

Influence of Foliar Application with Moringa (*Moringa oleifera* L.) Leaf Extract on Yield and Fruit Quality of Hollywood Plum Cultivar

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Received date: February 06, 2017; Accepted date: February 21, 2017; Published date: February 28, 2017

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Abstract

This investigation was carried out during 2015 and 2016 seasons on twelve trees five years old of "Hollywood" plum trees (*Prunus salicina* Lindl.) grown in loamy clay soil in a private orchard at Ashmoun, Monofia, governorate, Egypt. Trees were sprayed with (0%, 4%, 5%, 6%) moringa (*Moringa oleifera* L.) leaf aqueous extract at full bloom stage+fruit setting stage+two weeks after fruit setting stage. Plums treated with 6% moringa leaf aqueous extract exhibited significantly higher setting, yield, fruit weight, firmness, color, Soluble Solid Content (SSC), Titrable Acidity (TA) ratio, ascorbic acid, anthocyanin content, antioxidant activity contents and reducing titrable acidity with reduced fruit drop as compared to all other treatments. It can be concluded that, foliar applications of 6% moringa leaf aqueous extract can be used effectively to improve fruit set, yield, fruit weight, firmness, color, soluble solids content, vitamin C, anthocyanin content and antioxidant activity of "Hollywood" plum.

Keywords: Aqueous extract; Spraying; Fruit set; Ascorbic acid; Anthocyanin; Antioxidant

Introduction

Plums belong to the Rosaceae family and include the European species (*Prunus domestica* L.), which is consumed fresh or dried, and the Japanese species (*Prunus salicina* L.), mainly freshly consumed. Hollywood (*P. salicina* L.) plum is one of the Japanese varieties that grow well under the Egyptian environmental conditions due to their low chilling requirements compared to the European plums. The total area of plum in Egypt was 1693 hectares in 2000 and reached 1034 hectares in 2012, and the average yield productivity was 10.337 ton ha⁻¹ in 2000, and reached 7.133 ton ha⁻¹ in 2012 [1]. Plum is a highly rich in vitamins A, C and E, fibre, mineral nutrients (potassium, fluoride and iron), flavonoids and phenolic acids, which may function as an effective natural antioxidant beside low calorie content. These phytochemical compounds have great contributions to colour, taste and flavor of plum. Most of them also have anti-oxidative, anti-carcinogenic, anti-microbial, anti-allergic, anti-mutagenic and anti-inflammatory impacts [2-4]. Therefore, consumers should prefer the foods with high phytochemical contents such as plums in their daily diets. Increase in its yield is very vital. One of the strategies in achieving yield increase is the use of biostimulants.

The nutritional quality of food raised by organic farming in comparison to traditional farming is a current issue that continues to attract interest and generate discussion. Consumers regard organic foods not only as better, but also a safe, more hygienic, and free of chemical residues and artificial ingredients [5]. Organic fertilizers not only increase physical (porosity, structure and water-holding capacity) and chemical properties of soil but also increase mineral deposition, which is essential for proper development of plants [6]. Therefore, application of organic fertilizer has received great attention especially among the farmers [7]. In fact, nutrient management through organic resources is very essential for crops [8].

Nowadays farmers are well aware about the application of organic fertilizer to improve their production as well as farming land [6]. In order to fill the demand of organic fertilizer, one of such option is use of *Moringa oleifera* leaf extracts as fertilizer [9].

M. oleifera is one of the 13 species of genus *Moringa* and family Moringaceae. It is well known vegetable in Africa, Arabia, India, America and Pakistan [10]. Moringa leaves are potential source of vitamins (A, B, C), essential minerals (K, Ca, Fe) and amino acids [11]. Its leaves contain powerful natural antioxidants (Ascorbate, Phenolics) [12]. Moreover, moringa leaf extract is enriched with cytokinins, auxins and Abscisic Acid (ABA) like growth substances [13]. Hence, its leaf extract in water contains growth enhancing substances and can be used as biostimulants [14].

Many investigations proved that foliar application of *M. oleifera* leaf extract beneficial for the vigour growth [7], deeper root development and better seed germination [8], delay of fruit senescence and improve yield quality/quantity [15-18] and also impart on the ability of crops to withstand adverse environmental conditions [7]. Moreover, plants treated with *Moringa oleifera* leaf extract exhibited more pest and disease resistance [19]. Hence, this study aimed to study evaluates foliar application of *M. oleifera* leaf extract in enhancing fruit set, yield and quality of Hollywood plum.

