronjé et al. 2014, Juan and Jiezhong 2017). Among these factors, the use of property plant nutrition programs significantly affects fruit cracking and also fruit quality characteristics. In recent years, foliar application of nutrients is the most popular way among gardeners that could provide better plant nutrient requirements, amend the lack of nutrient elements as sometimes soil utilization of nutrients is not useful due to adaphic and environmental risks (Shiri et al. 2014, Shiri et al. 2016 a,b, Gaaliche et al. 2019).

The potassium (K) is identified as a vital nutrient element for all steps of protein production that related to all plant growth processes. K manages numerous enzymes activities in plant cells, by the compilation of photosynthesis level as well as an increment in the translocation amount from leaves within the phloem to storage tissue, leading to enhance the yield and fruit

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quality attributes (Gaaliche et al. 2019). Furthermore, K can cause higher osmotic and turgor pressure, which can give power for cell division, cell wall extension, and cell expansion to stimulate cell growth rate. A high K content could enhance the cell and fruit size, and make peel smooth; in contrast, when the peel K amount is low, fruit splitting and dropping will happen quickly, following in less and smaller fruit, thinner peel and the decline of SSC, organic acids, and vitamin C (Alva et al. 2006). K can stimulate cell division in citrus, and diminish fruit splitting. Using K in the spring season or during the early fruit development stages can raise fruit peel development, enhance peel thickness, increase the fruit cracking resistance capacity, and diminish preharvest fruit splitting (Ali et al. 2000). However, the extra utilization of K during the late fruit development stages has a slight impact on the decline of fruit splitting (Juan and Jiezhong 2017). It was reported that spraying of 1% KNO_3 significantly reduced more than 50% of fruit splitting in pomegranate (Singh, Sharma and Awasthi 1993).

On the other hand, 2,4-D as an artificially synthetic plant growth regulators (PGRs) are usually applied in the citrus fruit production systems. However, regarding its impact on environmental factors and food safety and security, its utilization in citrus fruits is still contentious in the world. Therefore, seeking safer and more efficient new preservatives materials to replace 2,4-D is a general subject to be decided in several citrus-producing countries. 2,4-D has biphasic impacts on plant growth processes. At a low amount, 2,4-D induces cell division and elongation, while at the high amount it forbids plant growth (Grossmann 2000, Raghavan et al. 2005, Ma et al. 2015).

2,4-D is used for controlling preharvest fruit drop, improving fruit size and fruit quality (Anthony and Coggins Jr 1999). It was mentioned that the foliar uses of 2,4-D during the flowering phase could enhance the fruit peel thickness so that citrus fruit splitting can be dramatically diminished (Garcia-Luis et al. 2001). Additionally, foliar application of 2,4-D after physiological fruit drop alone or in combination with K, increased rind thickness and significantly reduced fruit splitting of 'Marisol' and 'Mor' mandarin cultivars more than 50% as compared with control (Stander et al. 2014).

Foliar treatment of 10 mg/L 2,4-D directly (APFD) compared with later dates in January and February, either alone or in combination with K, increased rind thickness and reduced fruit splitting of 'Marisol' and 'Mor' by up to 50%, without negatively affecting internal fruit quality. Additionally, foliar application of 2,4-D in combination with GA_3 at 30 and 60 days before the predicted fruit splitting course gives more beneficial results in decreasing of navel orange splitting (Agusti et al. 1994).

Mandarin cv. 'Page' [*Citrus clementina* × (*C. paradisi* × *C. reticulata*)] is one of the most cultivated fruit among different citrus species due to its delicious taste and high nutritional quality. Sensitivity to fruit splitting is one of the serious limitations of its cultivation in North of Iran, due to a decrease of annual yield up to half percentage. Therefore, as the first research about foliar application of chemical treatments to reduce of citrus fruit splitting in Northern Iran, this study was conducted to evaluated 1) the effects of foliar application of KNO_3 and 2,4-D on some fruit splitting related characteristics [fruit splitting severity, fruit size, peel firmness, the activity of β-galactosidase and polygalacturonase (PG)] and biochemical traits [SSC, titratable acidity (TA), SSC/TA, vitamin C, total phenolic content (TPC) and total antioxidant capacity (TAC)] of mandarin cv. 'Page', and 2) achieve the most effective time to foliar application of KNO_3 and 2,4-D in order to reduce the fruit splitting of mandarin cv. 'Page'.

