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Full Length Article

Integrative Application of Biostimulants and Nutrients Improves Vegetative Growth of 'Kinnow' Mandarin

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Abstract

Vegetative growth is a very vital factor to determine the health of tree. However, vegetative growth is little focused area by researchers. The present study evaluated the effect of foliar applied moringa leaf extract (MLE), ascorbic acid, cytokinins (BAP), potassium (K) and zinc (Zn) on vegetative growth [plant height (cm), plant width (cm), trunk size (cm), leaf area (cm^2), leaf length (cm), leaf width (cm), flush length (cm), and leaf age (number of days)] of 'Kinnow' mandarin trees. Four experiments were conducted during year 2012–2015. In experiment I, 3% MLE was sprayed at different growth stages *i.e.*, before flowering, at full bloom stage, before flowering + fruit set stage and at pre- mature stage. While in experiment-II, 3% MLE was applied before flowering + fruit set stage and at pre- mature stage, 300 mg L^{-1} ascorbic acid and 800 mg L^{-1} BAP at fruit set stage. In experiment-III, 3% MLE before flowering + fruit set stage and pre-mature stage, Zn (0.6%) and K (0.25%) at fruit set stage) and integrative MLE (3%) + K (0.6%) + Zn (0.25%) at fruit set stage were sprayed. Treatments selected for experiment-IV were the best treatments from experiment I, II and III, respectively. The results from experiment-I showed that maximum trunk diameter and leaf age was observed in trees applied with 3% MLE at before flowering + fruit set stage. In experiment-II, highest increase in tree height and trunk diameter was observed in trees treated with BAP, whereas increase in tree width and leaf age was found in trees treated with MLE at before flowering + fruit set stage. In experiment-III and IV highest tree height, tree width, leaf length, leaf area, flush length and leaf age were found in trees sprayed with MLE + K +Zn at fruit set stage, respectively. Conclusively, it can be suggested that foliar application of integrative 3% MLE + 0.25% K + 0.6% Zn at fruit set stage can improve the vegetative growth of 'Kinnow' mandarin trees.

Keywords: Ascorbic acid; BAP; 'Kinnow' mandarin; Moringa; Potassium; Vegetative growth; Zinc

Introduction

Pakistan is blessed with agro-climatic condition, suitable for cultivation of variety of horticultural crops. Among various fruits crops such as citrus, apple, date palm, guava and banana, etc. grown throughout the country with citrus predominant fruit crop covering an area of 0.45 million acres (GOP, 2015). Different citrus varieties grown in Punjab includes 'Kinnow' mandarin, 'Feutrell Early', musambi, sweet orange, 'Blood Red' orange, grapefruit, sweet lime, 'Kaghzi' lime and 'Lisbon' lemon (Naseer, 2010). 'Kinnow' mandarin (*Citrus nobilis* Lour. × *Citrus deliciosa* Tenora.) is also known as "King of all mandarins", because of its likable taste, aroma, high juice content and nutritional composition (Memon, 2014).

Although, 'Kinnow' mandarin is the main commercial citrus crop of Pakistan, but it does not reach to its maximum production potential. Because of its unhealthy growth and poor orchard management citrus trees suffer from low productivity (Ashraf *et al.*, 2012). There are various factors that affect its growth and the major one is nutritional status of tree (Ibrahim *et al.*, 2007; Khan *et al.*, 2015). Management of tree nutrition is vital for healthy growth and production (Ioannis *et al.*, 2004). Usually, tree nutrition is managed by application of synthetic nutrients (Ashraf *et al.*, 2012; Khan *et al.*, 2015). But the hazardous effects of synthetic fertilizers is being supplemented in the field with natural products which are cost effective, environmental friendly and beneficial for plants. In this aspect plant hormones have been already used for citrus cultivation (Nickell, 1982; Saleem *et al.*, 2008). Furthermore, discovery of moringa as a natural biostimulant open up a new era for agriculture sector and its application improved growth and productivity of various crops (Yasmeen *et al.*, 2013).

