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Foliar application of moringa leaf extract, potassium and zinc influence yield and fruit quality of 'Kinnow' mandarin

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ABSTRACT

'Kinnow' mandarin (*Citrus nobilis* L. × *Citrus deliciosa* T.) is the most important commercial citrus cultivar grown in Pakistan. Poor nutrient management practices in citrus orchards had significantly reduced its yield and fruit quality. Recent reports of *Moringa olifera* as a promising growth enhancer showed its potential for application in agriculture sector. Moringa leaf extract (MLE) is enriched with phytohormones, phenolics and minerals. Hence, present study was conducted to evaluate efficacy of MLE alone or in combination with zinc (Zn) (as $ZnSO_4$) and potassium (K) (as K_2SO_4) on 'Kinnow' mandarin during two consecutive years (2013–2015). In first experiment, trees were sprayed with 3% MLE, 0.6% $ZnSO_4$ and 0.25% K_2SO_4 alone and in combination with 3% MLE at fruit set stage (year-I); whilst, in second experiment trees were sprayed with 3% MLE at pre-mature stage and 3% MLE, 0.6% $ZnSO_4$ and 0.25% K_2SO_4 at fruit set stage (year-II). Data were collected regarding leaf nutrient and ascorbic acid contents, yield and fruit quality. Leaf nitrogen (N), phosphorous (P), K, calcium (Ca), manganese (Mn) and Zn were significantly increased with all treatments in both experiments. Combined application of MLE, K and Zn at fruit set stage in both experiments resulted in significantly lower fruit drop and higher fruit set, yield, fruit weight, juice weight, soluble solid contents (SSC), vitamin C, sugars, total antioxidants and total phenolic contents. Activities of SOD and CAT enzymes in fruit juice were significantly increased with 3% MLE application in both experiments. Conclusively, combined foliar application of 3% MLE, 0.6% $ZnSO_4$ and 0.25% K_2SO_4 at fruit set stage can be used effectively to improve leaf nutrient status, fruit yield and quality of 'Kinnow' mandarin trees.

1. Introduction

Citrus is the second most important fruit crop in the world after grapes and has tremendous economic and social impact on the society. Presently, it is cultivated in almost 125 countries of the world with 1155 million tones production (FAOSTAT, 2012). Pakistan is the 12th largest producer of citrus in the world with 199.4 thousand ha area and 2.29 million tones production (FAOSTAT, 2012). Among all fruits (mango, apple and dates etc.) produced in Pakistan, share of citrus is 40% (Khalid et al., 2012), and 'Kinnow' mandarin (*Citrus nobilis* L. × *Citrus deliciosa* T.) is the most preferred commercial fruit crop. Currently Pakistan is the largest producer of 'Kinnow' mandarin in the world (FAOSTAT, 2012; GOP, 2011).

Although citrus is the leading fruit crop of Pakistan, but there is vast difference between its average yield (12.78 tons ha^{-1}) and potential yield (18–20 t ha^{-1}) (Anonymous, 2006). Low productivity is attributed to various factors such as improper cultural practices like irrigation, pruning, thinning (Iglesias et al., 2007) and nutrient deficiency (N, K, P, Zn and Mn) (Khan et al., 2009). Properly nourished citrus trees have been reported to perform well and produce better quality fruits (Ioannis et al., 2004). Therefore, to improve the health and performance of citrus, trees should be supplied with balanced nutrients (N, K, P, Zn and Mn) (Khan et al., 2009) along with growth promoter such as salicylic acid (Ashraf et al., 2012); growth hormones (auxins, gibberelins, cytokinins) (Saleem et al., 2008; Khalid et al., 2012) and seaweed (Selvam and Sivakumar, 2014).

Recently discovery of moringa (*Moringa oleifera*) as a biostimulants for agricultural crops has gained importance (Rady and Mohamed, 2015). The Moringa tree known as the "Miracle Tree" is native to northern India, Pakistan and Nepal. It is used for multiple purposes such as fresh vegetable, livestock fodder, green manure, biogas, medicine, bio pesticide, and seed priming agent (Fuglie, 1999). Leaves of *Moringa oleifera* are rich in phytohormones *i.e.*, zeatin (cytokinins) and auxins in addition to other growth enhanc-

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ing compounds like ascorbate, phenolics and minerals [Ca, K, Zn and iron (Fe)]. Balanced composition of nutrients, antioxidants and phytohormones in moringa leaves makes it an excellent natural plant growth promoter (Yasmeen, 2011). Research work on MLE as a growth stimulator to increase the plant growth and productivity had already been reported on various field crops such as Maize (*Zea mays*) (Ali et al., 2011), sugarcane (*Sacchrum officinarum*), coffee (*Coffea arabica*) (Foidl et al., 2001), black gram (*Vigna mungo*) (Makkar and Becker, 1996) and sacred basil (*Ocimum sanctum*) (Prabhu et al., 2010). MLE increased the fruit size and sugar content when sprayed to cantaloupe (*Cucumis melo* var. cantalupensis) and soya bean (*Glycine max*) (Foidl et al., 2001). Foliar spray of MLE along with soil application of FYM increased the N, P, and K content of *Solanum nigrum* leaves (Sivakumar and Ponnusami, 2011). Thus, foliar application of MLE has potential to improve yield and quality of various crops.

Application of K is not given much importance by local Kinnow growers; although K is removed by citrus fruit more than any other macronutrient (Alva and Tucker, 1999). It is involved in several physiological processes like osmoregulation, cell division, growth, neutralization of organic acids, formation of starch and sugar, synthesis of protein, transport of sugars and photo assimilates into sink (Liu et al., 2000). It had been reported that foliar application of K improved fruit size, colour (Tiwari, 2005) and acidity (Alva et al., 2006) of citrus fruits. Lester et al. (2010) also found increased yield, sugars and ascorbic acid contents in sweet orange (*Citrus sinensis* Osbeck.) and mandarin (*Citrus reticulate* Blanco.) fruits through foliar application of K.

Due to high soil pH (8–8.5), micro nutrient deficiency especially Zn, is often observed in Kinnow orchards. Zn is considered as essential micronutrient, involved in various biological processes of plants (Broadley et al., 2007) including synthesis of tryptophan (precursor of indole acetic acid), amino acid, protein, carbohydrates/starch and act as a co-factor for many enzymes (Marschner, 1996). Previously, foliar application of Zn significantly improved leaf nutrient content (N, P, K, Zn and Mn), production and physico-biochemical quality of 'Washington Navel' oranges (Dawood et al., 2001), 'Balady' (El-Baz, 2003) and 'Kinnow' (Razzaq et al., 2013) mandarins. Moreover, combined foliar application of Zn and K improved N, P, K and Zn level in 'Washington Navel' leaves (Hafez and El-Metwally, 2007) and effectively controlled fruit drop, increased yield, juice volume, SSC and vitamin C in 'Kinnow' mandarins (Ashraf et al., 2012). Hence, the foliar application of K and Zn alone or in combination can improve the yield and quality citrus fruit.