MATERIAL AND METHODS

Plant material and treatments

This experiment was performed in commercial mandarin cv. 'Page' orchards in the north of Iran during two 2016 and 2017 growing seasons. The orchard was located at the Iran Citrus and Subtropical fruits Research Center in Ramsar (the latitude of 36°90 N, the longitude of 50°65 E, 21 m altitude, 21ºC mean annual temperature and 1,200 mm rainfall per year). The 22-year-old trees were spaced 6×4 m (417 trees/ha). The soil texture was clay loam (26% sand, 43% silt, and 31% clay) and a pH of 7.1. The trees were managed according to standard local commercial practices, pruned annually, and water was supplied based on the demand due to evaporation. Tilling and mowing kept the alleyways mostly weed-free. Pest populations were kept under control following a recommended pest management program (Abbas and Fares 2009).

One hundred sixty-two uniform (in age, height, and vigor) trees were selected for preharvest KNO_3 (0, 1 and 2%) and 2,4-D (0, 50 and 100 mg/L) spray. Foliar application of treatments was done at three stages (full bloom, end of petal fall, and end of June drop) during two growing seasons. Treatments were established in two factorial randomized block design and each treatment was replicated thrice. Two trees served as a treatment unit. To prevent any sunburn injury, all sprays were done early in the morning (at 7 am) using a handheld sprayer until complete runoff. All the trees and the soil were managed based on standard cultural methods. Tilling and mowing kept the alleyways regularly weed-free. Fruits uniform in color and size and free from any visible injury or blemishes were harvested at the commercially mature stage for the next experiments.

Fruit splitting related characteristics

Twenty uniform and defect-free fruits from each replication were subjected to quantitative and qualitative

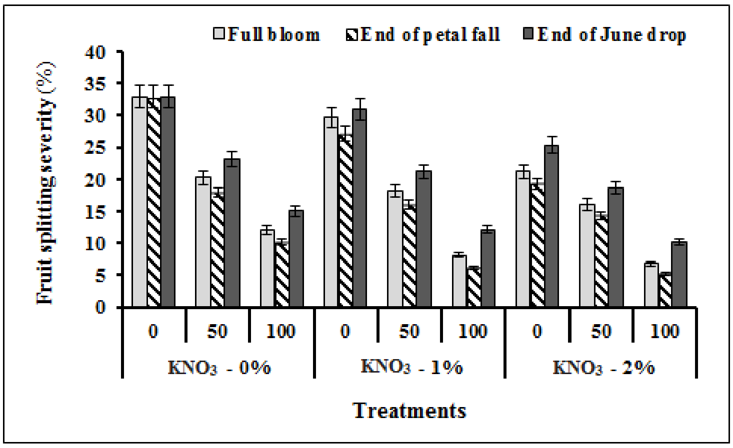

Fig. 1. Effect of foliar application of KNO_3 (0, 1 and 2%) and 2,4-D (0, 50 and 100 mg/L) at three stages (full bloom, end of petal fall, and end of June drop) on the fruit splitting severity of mandarin cv. 'Page'. The values are the means (n = 3) ± standard error. Means are the average of obtained data in two years (2016 and 2017)

analysis. Immediately fruit size was measured by a digital caliper (Digit-CAL. SI, TESA, Switzerland) and then peel firmness was determined on both parties of the fruit after elimination of the peel using a penetrometer (FT 327, Tokyo, Japan). To determine the severity of fruit splitting, the number of the split fruits on each tree was counted daily from the start of splitting until harvest. The split fruits were then removed from the tree. The number was compared with the total number of the picked fruits at harvest date to find the percentage of split fruits (severity) on each tree.

The β-galactosidase activity was evaluated according to the technique described in Moctezuma, Smith and Gross (2003), where *p*-nitrophenol-β-D-galactopyranoside (PNP-gal) was used as a substrate. PG activity was analyzed at 274 nm with a spectrophotometer (Shimadzu, UV-1800, Japan) based on Faize et al. (2003) methods, where polygalacturonic acid (1.5% w/v) was used as substrate.

Fruit biochemical traits

SSC, TA and SSC /TA were determined with juice extracted from 10 fruits from per replication. SSC was assayed with a digital refractometer (Euromex RD 635, Arnhem, Netherlands). TA expressed as percent citric acid was determined as titrating (0.1 M NaOH) methods using a digital titrometer (Shiri et al. 2011).

The vitamin C was determined by titration of 15 mL fruit juice with 2,6-dichlorophenol indophenol (DCIP) containing $NaHCO_3$ and finally expressed as mg ascorbic acid/100 g fresh weight (FW). TPC was assayed by the Folin–Ciocalteu method according to Singleton, Orthofer and Lamuela-Raventos (1999) at 765 nm, where gallic acid was used as a standard. TPC was expressed as milligram of gallic acid equivalent (mg GAE) per gram of fruit FW. TAC was measured as described by Brand-Williams, Cuvelier and Berset (1995) at 515 nm according to 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging method.