Moringa oleifera (L.) have recently gained much popularity in agricultural sector as a natural growth promoter for plants (Yasmeen *et al.*, 2013; El-Hamied and El-Amary, 2015). Moringa leaves are rich in nutrients, vitamins, phenolics, organic acid (oxalic acid) and growth regulators (zeatin and auxins) (Lachance *et al.*, 2001;

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Shindano and Kasase, 2009). Individually these components have been reported to increase growth of plants (Fayed, 2010; Yasmeen *et al.,* 2013; El-Hamied and El-Amary, 2015). Earlier research showed that foliar application of MLE improved plant growth, number of leaves and yield in 'Le Conte' pear (*Pyrus communis* × *Pyrus serotina*) (El-Hamied and El-Amary, 2015; Khalid *et al.*, 2016) and tomatoes (*Lycopersicum esculentum)* (Culver *et al.*, 2012). Spraying pea (*Pisum sativum)* and tomato with MLE significantly improved plant height, shoot length, root length, leaf area and yield, respectively (Yasmeen, 2011).

Ascorbic acid is the simplest and most abundant antioxidant (vitamin) in plants (Davey *et al.*, 2000). It participates in a variety of physiological process that occurs within plants (Smirnoff and Wheeler, 2000) and is also involved in vegetative and reproductive growth, fruit maturation, ripening and senescence (Barth *et al.*, 2006). Foliar application of ascorbic acid increased primary root length and plant height in almond (*Prunus amygdalus* L.) (Amira, 2013), growth and yield of pomegranate trees (*Punica granatum*) (Fayed, 2010) and 'Flame' seedless grapevines (Wassel *et al.*, 2007).

Among growth regulators, cytokinins have wide range of application as it is involved in plant growth and developmental processes (Frankenberger and Arshad, 1995) as well chlorophyll biosynthesis and photosynthate partitioning (Taiz and Zeiger, 2006). It was found that citrus trees treated with cytokinins have higher yield, improved juice volume and better biochemical fruit quality (Lovatt *et al*., 2011). Lashari *et al.* (2008) found that application of zeatin to cotton plant increased leaf age and yield. The application of cytogen (commercial cytokinin containing product) increased yields of corn, rice, pepper, cucumber and cantaloupe (Mayeux *et al.*, 1983).

Among nutrients, K is required in large amount as compared to other macronutrients (Alva and Tucker, 1999), as it is involved in formation of sugars, synthesis of proteins, cell division, growth, neutralization of organic acids, photosynthesis and transportation of metabolites (Liu *et al.*, 2000). K improves plant health and increases resistance to disease and insects (Zekri and Obreza, 2010). Previous research showed that foliar application of K significantly increased leaf mineral content, yield and fruit quality 'Navel' oranges (Tiwari, 2005), mandarin (Shawky *et al*., 2000; Gill *et al.*, 2005) and oranges (El-Safty *et al.*, 1998). Zn as an essential micronutrient is involved in synthesis of protein, auxins, cell division, sexual fertilization, regulates starch formation and maintains membrane structure (Zekri and Obreza, 2003; Wassel *et al.*, 2007; Rehman *et al.,* 2012). Foliar application of Zn significantly increased number of leaves, stem diameter, root diameter and secondary root per plant in 'Khasi' mandarin (*Citrus reticulate)* and *Citrus volkameriana* seedlings (Singh and Sheo, 2001). Increase in height and trunk spread was found in mandarin cv. Darjeeling after foliar application of Zn (Rodríguez *et al.*, 2005).

Therefore, the present study was planned to find out the effect of MLE, ascorbic acid, cytokinins and nutrients (K and Zn) in individual or in combination applied at different growth stages on the vegetative growth of 'Kinnow' mandarin.

Materials and Methods

The study was carried out in the Experimental Orchard Square # 9 (31°25'N; 73°09'E), Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan during the year 2012–2015. 'Kinnow' mandarin trees 13–15 year old were selected for the experiment budded on Rough Lemon rootstock (*Citrus jambhiri* Lush); planted in square system at 6.1 × 6.1 m row to row and plant to plant distance. Selected trees were of uniform size, health and vigour, being grown under similar agro-climatic conditions and subjected to similar cultural practices like fertilizer, irrigation, insect pest control and pruning.