Since, biostimulants are reported to help in nutrients uptake or efficiency. It is important to study the effect of combine application of MLE, K and Zn on citrus. To best of our knowledge no research has been reported on foliar application of MLE along with K and Zn affecting yield and fruit quality of 'Kinnow' mandarin. MLE with its growth promoting properties can be a good substitute to expensive growth stimulators such as seaweed extract, algae extract and synthetic phytohormones. Therefore, it was hypothesized that foliar application of MLE, K and Zn alone or in combination can improve the growth, production and fruit quality of 'Kinnow' mandarin. Hence, the present research work was conducted to evaluate the effect of foliar application of MLE, K and Zn alone or in combination on nutrient uptake, yield and quality of 'Kinnow' mandarin fruit.

2. Material and methods

2.1. Plant material

To find out the effect of foliar application of MLE, K and Zn, two experiments were carried out during year 2013-15 in the Experimental Fruit Orchard Square # 9, Institute of Horticultural Sciences, University of Agriculture, Faisalabad (31°25′N; 73°09′E) on 13 years old 'Kinnow' mandarin trees. Trees budded on Rough Lemon (*Citrus jambhari* L.) rootstock were grown at 6.1 × 6.1 m distance in rows and between rows. Healthy, uniform size, disease free trees were selected for the experiment and subjected to similar cultural practices like weeding, recommended fertilizer application [*i.e.,* 1000 g N: 500 g P: 500 g K (excluding treatment)], irrigation, insect pest control and pruning.

2.2. Treatments

First experiment includes treatments *viz.,* T1 = control; T2 = 3% MLE at pre mature stage; T3 = Zn [0.6% zinc sulphate ($ZnSO_4$)] at fruit set stage; T4 = K [0.25% potassium sulphate (K_2SO_4)] at fruit set stage and T5 = 3% MLE + 0.25% K (K_2SO_4) + 0.6% Zn ($ZnSO_4$) at fruit set stage. MLE was extracted according to the method illustrated by Yasmeen (2011). Surfactant (0.01% Tween 20) was added in all treatments. Foliar spray was done early in the morning (at 7 am) using a hand held sprayer until complete run off.

The experiment was laid out according to Randomized Complete Block Design (RCBD); where, single tree was taken as an experimental unit replicated four times. Leaf nutrient (N, P, K, Ca, Zn, Mn and Fe) and ascorbic acid contents were analyzed after 15 days of foliar application of treatments. Reproductive growth (fruit set, fruit drop, number of fruits, number of marketable and unmarketable fruits, fruit yield) was determined during various fruit growth stages. Physical (fruit size, fruit weight, peel thickness, peel weight, seed weight, seed number, pulp weight, juice weight, and peel: pulp ratio) and biochemical fruit quality [SSC, titrateable acidity (TA), SSC:TA ratio, vitamin C, total sugars, non-reducing sugars, reducing sugars, total antioxidants, total phenolic content (TPC), antioxidative enzymes {superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT)}] parameters were determined at harvest.

The second experiment was repetition/confirmation of best treatments of first experiment and includes treatment *viz.,* T1 = control; T2 = 3% MLE at pre mature stage and T3 = 3% MLE + 0.25% K (K_2SO_4) + 0.6% Zn ($ZnSO_4$) at fruit set stage. Experimental layout and parameters studied were similar as in first experiment.

2.3. Leaf analysis

For determination of leaf mineral contents (N, P, K, Ca, Fe, Zn and Mn), fifty 5–7 month old leaves were selected from non-fruiting terminals of spring growth cycle at random. Leaf samples were washed with ordinary detergent, rinsed well with distilled water and dried in oven at 65 °C for 48 h. After drying, leaves were grinded into fine powder using electric grinder. To determine total N in leaves micro kjeldhal apparatus was used following method illustrated by Khan et al. (2015) and N was expressed as percent (%).

To estimate leaf P, K, Ca, Fe, Zn and Mn wet digestion method was used as explained by Razzaq et al. (2013). In a 100 mL beaker, 1 g leaf sample and 10 mL of tri acid mixture [nitric acid (HNO_3): per chloric acid ($HClO_4$): sulphuric acid (H_2SO_4); 5:2:1] were added. The beaker was covered with watch glass and allowed to stand for at least 4 h until all the initial reaction subsided. The beaker was transferred to hot plate and heated vigorously until colourless solution resulted and volume was reduced to 1.5 mL, then it was removed, cooled and volume was made up to 100 mL by adding distilled water. P was determined on visible spectrophotometer (GmbH, Geesthacht, Germany); while, K and Ca were determined on flame photometer (Sherwood Scientific Ltd., Cambridge, UK) and were expressed as %. Metal elements (Zn, Mn and Fe) were determined on atomic absorption spectrophotometer (2-8200 Series Polarized

Zeeman, Hitachi, Kyoto, Japan) by using specific lamp for specific nutrient and were expressed as mg/kg.

Ascorbic acid contents in leaves were determined by using 10 g of fresh leaves, and extraction was done as illustrated by Yasmeen (2011). Ten mL of leaf extract was taken in a 100 mL volumetric flask, and volume was made up to mark by adding 0.4% oxalic acid. From the prepared solution, 5 mL of aliquot was taken and titrated against 2, 6 dichlorophenolindophenol dye, to light pink colour end point and was expressed as mg/100 g (Ullah et al., 2012).

2.4. Reproductive growth

To study the effect of experimental treatments on the reproductive growth, four branches of two inches diameter from all four sides (north, south, east and west) on the experimental tree were tagged. Data regarding reproductive behaviour including fruit set, fruit drop and yield were taken at different growth stages during fruit development. Fruit set and fruit drop were calculated as explained by Saleem et al. (2008) and average values were expressed as %, respectively. At harvest, average yield including total weight (kg) and total number of fruit per experimental trees were calculated. Total number of marketable (more than 8 cm diameter) and unmarketable fruit (less than 8 cm diameter) were also counted per experimental tree and their respective percentages were calculated by dividing the number of marketable/unmarketable fruit to total number of fruit and multiplied by hundred (Khan et al., 2009).

2.5. Physical fruit quality

To determine physical fruit quality, 10 fruit per experimental tree were harvested from all sides of the 'Kinnow' mandarin trees at random. Fruit size was calculated by multiplying the diameter and length of 10 selected fruit with a digital vernier caliper and expressed as cm^2. Average fruit weight was determined by weighing 10 fruit on digital electric balance and expressed as g. Peel thickness was measured with digital Vernier caliper and was expressed as mm. Peel weight, pulp weight, seed weight and juice weight was calculated on electric balance and their averages were calculated and expressed as g. Peel: pulp ratio was calculated by dividing the peel weight with their respective pulp weight.