Statistical analysis

The experiment was conducted in two factorial randomized block design with three replications. The data were analyzed by the PROC ANOVA procedure by SAS software (ver. 9.1 2002–2003, SAS Institute, Cary, NC). The least significant difference (LSD) at $P \leq 0.05$ was calculated to compare the differences between means following a significant ANOVA effect. It should be noted that although the mandarin cv. 'Page' shows a little alternate bearing (mostly depends on climatic conditions and management programs such as fertilization and pruning methods), in the North of Iran, this cultivar had no alternate bearing under traditional management programs. In this regard, due to the main effect of year was not significant, the means are the average of obtained data in two years (2016 and 2017). Moreover, during means comparison, slicing was performed based on the foliar application times.

RESULTS

Fruit splitting related characteristics

The results showed that all fruit splitting related characteristics (fruit splitting severity, fruit size, peel firmness, the activity of β-galactosidase and PG) significantly affected by the simple and interaction effects of foliar application time and chemical treatments (KNO_3 and 2,4-D).

According to **Fig. 1**, treated fruits at the end of the petal fall stage had the lowest fruit splitting severity as compared with other times. Fruit splitting severity significantly reduced along with increasing the concentration of KNO_3 and 2,4-D treatments as at each application time the lowest fruit splitting severity was found in treated fruits with KNO_3 2% + 2,4-D 100 mg/L (**Fig. 1**).

Generally, the biggest fruits (72.11 mm) were obtained when fruits treated at the end of the petal fall stage (**Table 1**). Both KNO_3 and 2,4-D treatments

Table 1. Effect of foliar application of potassium nitrate (KNO_3) and 2,4-dichlorophenoxyacetic acid (2,4-D) at three stages (full bloom, end of petal fall, and end of June drop) on some physicochemical characteristics of mandarin cv. 'Page'.

KNO_3 (%)	2,4-D (mg/L)	Fruit size (mm)	Peel firmness (N)	SSC (ºBrix)	TA (mg/100g)	SSC/TA
			Full bloom			
0	**0**	65.7f*	3.46a	9.32fg	0.68d	13.70a
	50	68.9cd	3.40ab	9.39f	0.68d	13.81a
	100	68.2d	3.38b	9.37f	0.70cd	13.38b
1	**0**	67.5e	3.29c	9.56de	0.72c	13.28b
	50	69.9c	3.14e	9.67cd	0.76b	12.72c
	100	69.3c	3.23d	9.78c	0.77b	12.70c
2	**0**	68.4d	3.26cd	9.59d	0.73bc	13.14b
	50	72.3ab	3.07f	9.84b	0.81a	12.30e
	100	73.5a	2.85g	10.05a	0.80a	12.56d
Mean		**69.30B****	**3.23A**	**9.62B**	**0.74B**	**13.07A**
			End of petal fall			
0	**0**	65.7e	3.46a	9.32f	0.68e	13.70a
	50	70.9cd	3.25c	9.75cd	0.77c	12.66c
	100	71.5c	3.20cd	9.80c	0.82bc	11.95d
1	**0**	69.3d	3.32b	9.54de	0.71de	13.44b
	50	73.8b	3.11ef	10.11b	0.81bc	12.48c
	100	73.9b	3.13e	10.08b	0.85b	11.86d
2	**0**	71.8c	3.06f	9.60d	0.76cd	12.63c
	50	75.6a	2.70g	10.53ab	0.96a	10.97e
	100	76.5a	2.71g	11.26a	0.98a	11.49d
Mean		**72.11A**	**3.10C**	**10.00A**	**0.82A**	**12.35B**
			End of June drop			
0	**0**	65.7e	3.46a	9.32e	0.68d	13.70a
	50	67.2d	3.33b	9.40de	0.69d	13.62a
	100	68.3c	3.30b	9.55cd	0.75bc	12.73c
1	**0**	66.5de	3.21c	9.47d	0.74c	12.80c
	50	68.6c	3.19cd	9.81b	0.79b	12.42cd
	100	69.9b	3.04e	9.99ab	0.83a	12.04d
2	**0**	69.4b	3.16d	9.63c	0.73c	13.19b
	50	72.8a	2.89f	10.04a	0.84a	12.10d
	100	72.1a	2.82f	10.13a	0.85a	11.92e
Mean		**68.94B**	**3.16B**	**9.70B**	**0.77B**	**12.72AB**

* For each column means followed with the same lowercase letters are not significantly different at P ≤ 0.05 according to the LSD test. Slicing was performed based on orchard location and sampling time. It should be noted that slicing was performed based on the foliar application times.
** In each column, means followed with the same bold uppercase letters show the non-significant difference of the simple effect of foliar application time.
Means are the average of obtained data in two years (2016 and 2017).

enhanced fruit size and showed synergistic effects as the biggest fruits were found in KNO_3 2% + 2,4-D 100 mg/L treatment.