Treatments

Experiment-I: Treatments selected were control (no water or surfactant was sprayed), water spray, 3% MLE was sprayed before flowering, at full bloom stage, before flowering + fruit set stage, at pre-mature stage (3rd week of September) and in combination before flowering + full bloom stage + pre- mature stage.

Experiment-II: Treatments selected were control (water), 3% MLE sprayed before flowering + fruit set stage, and at pre- mature stage, 300 mg L^{-1} ascorbic acid and BAP (800 mg L^{-1}) were sprayed at fruit set stage.

Experiment-III: Treatments were control, 3% MLE before flowering + fruit set stage and at pre-mature stage, Zn (0.6% Zn SO_4) at fruit set stage, K (0.25% K_2SO_4) at fruit set stage and integrative spray (3% MLE + 0.25% K+ 0.6% Zn) at fruit set stage.

Experiment-IV: Treatments selected for this experiment were the best treatments from experiment I, II and III, respectively. They are control, 3% MLE at pre-mature stage, 300 mg L^{-1} ascorbic acid and integrative spray (3% MLE + 0.25% K+ 0.6% Zn) at fruit set stage.

Surfactant Tween-20® (0.01%) was added in all treatments before spray and foliar sprays were done with hand held sprayer until run off early in the morning (7 a.m.). The experiment was laid out according to Randomized Complete Block Design (RCBD) where single tree was taken as an experimental unit replicated four times. Parameters studied included tree height and width, trunk diameter, leaf length and width, leaf area, flush length and leaf age.

Vegetative Growth

To study the vegetative growth pattern of 'Kinnow' mandarin trees, the calculations were made before the start of the experiment and after harvesting the fruits.

Tree height (cm) was measured with telescopic rod by placing it close to the tree trunk and increase in tree height was measured using following formula:

$$Increase\ in\ tree\ height\ (cm) = final\ tree\ height - initial\ tree\ height$$

Tree width (cm) was taken by using the measuring tape. The measurements were made on two directions *i.e.,* North-South and East-West and their mean was calculated as actual tree width, using the following formula:

$$Increased\ in\ tree\ width\ (cm) = final\ tree\ width - initial\ tree\ width$$

Trunk diameter was measured by placing the measuring tape 10 cm above the point of bud union, and the diameter was calculated by the following formula:

$$D = C \div \mu$$

Where,
D = diameter of tree trunk (cm)
C = Circumference (girth) of the tree trunk (cm)
μ = 3.14
The increase in diameter was calculated using the following formula:

$$Increase\ in\ trunk\ diameter\ (cm) = final\ trunk\ diameter - initial\ tree\ diameter$$

Flush length (cm) was measured using the regular scale, by tagging four branches of almost 2 inch of diameter from all the four sides (North, South, East and West) of tree and newly emerged six flushes on each branch.

Leaf area (cm^2) was taken by measuring the width and length of twenty leaves of tagged branches, using the following formula:

$$Average\ leaf\ size\ (\text{cm2}) = average\ leaf\ length \times average\ leaf\ width$$

Leaf age was calculated by counting the number of leaves from tagged branches (newly emerged flushes) in month of March and number of leaves remain attached to the flushes.

Statistical Analysis

The data collected were statistically analyzed using software Statistix 10 (Analytical software, Tallahasee, FL 32317, USA) (Ullah *et al.,* 2013). Analysis of variance (ANOVA) techniques were employed to test the overall significance of the data, while the Least Significant Difference (Fisher's LSD) test at $P \leq 0.05$ was used to compare the differences among treatment means, where the F test was significant (Steel *et al.,* 1997). Pearson correlation analysis was done using same software to determine the relationship between vegetative growth of 'Kinnow' mandarin at $P \leq 0.05$.

Results

Experiment-I: The results showed that exogenous application of 3% MLE at different growth stages does not significantly improved the vegetative growth (increase in height, increase in width, leaf area, leaf length, leaf width and length of flush) of 'Kinnow' mandarin trees, excluding the trunk diameter and leaf age (Table 1 and Fig. 1). Maximum increase in trunk diameter was found in trees sprayed with 3% MLE at before flowering + fruit set stage (0.24 cm) and 3% MLE at pre- mature stage (0.24 cm), respectively. The increase in trunk diameter was almost 3-fold than untreated trees in both the treatments, respectively (Table 1). Highest leaf age (315 days) was found in trees treated with 3% MLE before flowering + at full bloom stage + pre-mature stage. The increase was almost 1.65-fold than the control trees (Fig. 1).