2.6. Biochemical fruit quality

Biochemical fruit quality was determined from the juice extracted from 10 fruit. The SSC was estimated using digital refractometer (ATAGO, RX 5000, Tokyo, Japan) and was expressed as °Brix. TA was determined by titrating 5 mL of juice against 0.1 N NaOH and expressed set %. SSC: TA ratio was calculated by dividing the SSC with their respective TA (Khan et al., 2009). Vitamin C contents (mg/100 g) were determined as described earlier by Ullah et al. (2012). Total sugars, reducing sugars and non-reducing sugars were determined according to the method explained by Khan et al. (2009) and were expressed as %. Total phenolic contents were determined from fruit juice using method reported by Ainsworth and Gillespie (2007) with few modifications as described by Ullah et al. (2012) using a standard curve of gallic acid at 0.02–0.1 mg/ mL concentrations and were expressed as gallic acid equivalents (GAE) mg/100 mL fresh weight (FW). Total antioxidants were determined following the procedure outlined by Razzaq et al. (2013), and expressed as % inhibition. For determination of fruit antioxidative enzymes, frozen fruit juice samples (1 g) were homogenized with 2 mL phosphate buffer (pH 7.2) at room temperature and then centrifuged at 10,000 × g for 5 min at 4 °C. The supernatant was collected and used for determination of antioxidative enzymes [catalase (CAT, EC 1.11.1.6), peroxidase (POD, EC 1.11.1.7) and superoxide dismutase (SOD, EC1.15.1.1)] and were expressed as U/mg protein (Razzaq et al., 2013).

2.7. Statistical analysis

The data were statistically analyzed using MSTAT-C computer software (Michigan State University, East Lansing, MI, USA). ANOVA (analysis of variance) were used to test the overall significance of data, while the LSD test (least significant difference) was used to compare treatment means at $P \leq 0.05$ (Steel et al., 1997). Pearson correlations were performed using same software to determine the relationship between nutrient content in leaves and reproductive growth at $P \leq 0.05$.

3. Results

3.1. Leaf nutrient contents

Leaves macronutrient contents were significantly ($P \leq 0.05$) increased after foliar application of MLE alone or in combination with K and Zn. Highest N contents were found in trees treated with combine application of 3% MLE, 0.6% $ZnSO_4$ and 0.25% K_2SO_4 at fruit set stage both in first (2.34%) and second year (2.23%) experiments. The increase was 1.35-fold and 1.42-fold higher than control trees in both experiments, respectively (Table 1). Also, leaf N contents exhibited significant ($P \leq 0.05$) positive correlations with leaf P (r = 0.75), K (r = 0.46), Ca (r = 0.7), Zn (r = 0.56), Mn (r = 0.67) and Fe (r = 0.53) contents (Table 2). P level in leaves was increased in all treated trees, as compared with control trees. Maximum increase in P contents was recorded in leaves treated with 3% MLE at premature stage in both experiments, which was 1.09-fold and 1.07-fold higher than control trees, respectively. Furthermore, leaf P level showed positive significant ($P \leq 0.05$) correlations with leaf K (r = 0.51), Ca (r = 0.54) and Mn (r = 0.58) contents (Table 2). Leaf K contents were significantly increased with combined foliar application of 3% MLE, 0.6% $ZnSO_4$ and 0.25% K_2SO_4 at fruit set stage, about 1.89-fold and 1.52-fold higher as compared with control trees, in both year experiments, respectively. Moreover, leaf K contents showed significant ($P \leq 0.05$) positive correlations with leaf Ca (r = 0.49), Zn (r = 0.52) and Mn (r = 0.74) contents (Table 2). Maximum Ca (3.75%) contents in 'Kinnow' mandarin leaves were found with combined application of 3% MLE, 0.6% $ZnSO_4$ and 0.25% K_2SO_4 at fruit set stage in first year experiment. While, in second year experiment, highest leaf Ca concentration (4.26%) was recorded in trees treated with 3% MLE at premature stage (Table 1). Also, leaf Ca contents showed significant ($P \leq 0.05$) positive correlation with leaf Zn (r = 0.4), Mn (r = 0.68) and Fe (r = 0.48) concentration (Table 2).

The results revealed that micronutrient contents (Zn and Mn) were significantly improved in 'Kinnow' mandarin leaves after foliar application of MLE K and Zn (Table 1). Zn concentration in 'Kinnow' mandarin leaves were significantly ($P \leq 0.05$) increased with combined foliar application of 3% MLE, 0.6% $ZnSO_4$ and 0.25% K_2SO_4 at fruit set stage in both experiments. Increase in leaf Zn contents (27.28 mg/kg) during first year experiment was 1.34-fold; while, in second year experiment (27.4 mg/kg) it was 1.14-fold higher than control trees, respectively. Pearson correlation showed that leaf Zn contents were significantly ($P \leq 0.05$) positively correlated with Mn contents (r = 0.53) and non-significantly with Fe contents in leaves (r = 0.24) (Table 2). Similarly, highest Mn concentration was found in leaves treated with 3% MLE + 0.6% $ZnSO_4$ + 0.25% K_2SO_4 at fruit set stage *i.e.*, 24.28 mg/kg and 23.37 mg/kg in first and second year experiments, respectively. However, no significant change was observed in Fe contents of 'Kinnow' mandarin leaves during the first year experiment; while, in second year experiment leaf Fe contents were signifi-

Table 1
Effect of foliar application of moringa leaf extract (MLE), zinc sulphate ($ZnSO_4$) and potassium sulphate (K_2SO_4) on leaf nutrient content of 'Kinnow' mandarin trees.

Treatments	N (%)	P (%)	K (%)	Ca (%)	Zn (mg/kg)	Mn (mg/kg)	Fe (mg/kg)
	Year I						
Control	1.7d	0.07b	0.38c	3b	20.34d	21.25d	90.5
3% MLE	2.17ab	0.12a	0.52b	3.65a	23.42bc	23.25b	89.8
0.6% $ZnSO_4$	2.1bc	0.1a	0.5b	3.5ab	26.93a	22.62bc	92.46
0.25% K_2SO_4	1.96c	0.11a	0.65a	3.5ab	24.22b	24.25a	86.81
3% MLE + 0.6% $ZnSO_4$ + 0.25% K_2SO_4	2.31a	0.11a	0.72a	3.75a	27.28a	24.28a	89.24
LSD ($P \leq 0.05$)	0.15	0.01	0.08	0.52	1.32	0.83	NS
	Year II						
Control	1.56b	0.08b	0.42c	3.32b	23.87b	21b	68.75b
3% MLE	1.83ab	0.11a	0.51b	4.26a	26.68a	23.25a	77.53a
3% MLE + 0.6% $ZnSO_4$ + 0.25% K_2SO_4	2.23a	0.1a	0.66a	3.67ab	27.4a	23.37a	77.4a
LSD ($P \leq 0.05$)	0.39	0.06	0.12	0.28	0.49	1.06	2.18

Means within a column followed by different letters are significant at $P \leq 0.05$. NS represents not significant.