As shown in **Table 1**, treated fruits at the full bloom stage had the highest peel firmness (3.23 N) as compared with treated fruits at the end of June drop (3.16 N) and the end of the petal fall stage (3.10 N). Peel firmness reduced in response to KNO_3 and 2,4-D treatments as untreated fruits (controls) showed the highest peel firmness at all spraying times (**Table 1**).

PG activity of mandarin cv. 'Page' fruits showed the highest level (83.54 µg/min g FW) when fruits treated at the end of the petal fall stage (**Fig. 2**). KNO_3 and 2,4-D treatments enhanced PG activity, and also a synergistic effect was found between KNO_3 and 2,4-D treatments as the highest PG activity was recorded when fruits treated with KNO_3 2% + 2,4-D 100 mg/L (**Fig. 2**). Furthermore, according to **Fig. 3**, a negative correlation was revealed between increasing of PG activity and reducing of fruit splitting severity at each KNO_3 and 2,4-D concentrations. Fruits with higher PG activity showed lower fruit splitting severity.

The results indicated that treated fruits at the end of the petal fall stage had the highest β-galactosidase activity (129.18 µg/min g FW) as compared when mandarin cv. 'Page' fruits treated at full bloom (117.54 µg/min g FW) and at the end of June drop (99.71 µg/min g FW) stages (**Fig. 4**). At all spraying time, β-galactosidase activity increased in response to KNO_3 and 2,4-D treatments. Moreover, KNO_3 and 2,4-D treatments showed a synergistic effect when applied together as the highest β-galactosidase activity was obtained in KNO_3 2% + 2,4-D 100 mg/L treatment (**Fig. 4**). At all KNO_3 and 2,4-D treatments, a negative correlation was found between β-galactosidase activity and fruit splitting severity, as fruits with the highest β-

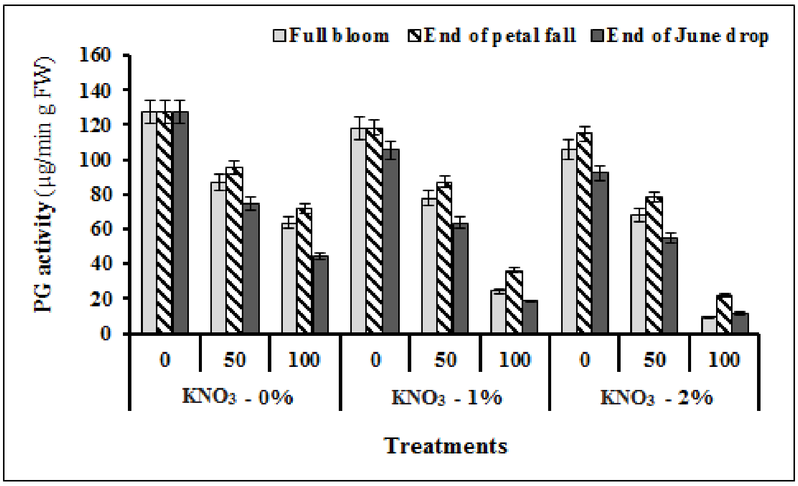

Fig. 2. Effect of foliar application of KNO_3 (0, 1 and 2%) and 2,4-D (0, 50 and 100 mg/L) at three stages (full bloom, end of petal fall, and end of June drop) on the PG enzyme activity of mandarin cv. 'Page'. The values are the means (n = 3) ± standard error. Means are the average of obtained data in two years (2016 and 2017)