Pearson correlation analysis exhibited that tree diameter had significant positive correlation with tree height (r = 0.45) (Table 2). Leaf length is positively correlated to leaf width (r = 0.78), leaf area (r = 0.94) and flush length (r = 0.46) (Table 2). Leaf width also showed significant positive correlation with leaf area (r = 0.93) (Table 2).

Experiment-II: The results revealed that foliar application of MLE, ascorbic acid and BAP (cytokinins) significantly increased the tree height, trunk diameter and tree width (Table 3). Maximum increase in tree height (57.51 cm) and trunk diameter (7.54 cm) was found in trees treated with BAP at fruit set stage, and the increase was almost 2.80-fold and 4.56-fold than control trees, respectively. Whereas, highest increase in tree width (11.39 cm) was found in trees treated with 3% MLE (before flowering + fruit set stage). However, there was no significant increase observed in leaf length, leaf width, leaf area and flush length after foliar application of MLE, BAP and ascorbic acid (Table 3). Leaf age in 'Kinnow' mandarin trees was significantly increased and maximum leaf age (256 days) was found in trees treated with BAP and the increase was 1.34-fold higher than the untreated trees (Fig. 2).

Pearson correlation analysis showed that tree height exhibited significant positive correlation (r = 0.54) with leaf length (Table 4). However, tree diameter had positive correlation with leaf age (r = 0.71) (Table 4). Leaf area of 'Kinnow' mandarin trees showed significant positive correlation with leaf length (r = 0.79) and leaf width (r = 0.92) (Table 4).

Experiment-III: Foliar application of moringa and nutrients (K and Zn) significantly increased the vegetative growth (tree height, tree width, leaf length, leaf width, leaf area and leaf age) of 'Kinnow' mandarin trees (Table 5). Maximum increase in tree height (5.34 cm) and tree width (21.81 cm) was found with combined application of MLE, K and Zn and the increase was 1.11-fold and 1.95-fold higher than control trees, respectively (Table 5). No significant change was found in trunk diameter of 'Kinnow' mandarin trees after foliar application of MLE, K and Zn (Table 5). Similarly, increase in leaf length (7.53 cm) was found with combined application of MLE, K and Zn and that is almost 1.33-fold higher than control trees (Table 5). Maximum leaf width (4.65 cm) was noted in trees treated with 3% MLE at premature stage (Table 5).

Table 1: Influence of exogenous application of 3% MLE (moringa leaf extract) at different growth stages on vegetative growth of 'Kinnow' mandarin trees

Treatments	Increase in height (cm)	Increase in tree width (cm)	Increase in trunk diameter (cm)	Leaf area (cm^2)	Leaf length (cm)	Leaf width (cm)	Flush length (cm)
Control	0.125	1.53	0.08b	16.98	6.57	2.87	12.08
Water spray	0.175	1.51	0.11b	19.04	6.42	2.95	12.79
3% MLE (before flowering)	0.175	1.70	0.1ab	24.33	7.6	3.25	14.25
3% MLE (full bloom stage)	0.225	1.77	0.2ab	19.98	6.65	2.97	13.28
3% MLE (before flowering + fruit set stage)	0.325	1.63	0.24a	24.89	7.52	3.25	15.38
3% MLE (pre-mature stage)	0.312	1.58	0.24a	22.23	7.15	3.1	15.25
3% MLE (before flowering + full bloom stage + pre- mature stage)	0.175	1.53	0.1ab	19.88	6.1	2.75	15.79
LSD($P \leq 0.05$)	NS	NS	0.11	NS	NS	NS	NS

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

Table 2: Pearson correlation analysis of vegetative growth of 'Kinnow' mandarin trees after foliar application of 3% MLE at different growth stages (n = 24)