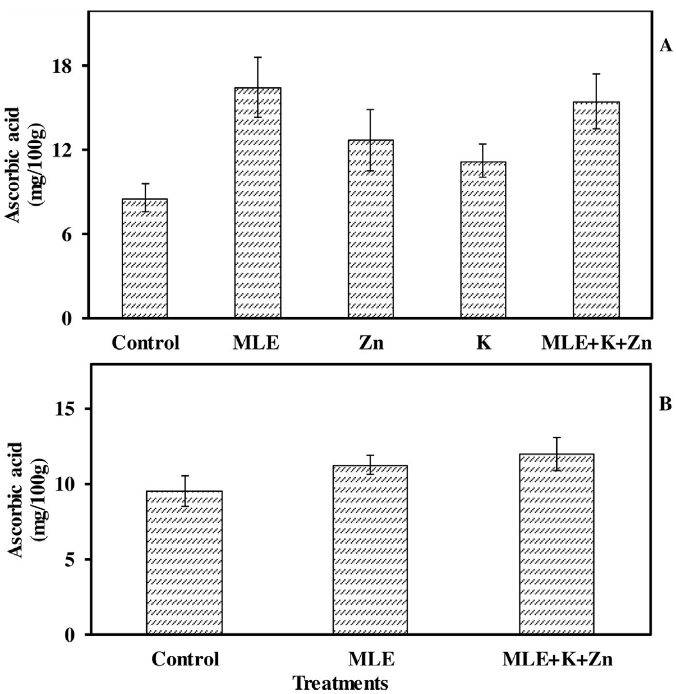

Fig. 1. Effect of exogenous application of 3% MLE (moringa leaf extract) alone or in combination with 0.6% $ZnSO_4$ and 0.25% K_2SO_4 on the ascorbic acid contents of 'Kinnow' mandarin leaves in first year (A) and second year (B) experiments, respectively. Vertical bars represent the ± SE of the means. n = 4 replicates.

cantly increased with foliar application of 3% MLE at premature stage (77.53 mg/kg) (Table 1).

3.2. Leaf ascorbic acid contents

Leaf ascorbic acid contents were significantly ($P \leq 0.05$) increased in all treatments compared to control trees (Fig. 1). In first year experiment, maximum ascorbic acid contents (17 mg/100 g) were recorded in trees sprayed with 3% MLE at premature stage about 1.63-fold higher than control (Fig. 1A). Whereas, in second year experiment, combined application of 3% MLE, 0.6% $ZnSO_4$ and 0.25% K_2SO_4 at fruit set stage showed about 1.33-fold increase in ascorbic acid contents of 'Kinnow' mandarin leaves, as compared with control trees (Fig. 1B). Also, the ascorbic acid contents in 'Kinnow' mandarin leaves exhibited positive significant ($P \leq 0.05$) correlations with leaf nutrient contents i.e., N ($r = 0.82$), P ($r = 0.48$), K ($r = 0.42$), Ca ($r = 0.77$), Zn ($r = 0.48$) and Mn ($r = 0.57$) (Table 2).

Table 2
Pearson correlation analysis of relationship between leaf nutrient and ascorbic acid contents of 'Kinnow' mandarin leaves after foliar application of moringa leaf extract (MLE), zinc sulphate ($ZnSO_4$) and potassium sulphate (K_2SO_4) (n = 24).

	N	P	K	Ca	Zn	Mn	Fe
P	0.75**						
K	0.46*	0.51*					
Ca	0.7**	0.54*	0.49*				
Zn	0.56*	0.31	0.52*	0.4*			
Mn	0.67**	0.58*	0.74**	0.69**	0.53*		
Fe	0.53*	0.33	0.04	0.48*	0.24	0.35	
Ascorbic acid	0.82***	0.48*	0.45*	0.77***	0.48*	0.57*	0.39

N = Nitrogen (%); P = Phosphorus (%); K = Potassium (%); Ca = Calcium (%); Zn = Zinc (mg/kg); Mn = Manganese (mg/kg); Fe = Iron (mg/kg).
* Indicate significant correlations at $P \leq 0.05$.
** Indicate significant correlations at $P \leq 0.01$.
*** Indicate significant correlations at $P \leq 0.001$.

3.3. Reproductive growth

Treated trees showed significant ($P \leq 0.05$) increase in fruit set and decrease in fruit drop as compared with control trees during both experiments (Fig. 2). Fruit set percent was increased in trees treated with combined foliar application of 3% MLE + 0.6% $ZnSO_4$ + 0.25% K_2SO_4 at fruit set stage *i.e.*, 1.48-fold and 1.29-fold higher than control trees in first and second year experiments, respectively. Also, fruit set % exhibited negative correlation with fruit drop ($r = -0.02$) and significant ($P \leq 0.05$) positive correlation with yield (kg) ($r = 0.49$), total number of fruit ($r = 0.66$) and number of marketable fruit ($r = 0.78$) (Table 4). Minimum fruit drop in first year experiment was recorded in trees treated with 3% MLE + 0.6% $ZnSO_4$ + 0.25% K_2SO_4 at fruit set stage (83%), as compared with maximum fruit drop (91%) in control trees (Fig. 2A). In second year experiment, fruit drop was minimized by foliar application of 3% MLE (82%) in 'Kinnow' mandarin trees (Fig. 2B). Also, fruit drop (%) in 'Kinnow' mandarin trees exhibited negative correlation with yield (kg) ($r = -0.1$), total number of fruit ($r = -0.01$), number of marketable fruit ($r = -0.11$) and positive correlation was found with number of unmarketable fruit ($r = 0.22$) (Table 4)

Fruit yield (weight and number of fruits) were significantly ($P \leq 0.05$) increased with foliar application of 3% MLE + 0.6% $ZnSO_4$ + 0.25% K_2SO_4 at fruit set stage in both experiments (Table 3). During first experiment, 3% MLE + 0.6% $ZnSO_4$ + 0.25% K_2SO_4 application at fruit set stage increased the yield by 65%, as compared with untreated trees. In second experiment, 3% MLE alone and in combination with 0.6% $ZnSO_4$ and 0.25% K_2SO_4 significantly increased yield by 51.9% and 52.09%, respectively in contrast to control trees (Table 3). Moreover, highly significant ($P \leq 0.05$) positive correlations were found between yield (kg) and total number of fruit ($r = 0.85$) and number of marketable fruit ($r = 0.81$) (Table 6). Sim-

Table 3
Effect of foliar application of moringa leaf extract (MLE), zinc sulphate ($ZnSO_4$) and potassium sulphate (K_2SO_4) on 'Kinnow' mandarin fruit yield.

Treatments	Yield		Marketable fruit		Unmarketable fruit	
	Fruit weight (kg)	Fruit number (No.)	(No.)	(%)	(No.)	(%)
	Year I					
Control	54.67c	327c	231c	70b	96	29a
3% MLE	81.45ab	492b	396bc	80a	95	19b
0.6% $ZnSO_4$	67.99bc	476b	369bc	77ab	107	22ab
0.25% K_2SO_4	72.38bc	484b	385b	79ab	99	20b
3% MLE + 0.6% $ZnSO_4$ + 0.25% K_2SO_4	81.66a	531a	444a	83a	87	16b
LSD ($P \leq 0.05$)	9.04	38.58	28.9	8.47	NS	8.44
	Year II					
Control	16.84b	148.25b	107b	71.18b	41.25	28.8a
3% MLE	32.92a	280.75a	247.75a	88.31a	33	11.68b
3% MLE + 0.6% $ZnSO_4$ + 0.25% K_2SO_4	32.09a	266.25a	233.25ab	87.72a	33	12.27b
LSD ($P \leq 0.05$)	5.96	46.77	39.97	6.31	NS	6.32

Means within a column followed by different letters are significant at $P \leq 0.05$. NS represents not significant.