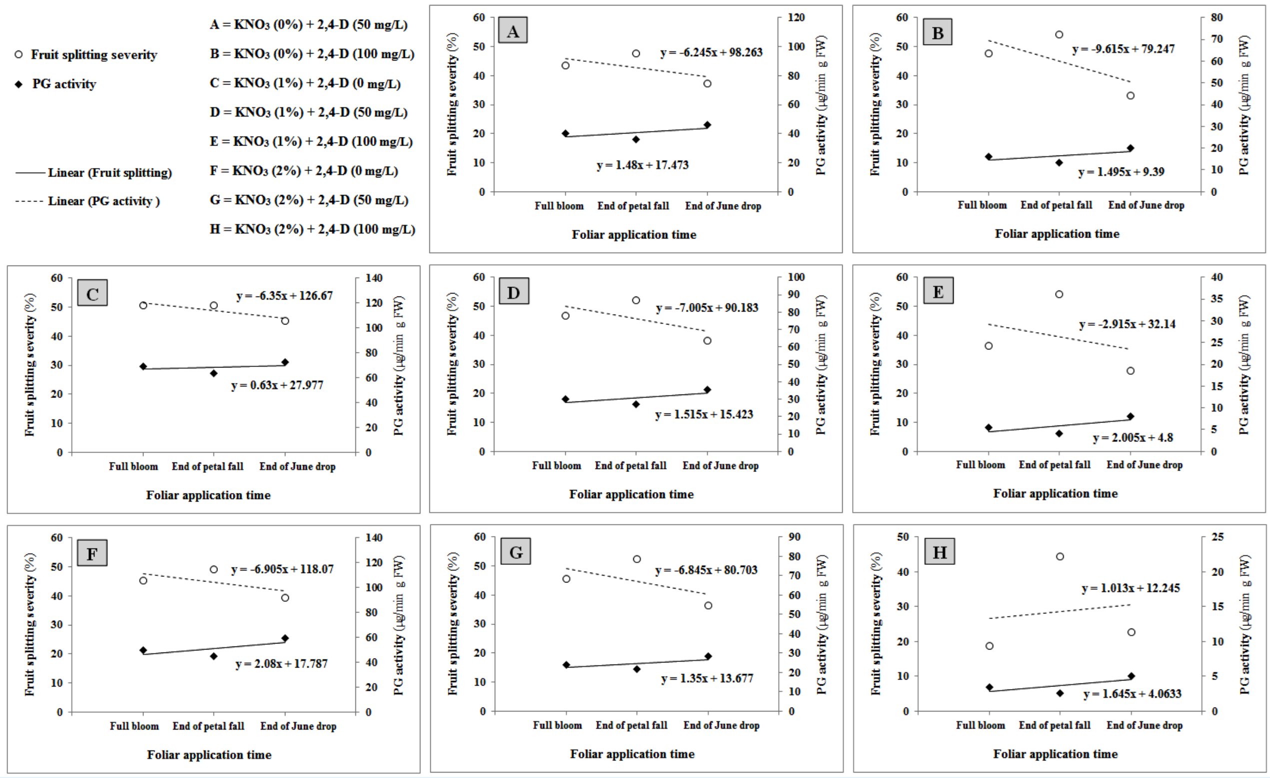

Fig. 3. Correlation between fruits splitting severity and PG enzyme activity at each KNO_3 and 2,4-D treatments (A-H)

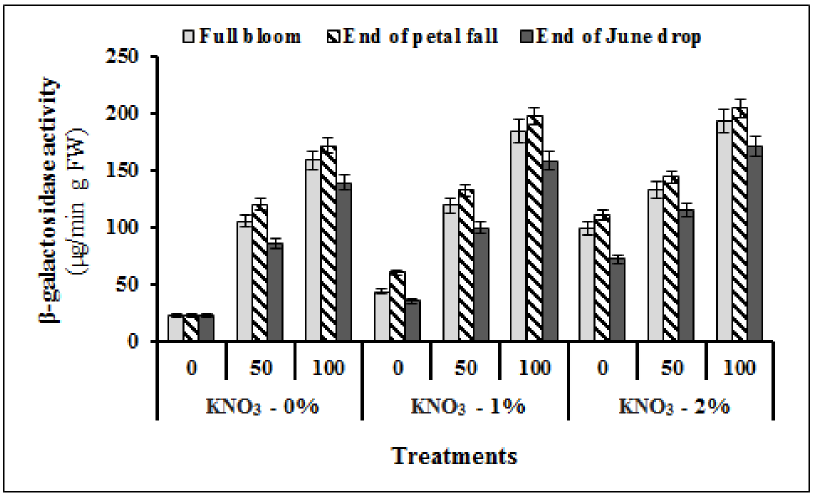

Fig. 4. Effect of foliar application of KNO_3 (0, 1 and 2%) and 2,4-D (0, 50 and 100 mg/L) at three stages (full bloom, end of petal fall, and end of June drop) on the β-galactosidase enzyme activity of mandarin cv. 'Page'. The values are the means (n = 3) ± standard error. Means are the average of obtained data in two years (2016 and 2017)

galactosidase activity had the lowest fruit splitting severity (**Fig. 5**).

Fruit biochemical traits

It was revealed that the highest SSC and TA content were obtained in treated fruits at the end of the petal fall stage (**Table 1**). Along with increasing the concentration of KNO_3 and 2,4-D treatments SSC and TA content gradually increased at all spraying times. Moreover, the synergistic effects of KNO_3 and 2,4-D treatments were found in treated fruits as the highest SSC and TA were recorded in KNO_3 2% + 2,4-D 100 mg/L treatment (**Table 1**).

According to **Table 1**, SSC/TA ratio changed according to the changes in SSC and TA content. The results mentioned that treated fruits at the full bloom stage had the highest SSC/TA ratio, whereas treated fruits at the end of the petal fall stage showed the lowest ratio (**Table 1**). Furthermore, KNO_3 and 2,4-D treatments reduced SSC/TA ratio at all spraying times.