Materials and Methods

Plant materials

This investigation was carried out during 2015 and 2016 seasons on twelve trees five years old of "Hollywood" plum (*Prunus salicina* Lindl.) budded on Marianna plum rootstock and planted at 5 × 5 m in loamy clay soil under surface irrigation system in a private orchard at Ashmoun, Monofia, governorate, Egypt. Trees were normal growth, uniform in vigour, trained on open vase training system and were received uniform management practices. Selected "Hollywood" plum

trees were sprayed at full bloom stage+fruit setting stage+two weeks after fruit setting stage with moringa (*Moringa oleifera* L.) leaf extract aqueous.

Chemical components	Leaf Powder
Moisture (%)	7.5
Protein (g)	27.1
Fat (g)	2.3
Carbohydrate (g)	38.2
Fiber (g)	19.2
Calcium (mg)	2.003
Magnesium (mg)	368
Phosphorus (mg)	204
Potassium (mg)	1.324
Copper (mg)	0.6
Iron (mg)	28.2
Sulfur(mg)	870
Vitamin A (β-carotene) (mg)	16.3
Vitamin B1 (thiamine) (mg)	2.6
Vitamin B2 (riboflavin) (mg)	20.5
Vitamin B3 (nicotinic acid) (mg)	8.2
Vitamin C (ascorbic acid) (mg)	17.3
Vitamin E (tochopherol acetate) (mg)	113
Arginine (g/16 g N)	1.33
Histidine (g/16 g N)	0.61
Lysine (g/16 g N)	1.32
Tryptophan (g/16 g N)	0.43
Phenylalanine (g/16 g N)	1.39
Methionine (g/16 g N)	2
Threonine (g/16 g N)	1.19
Leucine (g/16 g N)	1.95
Isoleucine (g/16 g N)	0.83
Valine (g/16 g N)	1.06

Table 1: Chemical analysis of 100 g *Moringa oleifera* leaves powder.

The aqueous extract of moringa leaves were prepared by soaking of 100 g powder air-dried *Moringa oleifera* leaves in 1 liter of water for 24 hrs and filtered out; then diluted with water in the following concentrations: 0%, 4%, 5%, 6% and sprayed directly onto trees thoroughly sprayed till run off. Tween-20 at 0.01% was added as a surfactant. The chemical analysis of *Moringa oleifera* dried leaf powder by Fuglie [20] as shown in Table 1. The experiment followed complete

randomized block design with three replicates for each treatment (4 treatments × 3 replicates=12 trees).

Fruit set percentage

Two years old shoot was selected from each tree for recording data of total number of flowers at full-bloom in March and number of set fruits in April of both years of study. These data were used in calculating the percentage of fruit set using the following equation:

$$\text{Fruit set (\%)} = \frac{\text{Number of developing fruitlet} \times 100}{\text{Total number of flowers}}$$

Fruit drop percentage: Fruit drop percentage was calculated by following formula:

$$\text{Fruit drop (\%)} = \frac{\text{Number of fruit at final harvest} \times 100}{\text{Total number of initial fruit set}}$$

Determination of yield: Fruits were harvested at maturity stage (the first week of June) from each tree of various replicates and yield was recorded as a number of fruits/tree and weight in Kilograms.

Quality assessments: Samples of 10 randomly mature fruits from each experimental unit were used for measuring the physical and chemical fruit quality.

Physical fruit quality

Fruit weight, length and diameters.

Fruit firmness: Fruit firmness was determined as (Lb/inch 2) by using fruit pressure tester mod. FT 327 (3-27 Lbs).

Fruit color: Ten fruits from each tree were used to determine the color characteristics [L*, a*, b* and hue angle (h°)]. Changes in fruit color characteristics were measured at opposite sides of each fruit with a colorimeter (Minolta Co. Ltd., Osaka, Japan) on the basis of the CIELAB Color system. Values of L*, a* and b* were used to define a three-dimensional color space and interpreted as follows: L* indicates lightness with values ranging from 0 (completely opaque or 'black') to 100 (completely transparent or 'white'), a* value indicates (green-red) and b* value indicates (blue - yellow). The hue angle (h°) expresses the color nuance and values are defined as follows: red-purple: 0°; yellow: 90°; bluish green: 180°; blue: 270°. The hue angle was calculated with the formula $h^\circ = \tan^{-1}(b^*/a^*)$ [21].