Table 4
Pearson correlation analysis of reproductive growth parameters of 'Kinnow' mandarin trees after foliar application of moringa leaf extract (MLE), zinc sulphate ($ZnSO_4$) and potassium sulphate (K_2SO_4) (n = 24).

	Fruit drop (%)	Fruit set (%)	Yield (kg)	Number of fruits	Number of marketable fruits
Fruit set	−0.02				
Yield	−0.1	0.49*			
Number of fruits	−0.01	0.66**	0.85***		
Number of marketable fruits	−0.11	0.78**	0.81***	0.9***	
Number of unmarketable fruits	0.22	−0.29	0.05	0.16	−0.27

* Indicate significant correlations at $P \leq 0.05$.
** Indicate significant correlations at $P \leq 0.01$.
*** Indicate significant correlations at $P \leq 0.001$.

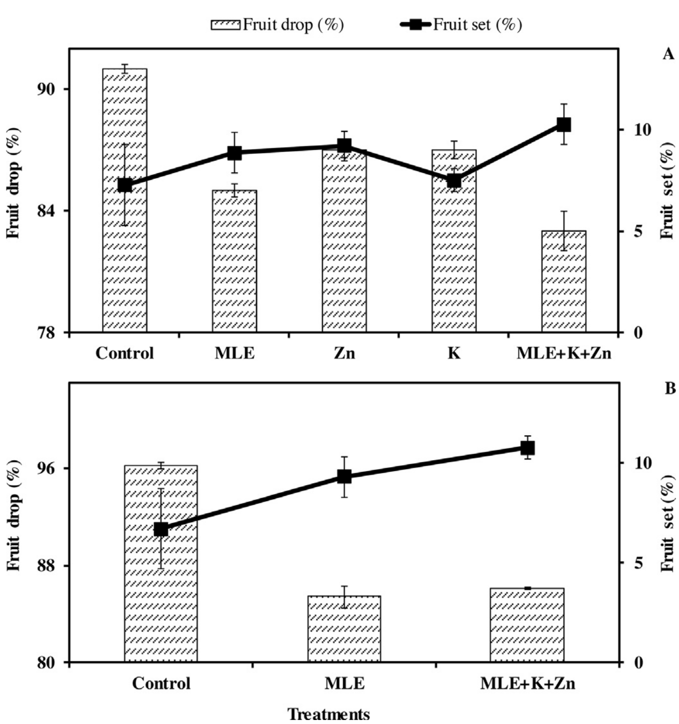

Fig. 2. Effect of exogenous application of 3% MLE (moringa leaf extract) alone or in combination with 0.6% $ZnSO_4$ and 0.25% K_2SO_4 on fruit set (%) and fruit drop (%) of 'Kinnow' mandarin trees during first year (A) and second year (B) experiments, respectively. Vertical bars represent the ± SE of the means. n = 4 replicates.

ilarly, total numbers of fruit per tree (531) were increased with foliar application of 3% MLE + 0.6% $ZnSO_4$ + 0.25% K_2SO_4 at fruit set stage in first experiment. In second experiment, maximum number of fruit per tree were found in trees treated with 3% MLE alone (280) or in combination with 0.6% $ZnSO_4$ and 0.25% K_2SO_4 (266) (Table 3). Maximum number of marketable fruit (444) was found in trees treated with 3% MLE + 0.6% $ZnSO_4$ + 0.25% K_2SO_4 during first year experiment, while in second year experiment with 3% MLE at premature stage treatment (247.75). Percentage of marketable fruit was highest in trees treated with 3% MLE + 0.6% $ZnSO_4$ + 0.25% K_2SO_4 at fruit set stage in first experiment (83%) and with 3% MLE at premature stage in second experiment (88.31%) (Table 3). Number of unmarketable fruit was not significantly affected by any treatment in both the experiments although the percent of unmarketable fruit was significantly higher in control trees for both experiments *i.e.*, 29% and 28.8%, respectively (Table 3).

3.4. Physical fruit quality

Average fruit weight was significantly ($P \leq 0.05$) increased in 'Kinnow' mandarin fruit harvested from the trees treated with MLE alone or in combination with K and Zn during both years experiments (Table 5). However, no significant change was found in fruit size, seed number and peel thickness during first year experiment. In second year experiment, maximum fruit size (461 cm^2) was found in fruit harvested from the trees treated with 3% MLE at premature stage. Minimum seed numbers (13.5) were exhibited in fruit harvested from the trees treated with combined application of 3% MLE, 0.6% $ZnSO_4$ and 0.25% K_2SO_4 at fruit set stage. No significant change was observed in peel weight during both years' experiments. Foliar application of 3% MLE at premature stage during second year experiment reduced fruit peel thickness by 1.33-fold, as compared with fruit harvested form treated trees (Table 5). Similarly, seed weight showed no significant difference among treatments during both years' experiments. Peel: pulp ratio was also not significantly affected by any treatment in first year experiment, while in second year experiment, minimum peel: pulp ratio was found in fruit treated with 3% MLE alone (0.7) or in combination with 0.6% $ZnSO_4$ and 0.25% K_2SO_4 (0.88) (Table 5).

Table 5
Effect of foliar application of MLE, zinc sulphate ($ZnSO_4$) and potassium sulphate (K_2SO_4) on physical quality of 'Kinnow' mandarin fruit.

Treatments	Fruit size (cm^2)	Fruit weight (g)	Seed weight (g)	Seed number	Peel weight (g)	Peel thickness (mm)	Peel:pulp ratio
	Year I						
Control	340	236b	1.24	16	20	4.02	0.33
3% MLE	413	278a	0.8	15.5	18	4.08	0.25
0.6% $ZnSO_4$	389	237b	1.04	14.77	21.3	4.89	0.38
0.25% K_2SO_4	404	250ab	0.69	12.32	22	3.97	0.36
3% MLE + 0.6% $ZnSO_4$ + 0.25% K_2SO_4	376	262a	0.89	13	21.3	3.96	0.34
LSD ($P \leq 0.05$)	NS	88.97	NS	NS	NS	NS	NS
	Year II						
Control	290b	191.85b	0.74	20a 16.5ab	33a	4.65a	1.33a
3% MLE	461a	222.01a	0.73		24b	3.82b	0.70b
3% MLE + 0.6% $ZnSO_4$ + 0.25% K_2SO_4	415ab	220.35a	0.68	13.5b	28ab	4.18ab	0.88b
LSD ($P \leq 0.05$)	33.19	5.86	NS	3.12	4.5	0.34	0.32

Means within a column followed by the same letter are not significant at $P \leq 0.05$. NS represents not significant.