Characters	Tree Height	Trunk Width	Tree diameter	Leaf length	Leaf width	Leaf area	Flush length
Trunk Width	0.29						
Tree diameter	0.45*	0.26					
Leaf length	0.37	0.02	0.31				
Leaf width	0.34	-0.26	0.24	0.78***			
Leaf area	0.39	-0.11	0.32	0.94***	0.93***		
Flush length	0.09	-0.09	0.22	0.46*	0.29	0.39	
Leaf age	0.19	-0.01	0.13	0.05	0.05	0.07	0.04

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

Table 3: Influence of exogenous application of MLE, ascorbic acid and BAP on vegetative growth of 'Kinnow' mandarin trees

Treatments	Increase in tree height (cm)	Increase in trunk diameter (cm)	Increase in tree width (cm)	Leaf length (cm)	Leaf width (cm)	Leaf area (cm^2)	Flush length (cm)
Control	20.47b	1.65b	9.77b	7.7	3.22	25.12	10.56
3% MLE (before flowering + fruit set stage)	29.56b	2b	11.39a	8.8	4	35.3	11.63
3% MLE (premature stage)	25.95b	4.36ab	10.37ab	8.5	3.8	32.77	11.77
300 mg L^{-1} Ascorbic acid (at fruit set stage)	34.63b	3.67b	10b	8.12	3.7	30.37	12.59
800 mg L^{-1} BAP (at fruit set stage)	57.51a	7.54a	10.52ab	9.47	3.8	36.1	11.74
LSD ($P \leq 0.05$)	18.62	2.79	0.78	NS	NS	NS	NS

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

Table 4: Pearson correlation analysis of vegetative growth of 'Kinnow' mandarin trees after foliar application of 3% MLE, ascorbic acid and BAP (n = 24)

Characters	Tree Height	Trunk Width	Tree diameter	Leaf length	Leaf width	Leaf area	Flush length
Trunk Width	0.15						
Tree diameter	0.00	0.03					
Leaf length	0.54*	0.42	0.15				
Leaf width	0.21	0.36	0.39	0.5*			
Leaf area	0.38	0.42	0.32	0.79***	0.92***		
Flush length	-0.09	-0.13	0.34	0.16	0.22	0.24	
Leaf age	-0.02	0.19	0.71***	-0.00	0.14	0.06	0.12

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

Table 5: Influence of exogenous application of MLE, K and Zn on vegetative growth of 'Kinnow' mandarin trees

Treatments	Tree height (cm)	Trunk diameter (cm)	Tree width (cm)	Leaf length (cm)	Leaf width (cm)	Leaf area (cm^2)	Flush length (cm)	Leaf age (No. of days)
Control	4.80b	3.1	10.72d	6.02d	3.57dd	21.80d	10.6	215b
3% MLE (before flowering + fruit set stage)	5.1ab	4.4	17.3bc	7.00b	4.07bc	28.67b	13.1	235ab
3% MLE (premature stage)	5.2ab	6.8	16.9bc	6.8bc	4.67a	31.97a	12.6	244a
0.6% Zn (fruit set stage)	5.1ab	3.5	15.81c	6.37cd	3.85cd	24.87c	11.2	239ab
0.25% K (fruit set stage)	4.9ab	4.1	20.1ab	7.00b	3.87cd	27.29bc	13.5	256a
3% MLE+ 0.25% K+ 0.6% Zn (fruit set stage)	5.34a	4.4	21.81a	7.53a	4.32ab	32.80a	12.1	252a
LSD ($P \leq 0.05$)	0.43	NS	3.52	0.21	0.19	1.25	NS	23.29

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

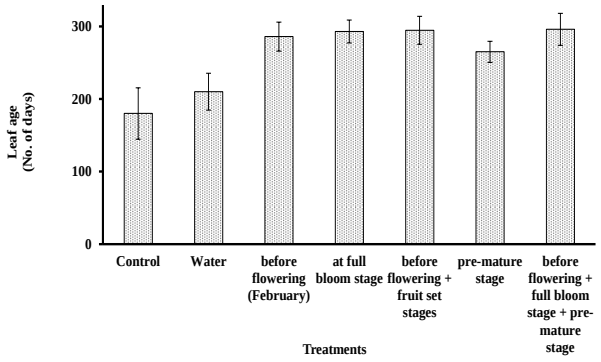

Fig. 1: Effect of exogenous application of 3% MLE at different growth stages on leaf age (number of days) of 'Kinnow' mandarin trees. Vertical bars represents ± SE of means, n = 4 replicates