It was found that foliar application of chemical treatments at the end of the petal fall stage produced

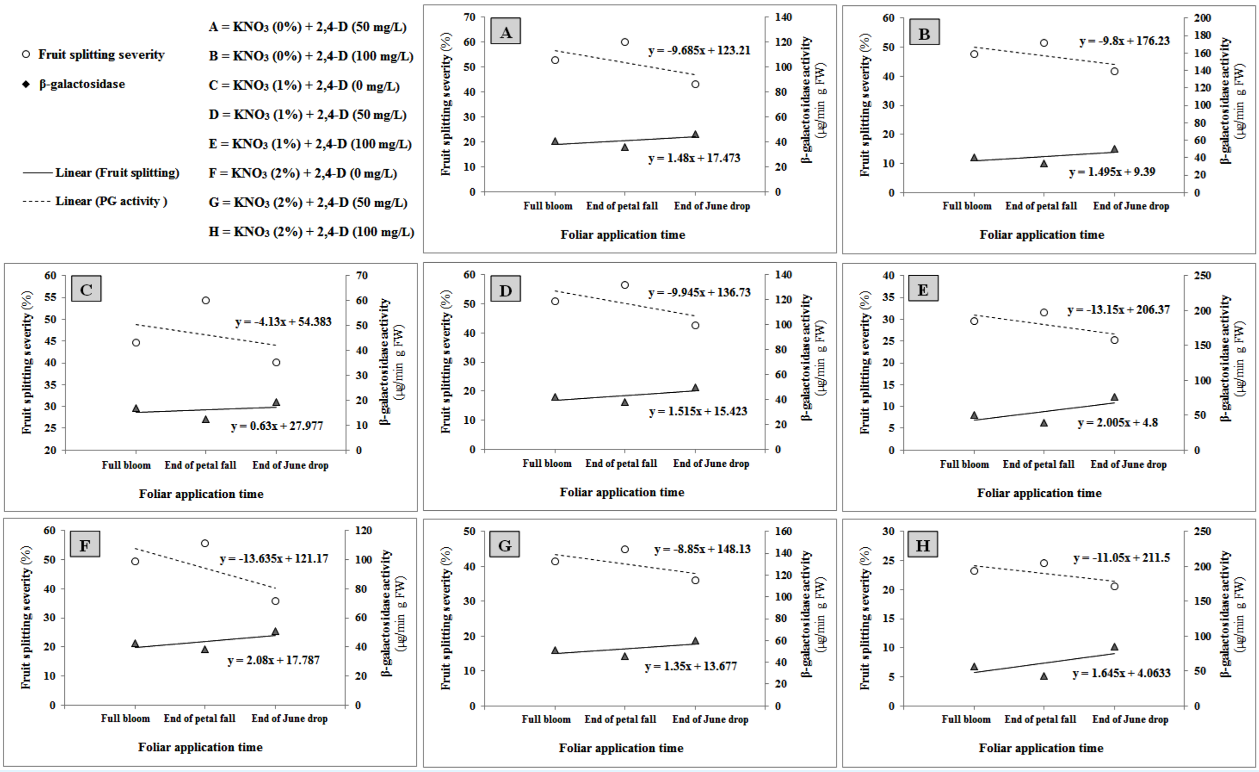

Fig. 5. Correlation between fruits splitting severity and β-galactosidase enzyme activity at each KNO3 and 2,4-D treatments (A-H)

mandarin cv. 'Page' fruits with the highest vitamin C content (29.11 mg/100g) (**Table 2**). While control fruits had the lowest vitamin C content, KNO_3 and 2,4-D treatments enhanced fruits vitamin C content as the highest content at all spraying times were obtained in treated fruits with KNO_3 2% + 2,4-D 100 mg/L.

As shown in **Table 2**, TPC significantly was higher in treated fruits at the end of the petal fall stage as compared with other spraying times. TPC significantly increased in response to KNO_3 and 2,4-D treatments. The synergistic effects of KNO_3 and 2,4-D treatments were found in treated fruits as the highest TPC was obtained in KNO_3 2% + 2,4-D 100 mg/L treatment (**Table 2**).

Our results indicated that mandarin cv. 'Page' treated fruits at the end of the petal fall stage had the highest TAC (44.60 %DPPHsc) as compared with treated fruits at the end of June drop (42.22 %DPPHsc) and full bloom (41.72 %DPPHsc) stages (**Table 2**). Generally, along with increasing the concentration of KNO_3 and 2,4-D treatments TAC gradually increased at all spraying times. Moreover, the synergistic effects of KNO_3 and 2,4-D treatments were found in treated fruits as KNO_3 2% + 2,4-D 100 mg/L treatment showed the highest TAC (**Table 2**).