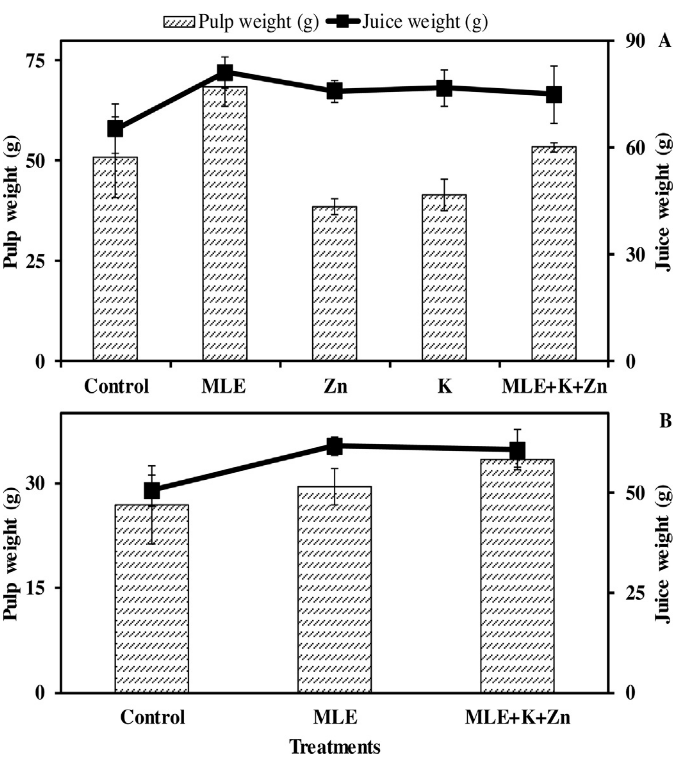

Fig. 3. Effect of exogenous application of 3% MLE (moringa leaf extract) alone or in combination with 0.6% $ZnSO_4$ and 0.25% K_2SO_4 on pulp weight (g) and juice weight (g) of 'Kinnow' mandarin fruits in first year (A) and second year (B) experiments, respectively. Vertical bars represent the ± SE of the means. n = 4 replicates.

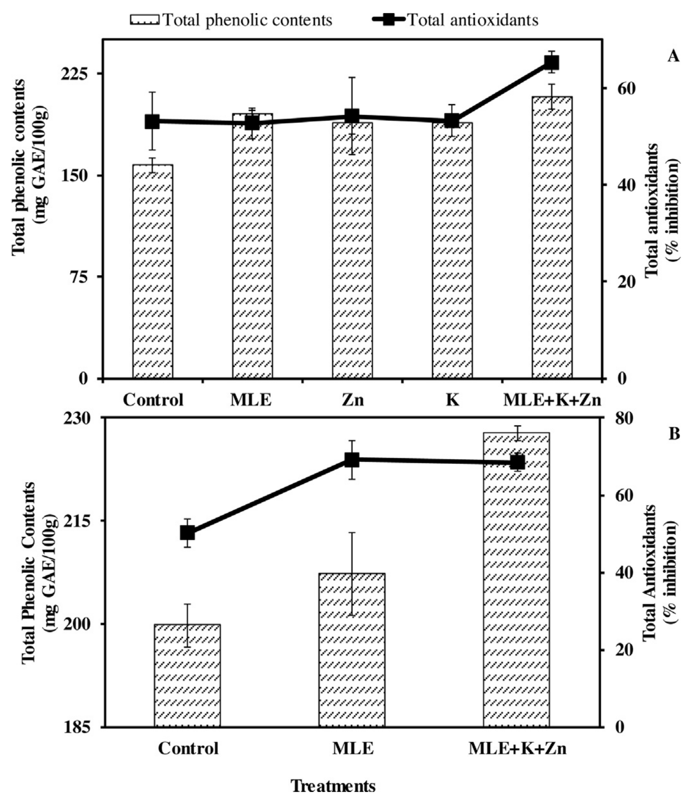

Fig. 4. Effect of exogenous application of 3% MLE (moringa leaf extract) alone or in combination with 0.6% $ZnSO_4$ and 0.25% K_2SO_4 on total phenolic contents (mg GAE/100 g) and total antioxidants (% inhibition) of 'Kinnow' mandarin fruits during first year (A) and second year (B) experiments, respectively. Vertical bars represent the ± SE of the means. n = 4 replicates.

The foliar application of MLE alone or in combination with K and Zn significantly increased the average pulp and juice weight of 'Kinnow' mandarin fruits (Fig. 3). Highest average pulp weight was recorded in fruit harvested form trees treated with 3% MLE at premature stage-treated fruits in first year experiment about 1.2-fold higher than untreated fruit. In second year experiment, maximum average pulp weight was found with combined application of 3% MLE, 0.25% K and 0.6% Zn at fruit set stage about 1,32-fold higher than control. Significant increase in the average juice weight was also observed in fruits harvested from the trees treated with 3% MLE at premature stage during both years experiments *i.e.*, 1.36-fold and 1.46-fold higher than untreated fruits, respectively (Fig. 3).

3.5. Biochemical fruit quality

Foliar application of different treatments significantly improved the 'Kinnow' mandarin fruit quality by increasing the SSC, TA, vitamin C, sugars (Table 6), total phenolics and total antioxidants (Fig. 4). The SSC of 'Kinnow' mandarin fruit juice was significantly ($P \leq 0.05$) increased in first (10.87 °Brix) and second year (10.62 °Brix) experiments with combined foliar application of 3% MLE, 0.6% $ZnSO_4$ and 0.25% K_2SO_4 at fruit set stage. The increase in SSC was 1.37-fold and 1.12-fold higher than untreated fruit during both years' experiments, respectively. TA of fruit juice was significantly increased in treated trees, as compared with control in both experiments. However, no significant change was observed in SSC: TA ratio of 'Kinnow' mandarin fruit juice (Table 6).

Vitamin C contents in 'Kinnow' mandarin fruit juice were significantly increased by 1.32-fold and 1.5 fold, as compared with control fruit with foliar application of 0.25% K alone and combined application of 3% MLE, 0.6% $ZnSO_4$ and 0.25% K_2SO_4 at fruit set stage during year I and II experiments, respectively.

Table 6
Effect of foliar application of moringa leaf extract (MLE), zinc sulphate ($ZnSO_4$) and potassium sulphate (K_2SO_4) on biochemical quality and enzymatic activity of 'Kinnow' mandarin fruits.

Treatments	SSC[a] (°Brix)	TA (%)	SSC:TA (Ratio)	Vit. C (mg/100 g)	TS (%)	NRS (%)	RS (%)	CAT (U/mg protein)	POD (U/mg protein)	SOD (U/mg protein)
	Year I									
Control	9.9b	0.75b	13.12	19.53b	13.8b	11.3b	1.95	14.24	0.67	113b
3% MLE	10.62a	0.75b	14.26	21.09ab	13.5b	11.1b	1.77	14.67	0.77	130a
0.6% $ZnSO_4$	10.37a	0.85a	12.19	24.215a	13.9b	11.4b	1.92	14.56	1.08	116b
0.25% K_2SO_4	10.87a	0.82a	13.31	25.79a	16.0a	14.0a	1.23	14.83	0.77	119ab
3% MLE + 0.6% $ZnSO_4$ + 0.25% K_2SO_4	10.82a	0.86a	12.56	21.87ab	15.3a	13.4a	1.86	13.28	0.87	112b
LSD ($P \leq 0.05$)	0.25	0.08	NS	3.96	1.7	1.54	NS	NS	NS	9.64
	Year II									
Control	9.75b	0.33b	29.59	25.5b	14.1b	9.89b	4.25b	16.23ab	0.79	110.8
3% MLE	10.62a	0.36ab	29.45	32.7a	15.3a	10.24a	5.08a	18.11a	0.94	132.58
3% MLE + 0.6% $ZnSO_4$ + 0.25% K_2SO_4	10.32a	0.45a	22.93	38.5a	15.5a	10.37a	5.12a	14.33b	0.92	125.85
LSD ($P \leq 0.05$)	3.4	0.04	NS	3.65	0.36	0.27	0.44	2.51	NS	NS

TA = titratble acidity; Vit. C = vitamin C; TS = total sugars; NRS = non-reducing sugars; RS = reducing sugars; CAT = catalase; POD = peroxidase and SOD = superoxide dismutase. Means within a column followed by different letters are significant at $P \leq 0.05$. NS represents not significant.
[a] SSC = soluble solid content.