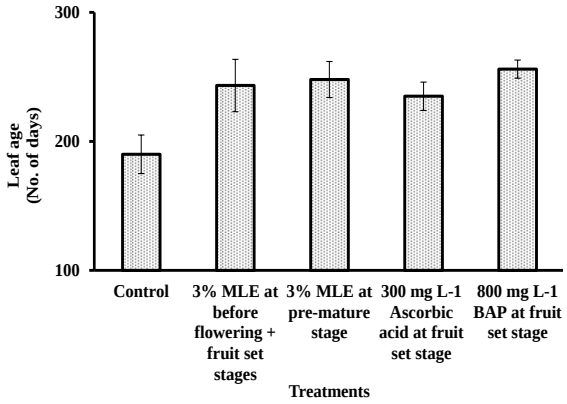

Fig. 2: Effect of foliar application of MLE, ascorbic acid and BAP on age of 'Kinnow' mandarin leaves. Vertical bars represent ± SE of means. n = 4 replicates

Highest leaf area (32.8 cm^2) was observed in trees treated with combined foliar application of MLE, K and Zn (Table 5). No significant change was observed in flush length of 'Kinnow' mandarin after treating with MLE, K and Zn. Maximum leaf age (252.35 number of days) in 'Kinnow' mandarin were found in trees treated with 3% MLE+ 0.25% K + 0.6% Zn (Table 5).

Tree height exhibited significant positive correlation with tree width (r = 0.59), leaf length (r = 0.61), width (r = 0.73), area (r = 0.75) and age (r = 0.66) (Table 6). Likewise, trunk width showed significant positive correlation with leaf length (r = 0.87), width (r = 0.63), area (r = 0.79) and age (r = 0.48) (Table 6). Leaf length in 'Kinnow' mandarin trees exhibited significant positive correlation with leaf width (r = 0.68), leaf area (r = 0.88) and leaf age (r = 0.53) (Table 6). Similarly, leaf width had shown positive significant correlation with leaf area (r = 0.94) and leaf age (r = 0.87) (Table 6). Significant positive correlation also existed between leaf area and leaf age (r = 0.81) (Table 6).

Experiment-IV: In 'Kinnow' mandarin tree height, trunk diameter, tree width and flush length were significantly increased after foliar application of MLE, ascorbic acid and combined spray of MLE, K and Zn (Table 7). Maximum increase in tree height was found in trees treated with ascorbic acid (11.15 cm) and combined application of MLE, K and Zn (11 cm); and the increase was 2.43-fold higher than untreated trees (Table 7). Increase in trunk diameter (3.76 cm) and tree width (47 cm) was recorded in trees treated with 3% MLE + 0.25% K + 0.6% Zn at fruit set stage *i.e.,* 2.87-fold and 1.08-fold higher than control trees (Table 6). Whereas, maximum flush length (8.46 cm) was measured in trees treated with ascorbic acid and the increase was 1.08-fold higher than control trees (Table 6). Similarly, no significant increase was observed in leaf length, leaf width and leaf area of 'Kinnow' mandarin trees after exogenous application of treatments compared to untreated tree (Table 7–8). Leaf age (number of days) showed significant ($P \leq 0.05$) improvement after exogenous application of MLE, ascorbic acid and MLE + K+ Zn. Maximum leaf age (368.25 days) was calculated in trees treated with 3% MLE+ 0.25% K+ 0.6% Zn at fruit set stage *i.e.,* about 1.3-fold higher than untreated trees (Table 7).

Person correlation analysis for tree height exhibited significant positive correlation with trunk width (r = 0.76), tree diameter (r = 0.67), leaf length (r = 0.66), leaf area (r = 0.64), flush length (r = 0.58) and leaf age (r = 0.67) (Table 9). Trunk width showed significant positive correlation with tree diameter (r = 0.9), leaf length (r = 0.53), leaf width (r = 0.65), leaf area (r = 0.68), flush length (r = 0.52) and leaf age (r = 0.82) (Table 9). Tree diameter exhibited significant positive correlation with leaf width (r = 0.62), leaf area (r = 0.55), flush length (r = 0.61) and leaf age (r = 0.67) (Table 9). Leaf area is significantly positively correlated with leaf length (r = 0.92) and leaf width (r = 0.78) (Table 9).