DISCUSSION

It was found that spraying time significantly affected the fruit splitting severity and also other evaluated characteristics as treated fruits at the end of the petal fall stage had the lowest fruit splitting severity and better fruit quality as compared with treated fruits at the full bloom and the end of June drop stages. These results are in agreement with Stander (2013), who mentioned that in different mandarin fruit time of the foliar application of 2,4-D along with calcium (Ca) and K significantly affected fruit splitting and fruit size, as treated fruit at the drop stage, significantly reduced fruit splitting levels and produced bigger fruits. They also showed that later foliar applications of chemical treatments in January and February had no significant effect. Greenberg et al. (2006) indicated that the early spray of 2,4-D and NAA reduced mandarin cv. 'Nova' fruit splitting and enhanced fruit size, whereas the late spray did not affect fruit splitting. It was mentioned that spraying of 2,4-D significantly diminished mandarin cv. 'Nova' fruit splitting by enhancing peel force, but not peel thickness, which could have revealed a likely strengthening effect of the citrus fruit peel (Almela et al. 1994). Erner, Goren and Monselise (1976) concluded that the foliar application of KNO_3 in early spring significantly reduced fruit splitting in mandarin cv. 'Nova'.

Different responses of mandarin cv. 'Page' fruits to different foliar application time might be related to this case fact that the main stage of peel cells development in citrus fruit happens at the end of the petal fall stage, however, it depends on numerous factors like climate conditions, orchard location, the type of cultivar, etc.

Table 2. Effect of foliar application of potassium nitrate (KNO3) and 2,4-dichlorophenoxyacetic acid (2,4-D) at three stages (full bloom, end of petal fall, and end of June drop) on Vitamin C, total phenolic content (TPC) and total antioxidant capacity (TAC) of mandarin cv. 'Page'

KNO_3 (%)	2,4-D (mg/L)	Vitamin C (mg/100g)	TPC (mg/g)	TAC (%DPPHsc)
		Full bloom		
0	**0**	24.7d	30.8de	38.6de
	50	26.9bc	30.9de	39.0d
	100	26.8bc	31.6cd	39.2cd
1	**0**	25.0d	31.2d	38.9d
	50	27.4b	32.7bc	43.2b
	100	27.1b	33.5b	43.1b
2	**0**	26.3bc	31.9c	40.3c
	50	31.7a	33.4b	46.0a
	100	33.1a	36.1a	47.2a
Mean		**27.67B**	**32.46B**	**41.72B**
		End of petal fall		
0	**0**	24.7f	30.8ef	38.6ef
	50	27.0d	31.4de	39.7de
	100	27.4d	31.6d	39.5e
1	**0**	25.6ef	31.3e	39.3e
	50	27.8cd	33.5c	46.2c
	100	28.2c	35.1b	46.9c
2	**0**	27.1d	32.7cd	41.4d
	50	35.3b	37.3a	52.0b
	100	38.9a	39.0a	57.8a
Mean		**29.11A**	**33.63A**	**44.60A**
		End of June drop		
0	**0**	24.7ef	30.8de	38.6e
	50	25.3e	31.0d	40.2d
	100	26.5d	31.4cd	40.0d
1	**0**	26.2d	32.1bc	39.3de
	50	28.1c	32.9b	42.7b
	100	28.0c	33.0b	43.0b
2	**0**	26.0de	32.3bc	41.1c
	50	30.9b	33.0b	47.2a
	100	34.2a	35.8a	47.9a
Mean		**27.77B**	**32.48B**	**42.22B**

* For each column means followed with the same lowercase letters are not significantly different at P ≤ 0.05 according to the LSD test. Slicing was performed based on orchard location and sampling time. It should be noted that slicing was performed based on the foliar application times.
** In each column, means followed with the same bold uppercase letters show the non-significant difference of the simple effect of foliar application time.
Means are the average of obtained data in two years (2016 and 2017).

(Spiegel-Roy and Goldschmidt 1996, Guardiola 2000, Ladanyia 2010). Furthermore, Shiri et al. (2016 a,b) reported that spraying time during the growing season significantly influenced kiwifruit nutrition elements and also fruit quality characteristics at the harvest time and during storage time. This might be due to the difference in cell capacity to absorption and uses of various foliar-applied of nutritional elements during the growing season.