Total sugars were significantly increased in 0.25% K_2SO_4 (16%) and 3% MLE + 0.6% $ZnSO_4$ + 0.25% K_2SO_4 (15.3%) treated fruits during first year experiment and the increase was 1.15-fold and 1.1-fold higher than control, respectively. In second year experiment maximum sugar was recorded in fruit harvested from trees treated with 3% MLE + 0.6% $ZnSO_4$ + 0.25% K_2SO_4 (15.5%) i.e., 1.09-fold higher than control fruit (Table 6). Similarly, non-reducing sugars were significantly increased with application of 0.25% K_2SO_4 (14%) and 3% MLE + 0.6% $ZnSO_4$ + 0.25% K_2SO_4 (13.4%) treatments in first year experiment; whereas, in second year experiment foliar application of 3% MLE + 0.6% $ZnSO_4$ + 0.25% K_2SO_4 showed highest non-reducing sugars (10.37%). No significant change was recorded in the reducing sugar percentage during the first year experiment; and in second year experiment maximum significant increase was observed in fruit harvested from the trees treated with 3% MLE + 0.6% $ZnSO_4$ + 0.25% K_2SO_4 at fruit set stage (5.12%) (Table 6).

Total phenolic contents and total antioxidants were significantly increased in fruit harvested from all treated trees, as compared with control in both experiments (Fig. 4). Total phenolic contents were increased by 1.34-fold and 1.29-fold in contrast with control fruit in first and second year experiments by application of 3% MLE + 0.6% $ZnSO_4$ + 0.25% K_2SO_4 at fruit set stage, respectively. Similarly, total antioxidants were significantly increased in fruit harvested from the trees treated with 3% MLE + 0.6% $ZnSO_4$ + 0.25% K_2SO_4 at fruit set stage by 1.34-fold and 1.75-fold in first and second years' experiment, respectively.

The activities of SOD enzymes were significantly increased up to 1.15-fold in fruit harvested from the trees treated with 3% MLE at premature stage in first year experiment; whereas, no significant change was recorded in second year experiment. Activity of CAT enzyme was not significantly affected by foliar application of MLE, K and Zn in first year experiment but in second year experiment it was increased by 1.26-fold than control in fruit harvested from the trees treated with 3% MLE at premature stage (Table 6). However, no significant change was observed in the activities of POD enzymes in 'Kinnow' mandarin fruit during both years' experiments (Table 6.)

4. Discussion

Results showed that foliar application of 3% MLE + 0.6% $ZnSO_4$ + 0.25% K_2SO_4 at fruit set stage increased N, P, K, Ca, Fe, Zn and Mn contents of 'Kinnow' mandarin leaves (Table 1). The increase in nutrient content of 'Kinnow' mandarin leaves may be due to fact that moringa itself is rich source of minerals (Yasmeen, 2011) and when applied along with nutrients such as K and Zn it increased the nutrients uptake in 'Kinnow' mandarin leaves. Similarly, significant increase in the leaves nutrient uptake (N, P, K, Ca and Fe mg/g.d.wt) of rocket plant (*Eruca vesicaria subsp. sativa*) (Abdalla, 2013) and 'Le Conte' pear tree (*Pyrus communis* × *Pyrus serotina*) (El-Hamied and El-Amray, 2015) was reported with foliar application of MLE. Koo and Mayo (1994) reported increase in mineral [N, magnesium (Mg), Mn, Zn, Cu, Fe and B] level of 'Washington Navel' orange, Carrizo citrange and sour orange (*Citrus aurantium*) leaves after foliar application of sea weed extract. Hafez and El-Metwally (2007) found significant increase in N, P, K, Mn and Zn content in leaves of 'Washington Navel' orange with foliar application of Zn and K.

Leaf ascorbic acid contents were significantly ($P \leq 0.05$) improved after foliar application of MLE alone or in combination with K and Zn (Fig. 1). Since moringa leaves are rich in ascorbate and phenolics, so its extract application increased the endogenous level of ascorbic acid in leaves. Increase in ascorbic acid ensures better photosynthetic activity of tree resulting in more photoassimilates that ultimately improves tree health and yield (Smirnoff, 1996). Earlier Abdalla (2013) also reported increase in ascorbic acid content of rocket plant after foliar application of MLE.

The application of MLE alone or in combination with K and Zn was effective in increasing the fruit set and reducing the fruit drop, as compared with untreated trees (Fig. 2). The increase in fruit set and reduction in fruit drop may be attributed to the presence of auxin, zeatin along with phenolics, antioxidants and minerals in MLE that might be involved in the production of endogenous hormones which regulate internal mechanism for controlling fruit set and abscission of ovaries (Talon and Zeevaart, 1992). K and Zn application enhanced the nutrient uptake and photosynthetic activity in leaves that consequently increased fruit set, reduced fruit drop and improved yield (Hafez and El-Metwally, 2007). Earlier Koo and Mayo (1994) found reduced premature fruit drop in 'Washington Navel' orange and grape fruit with use of seaweed extract. Foliar application of K, Zn or Zn + K were effective in improving the fruit retention per tree in 'Balady' mandarin (El-Baz, 2003), 'Washington Navel' orange (Hafez and El-Metwally, 2007) and 'Kinnow mandarin (Ashraf et al., 2010; Razzaq et al., 2013). Similarly, increased fruit set and reduced fruit drop in "Blood Red" sweet orange was found with foliar application of gibberellin and low biuret urea (Saleem et al., 2008). Ashraf et al. (2012) also observed increase in fruit set and reduction in fruit drop% after foliar application of Zn + K+ SA (salicylic acid) in 'Kinnow' mandarin.

Results showed significant increase in yield [weight (kg) and total number of fruits], total number and percentage of marketable fruit; and decrease in number and percentage unmarketable fruits with MLE alone or in combination with K and Zn in 'Kinnow' mandarin trees (Table 3). This can be ascribed to the fact that MLE is rich in mineral (K, Ca, Fe and Zn) and hormones (zeatin and auxin) which are directly/indirectly involved in fruit growth and development process (Swietlik, 1999; Abdalla, 2013). Moreover, better fruit set and reduced fruit drop resulted in higher yield in terms of fruit weight and fruit number. Previous studies showed that foliar application of MLE increased the yield of some crops like peanut, cantaloupe, onion, bell pepper and tomato by 20–35% (Foidl et al., 2001). Foliar application of K alone or in combination with Zn has also been reported to increase the yield of 'Kinnow' mandarin (Ashraf et al., 2010, 2012), 'Washington Navel' oranges (Hafez and El-Metwally, 2007) and 'Balady' mandarin (El-Baz, 2003). Fornes et al. (2002) also found increase in yield and number of marketable fruit of 'de Nules' clementine mandarin with application of GA_3 and seaweed extract.