Discussion

Vegetative growth in 'Kinnow' mandarin trees improved after foliar application of 3% MLE, ascorbic acid, nutrients (K and Zn) and combined application of MLE, K and Zn as compared to control trees (Table 1, 2, 3, 4, 5, 6, 7, 8 and 9; Fig. 1 and 2). Improvement in vegetative growth of 'Kinnow' mandarin trees can be attributed to the fact that moringa is highly rich in nutrients, phenolics, growth hormone, antioxidants, phytosterols and organic acids that play important role in cell growth, cell division and cell expansion (Yasmeen *et al.*, 2013; El-Hamied and El-Amary, 2015). Cytokinins (available in synthetic form as BAP) have direct effect on vegetative growth of trees as it directly involved in cell growth and division and stimulates xylem differentiation and vascular strand development, consequently resulting in more growth (Sorokin and Thiamnn, 1964; Frankenberger and Arshad, 1995). Ascorbic acid is associated with rapid cell division and cell wall extensibility (Smirnoff, 1996). While, K and Zn are among the most important nutrients required by citrus crop that influence activation of several enzymes which are

Table 6: Pearson correlation analysis of vegetative growth of 'Kinnow' mandarin trees after foliar application of 3% MLE, K and Zn (n = 24)

Characters	Tree height	Trunk width	Tree diameter	Leaf length	Leaf width	Leaf area	Flush length
Trunk Width	0.59**						
Tree diameter	-0.06	0.05					
Leaf length	0.61**	0.87***	0.12				
Leaf width	0.73***	0.63***	0.36	0.68***			
Leaf area	0.75***	0.79***	0.28	0.88***	0.94***		
Flush length	-0.29	-0.01	0.08	0.01	-0.05	-0.04	
Leaf age	0.66**	0.48*	0.34	0.53*	0.87***	0.81***	-0.12

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

Table 7: Influence of exogenous application of MLE, ascorbic acid and MLE+K+ Zn on vegetative growth of 'Kinnow' mandarin trees

Treatments	Increase in tree height (cm)	Increase in trunk diameter (cm)	Increase in tree width (cm)	Flush length (cm)
Control	4.52c	2.07d	16.8c	7.82c
3% MLE (premature stage)	7.53b	2.92b	34.8b	8.3ab
300 mg L^{-1} ascorbic acid (fruit set stage)	11.15a	2.61bc	32.2b	8.46a
3% MLE+ 0.25% K+ 0.6% Zn (fruit set stage)	11.00a	3.76a	47.0a	8.3ab
LSD ($P \leq 0.05$)	0.06	0.47	5.14	0.3

Means within a column followed by the same letter are not significant at $P \leq 0.05$. MLE = Moringa leaf extract

Table 8: Influence of exogenous application of MLE, ascorbic acid and MLE+K+ Zn on vegetative growth of 'Kinnow' mandarin trees

Treatments	Leaf length (cm)	Leaf width (cm)	Leaf area (cm^2)	Leaf age (No. of days)
Control	6.6	2.2	14.7	281.25b
3% MLE (premature stage)	6.8	2.3	15.7	352.75a
300 mg L^{-1} ascorbic acid (fruit set stage)	7.0	2.2	15.7	342.25a
3% MLE+ 0.25% K+ 0.6% Zn (fruit set stage)	7.0	2.2	16.2	368.25a
LSD ($P \leq 0.05$)	NS	NS	NS	13.96

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

Table 9: Pearson correlation analysis of vegetative growth of 'Kinnow' mandarin trees after foliar application of 3% MLE, ascorbic acid and MLE+K+ Zn (n = 24)