Our results indicated that the foliar application of KNO_3 and 2,4-D treatments significantly reduced fruit splitting severity (Figure 1), peel firmness and enhanced fruit size (**Table 1**) as compared with control. The positive effects of KNO_3 and 2,4-D treatments on the controlling of fruit splitting previously reported by Singh, Sharma and Awasthi (1993) in Kandhari and Beedana pomegranate, Almela et al. (1994) in mandarin cv. 'Nova', Greenberg et al. (2006) in mandarin cv. 'Nova', Stander (2013) in different cultivars of mandarin fruit, Juan and Jiezhong (2017) and Cronjé et al. (2014) in citrus fruits.

Natural and synthesized different auxin changes fruit growth during cell division and enlargement stage. Through the cell enlargement stage, auxin enhances photosynthesis and carbohydrate approachability inducing cell enlargement, change cell firmness, reduce splitting severity and also rises in final mandarin cv. Satsuma fruit size (Agusti et al. 1994). Raphael et al. (2007) found that spraying of auxin increased the carbohydrate content in the fruit and enhanced fruit size of Bing cherry. Modise et al. (2009) reported that 2,4-D treatment greatly enhanced the fruit weight by increasing fruit size in naval orange.

Additionally, K makes high osmotic pressure and turgor pressure, which can provide power for cell division, cell wall extension, cell expansion and finally, reduce fruit splitting (Alva et al. 2006, Marschner, 2012). Similarly, Ali et al. (2000) indicated that the use of K in spring or during the early fruit development stage can raise fruit peel development, enhance peel thickness, increase the fruit cracking resistance capacity, and diminish pre-harvest fruit splitting. Previous studies demonstrated that foliar spray of the nutrient perhaps reduced fruit split via rising peel elasticity and flexibility, as the increment of compounds and solutes such as K in the vacuole is needed for the osmotic potential required for cell expansion, enhancing cell wall elasticity (Chater and Garner 2018, Marschner 2012).

Our results revealed that PG and β-galactosidase activity of mandarin cv. 'Page' fruits significantly increased in response to KNO_3 and 2,4-D treatments (Figures 2 and 4). These results are in coincident with Mohamed et al. (2014), who concluded that spraying of some PGRs significantly changes the activities of the hydrolytic enzymes in date cv. 'Barhee'. Moreover, Marschner (2012) reported that K can influence the activity of different cell wall degradation enzymes. As regards a negative correlation between PG and β-galactosidase activity and fruit splitting severity (**Fig. 3 and 5**), it can conclude that in our study KNO_3 and 2,4-D treatments reduced fruit splitting severity and peel firmness by enhancing the activity of PG and β-galactosidase activity of mandarin cv. 'Page' fruits. Similar to our results, Moctezuma, Smith and Gross (2003) and Lu and Lin (2011) mentioned the correlation between the increase of PG and β-galactosidase activity and the decrease of the splitting.

Treated fruits with KNO_3 and 2,4-D treatments showed the highest SSC, TA (**Table 1**), vitamin C, TPC, and TAC (**Table 2**). Similarly, Baogang et al. (2008) revealed that 2,4-D biochemical compounds of mango fruits. Khandaker et al. (2012) indicated that 2,4-D treatment at different concentrations significantly enhanced enzyme activity, TPC and TAC in apple fruits.

Mohamed et al. (2014) revealed that PGRs treatments significantly changed the amount of TSS, acidity and antioxidant compounds of date cv. ‘Barhee’. Furthermore, it was mentioned that when pomegranate fruits treated with KNO_3 showed higher SSC and TPC as compared with untreated fruits (Chater 2015). Alva et al. (2006) concluded that citrus fruits with higher K content had higher SSC, organic acids, and vitamin C content. It was demonstrated that PGRs and nutrients elements had key roles in different plant growth processes, gene expression signaling, and some signal transduction pathways leading to activation of secondary metabolism ways, which can alter the amount of different biochemical compounds (Davies 1995, Marschner 2012).

CONCLUSION

Plant nutrition is one of the reliable methods to control fruit splitting and also improve fruit quality. Therefore, in the current study, we applied different concentrations of KNO_3 and 2,4-D at three stages on mandarin cv. ‘Page’ in two growing season (2016 and 2017). It was found that foliar spraying of chemical treatments at the end of the full bloom stage was more effective to reduced fruit splitting severity as well as enhancing fruit nutritional quality. Furthermore, fruit splitting severity significantly decreased in response to KNO_3 and 2,4-D treatments. Among chemical treatment, the combination of KNO_3 2% + 2,4-D 100 mg/L produced the biggest fruits with lowest fruit splitting severity, the highest β-galactosidase, and PG activity, SSC, TA, vitamin C, TPC, and TAC. Overall, hence this study is the first report about foliar application of chemical treatments to reduce of citrus fruit splitting in Northern Iran, therefore, the foliar application of KNO_3 2% + 2,4-D 100 mg/L especially at the end of the full bloom stage could be a recommended treatment in mandarin cv. ‘Page’ orchards.

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