Fruit weight, size, juice weight and pulp weight were significantly increased in fruit harvested from the trees treated with MLE alone or in combination with K and Zn (Table 5 and Fig. 3). This increase is ascribed to the fact that MLE is rich in minerals like K, Zn, antioxidant and zeatin. K is required by citrus in large quantities and is involved in starch/sugar accumulation and improves source sink relationship and Zn is precursor of tryptophan which is involved in synthesis of indole acetic acid that is required for fruit growth and development (Zekri and Obreza, 2009). Zeatin (important hormone of moringa) is involved in cell expansion and translocation of photo assimilates towards fruit (Yasmeen, 2011) thus ultimately increased the fruit size, weight and juice content. Similarly, foliar application of MLE have been found to increase fruit weight, size and volume in 'Le Conte' pear (El-Hamied and El-Amray, 2015) and fresh and dry weight of *Ocimum samctum* L. (Prabhu et al., 2010). Fruit weight was significantly increased by foliar spray of Zn alone and in combination with K or GA_3 in 'Washington Navel' oranges (Hafez and El-Metwally, 2007; Eman et al., 2007). Similarly, in 'Kinnow' mandarin fruit maximum juice and fruit size was found with foliar application of Zn and SA + Zn + K (Malik et al., 2000; Ashraf et al., 2012; Razzaq et al., 2013).

Applying MLE alone or in combination with K and Zn significantly improved fruit bio-chemical (SSC, TA, vitamin C and sugar contents) (Table 6). Since MLE have high sugar and starch content along with minerals such as K and Zn (Foidl et al., 2001) which when applied to 'Kinnow' mandarin increased SSC and TA. K is directly involved in translocation of photo assimilate (sugars and carbohydrates) toward fruit and has direct and strong role with juice acidity; more the K, higher will be acidity and vice versa (Zekri and Obreza, 2009). Zn plays an important role in photosynthesis, synthesis of starch, nucleic acid and promotes activation of various enzymes required in these biochemical reactions (Alloway, 2004). Hence, suggesting that when MLE, K and Zn were applied they were directly or indirectly involved in metabolic process that improved quality of 'Kinnow' mandarin. Similarly, exogenous application of MLE increased SSC in 'Le Conte' pear (El-Hamied and El-Amray, 2015). In another study, highest SSC was found with foliar application of Zn + K on sweet orange (Hafez and El-Metwally, 2007) and Zn + K + SA on 'Kinnow' mandarin trees (Ashraf et al., 2012). Khan et al. (2012) also reported increase in SSC and TA of 'Feutrell's Early' mandarin with foliar application of Zn. SSC:TA ratio was not altered by foliar application of moringa alone or in combination with K and Zn. In this case moringa, K and Zn had simultaneously increased SSC and TA but it did not affect their ratio showing that they had higher influence on fruit acidity.

Vitamin C contents were significantly increased in 'Kinnow' mandarin fruit by foliar application of MLE, K and Zn in both years (Table 6). Since moringa has ascorbate so its exogenous application might be triggered the endogenous production of ascorbate (Nouman et al., 2012); along with minerals such as K and Zn that were involved in sugar metabolism which are directly related to production of vitamin C (Mengel, 1997). Similarly, foliar spray of MLE increased vitamin C in 'Le Conte' pear (El-Hamied and El-Amary, 2015). Furthermore, foliar application of growth hormones (auxins, gibberelins and kinetin) (Saleem et al., 2008; Khalid et al., 2012), nutrients (Zn and K) (Eman et al., 2007; Khan et al., 2015), and combined application of plant growth regulators and nutrients (urea + GA_3; SA + Zn + K) (Ashraf et al., 2012) increased the vitamin C in citrus fruit juice.

Total sugars in 'Kinnow' mandarin fruit were increased with foliar application of 3% MLE, 0.6% $ZnSO_4$ and 0.25% K_2SO_4. The reason behind their increase might be that moringa contains zeatin which is directly involved in the translocation of sugars towards fruit as reported earlier by Foidl et al. (2001) who noticed increase in sugar levels of cantaloupe and sugar cane after foliar application of moringa as phytohormones. Khalid et al. (2012) also found increase in sugars after foliar application of kinetin in 'Kinnow' mandarin. In earlier studies, exogenous application of nutrients (Zn, K) improved the total sugar of 'Kinnow' mandarin (Khan et al., 2015). Thus, the foliar application of MLE alone or in combination with K and Zn improved 'Kinnow' mandarin fruit quality by increasing SSC, TA, vitamin C and sugars.

Total phenolic contents and total antioxidants were significantly increased in fruit treated with 3% MLE + 0.6% $ZnSO_4$ + 0.25% K_2SO_4 at fruit set stage (Fig. 4). The increase in phenolic contents and antioxidants might be due to fact that MLE possessed a wide spectrum of antioxidants (ascorbic acid), phenols, flavonoids and β-carotene (Jacob and Shenbagaraman, 2011); when applied exogenously to the tree, affect the metabolic process directly/indirectly in such a way that it increased the internal level of phenolics and antioxidants. Abdalla (2013) reported that aqueous extract of moringa increased the total phenolic contents and antioxidants of *Eruca vesicaria.* Similarly, foliar application Zn + B increased the phenolic content of 'Kinnow' mandarin fruits (Khan et al., 2015). Moreover, activities of antioxidative enzymes (SOD and CAT) were increased with foliar application of MLE alone or along with K and Zn; while activity of POD enzyme was not significantly affected by MLE treatment during both years' experiments (Table 6). Moringa leaves have been reported as a source of strong antioxidant system (Nouman et al., 2012) along with minerals and ascorbic acid which improved the antioxidative system of plants (Anwar et al., 2007). As reported earlier by Yasmeen (2011) who found an increase in antioxidative enzymes after foliar application of MLE in wheat (*Triticum aestivum*). Similarly, exogenous application of ascorbic acid on apple enhanced the activity of SOD and CAT enzymes (Bai et al., 2013).

5. Conclusion

MLE as a growth stimulator when applied exogenously with K and Zn on 'Kinnow' mandarin trees increased the nutrient content (N, P, K, Ca, Fe, Mn and Zn), ascorbic acid level of leaves, fruit yield (number of fruits and total weight), fruit size, juice weight and fruit biochemical quality (SSC, vitamin C, sugars, total phenolic contents and total antioxidants). Hence, it may be concluded that foliar application of 3% MLE along with 0.6% $ZnSO_4$ and 0.25% K_2SO_4 at fruit set stage can be used effectively on 'Kinnow' mandarin trees to improve leaves mineral status, ascorbic acid content, yield and fruit quality.

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