Characters	Tree height	Trunk Width	Tree diameter	Leaf length	Leaf width	Leaf area	Flush length
Trunk Width	0.76***						
Tree diameter	0.67**	0.9***					
Leaf length	0.66**	0.53*	0.36				
Leaf width	0.38	0.65**	0.62*	0.46			
Leaf area	0.64**	0.68**	0.55*	0.92***	0.78***		
Flush length	0.58*	0.52*	0.61*	0.39	0.68**	0.59	
Leaf age	0.67**	0.82***	0.67**	0.32	0.39	0.41	0.32

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

involved in photosynthesis (Alloway, 2008). It has been reported that reduction in photosynthesis, consequently reduced food reserves and adversely affect tree growth (Alloway, 2008; Ashraf *et al.*, 2010). However, among all the treatments best result regarding vegetative growth in 'Kinnow' mandarin was found in trees treated with combined foliar application of moringa leaf extract, K and Zn. Previous research showed that length, diameter of ten month old shoots and leaf area in pear trees were enhanced after foliar application of MLE (El-Hamiedand El-Amary, 2015). Similarly, exogenous application of MLE increased shoot length and leaf area in wheat (*Triticum aestivum* L.) (Yasmeen *et al.*, 2013); sacred basil (*Ocimum sanctum* L.) (Prabhu *et al.*, 2010); black nightshade (*Solanum nigrum* L.) (Sivakumar and Ponnusami, 2011), Cypress (*Cyperus rotundus* L.) (Ali *et al.*, 2015) and rocket plant (Abdalla, 2013), respectively. El-Shabasi *et al.* (2005) found that foliar application of amino acid and nutrients increased plant spread and number of leaves in 'Kinnow' mandarin. Foliar application of kinetin and ascorbic acid had also been reported to increase plant height, number of branches, number of leaves and stem diameter of croton plants (*Codiaeum varigaetum* L.) (Mazher *et al.*, 2011). Exogenous application of Zn and K had been found to increase vegetative growth of 'Washington Navel' orange, 'Valencia' orange and 'Balady' mandarin (Dawood *et al.*, 2001) and 'Kinnow' mandarin (Ashraf *et al.*, 2010; Khan *et al.*, 2015). Foliar application of

ascorbic acid had also been reported to increase plant height in almond tree (Amira, 2013). Similarly, increase in plant height and stem diameter was observed in *Adonis autumalis* with foliar spray of kinetin (Abdalla *et al.*, 1985). Thus, it can be concluded that exogenous application of MLE, K and Zn significantly improved the vegetative growth of 'Kinnow' mandarin trees.

Leaf age (number of days) in 'Kinnow' mandarin trees showed maximum increase with combined application of MLE, K and Zn as compared to other treatments. Leaf drop occurs due to chlorophyll degradation and elimination of Mg^{+2} from chlorophyllase (key enzyme in chlorophyll formation). Application of cytokinins prevents degradation of chlorophyll and increase chlorophyllase activity (Costa *et al.*, 2005). Ascorbates and phenolic compounds are primary antioxidants and direct scavengers of ROS (Buettner and Jurkiewicz, 1996), and reduction in ROS is directly related to delayed senescence. Since, MLE is an excellent natural source of cytokinins, ascorbate, phenolics and minerals (Moyo *et al.*, 2011), and when applied along with K and Zn it significantly improved leaf age in 'Kinnow' mandarin. Xu and Huang (2002) reported reduction in leaf senescence after application of cytokinins in bentgrass. Integrative application of maize grain extracts and Mg have been reported to improve sunflower vegetative growth (Rehman *et al.*, 2018). Increased leaf age (50% retention) has also been reported in 'Blood Red' sweet orange after spraying growth regulators (2-4, D + GA) (Saleem *et al.*, 2008). Xu *et al.* (2011) found that application of 6-BA delayed senescence in brocilli (*Brassica oleracea* L. ssp. *italica*). Hence, foliar application 3% MLE to 'Kinnow' mandarin trees improved vegetative growth.

Conclusion

Foliar application of 3% MLE is highly beneficial for vegetative growth of 'Kinnow' mandarin but when MLE is applied in combination with 0.25% K and 0.6% Zn at fruit set stage it improved the plant growth, leaf area, flush length and leaf age, respectively. Thus, it is recommended that foliar application of MLE + K + Zn at fruit set increase the vegetative growth and health of plant that later on directly influence the reproductive growth.

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