Biochemical fruit quality

Soluble Solids content (SSC%): Abbe refractometer was used to determine the percentage of total soluble solids in fruit juice.

Titrateable acidity% (TA): Titrateable acidity% was determined by titrating the juice against 0.1 N sodium hydroxide using phenolphthalein as an indicator. Results were expressed as percentage of malic acid in fresh pulp weight was determined according to A.O.A.C [22]. Ascorbic acid contents (Vitamin C): Ascorbic acid (mg/100 g FW) in fruit juice was determined by using the dye 2, 6-dichlorophenyl indophenols method described in A.O.A.C [22].

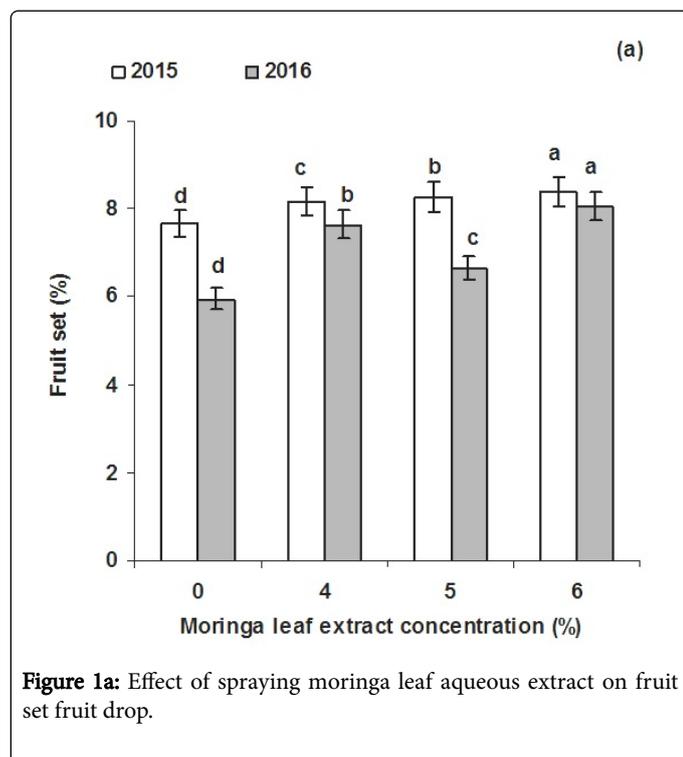
Anthocyanin content: Anthocyanin of plum peel was expressed as mg/100 g fresh weight according to Fuleki and Francis [23].

Antioxidant activity: Antioxidant activity was determined by the DPPH (1, 1-diphenyl-2- pycrylhydrazyl) method described by Ismail et al. [24].

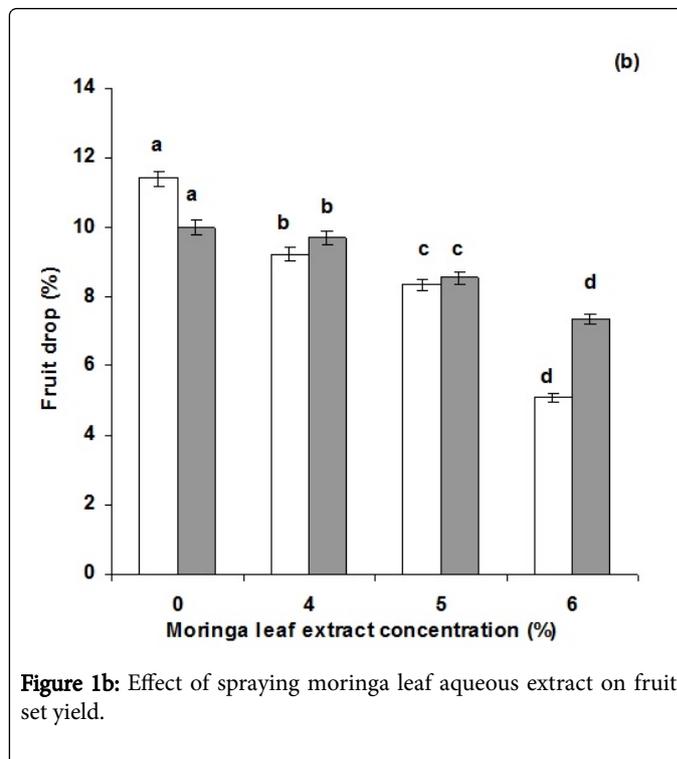
Statistical analysis: The data were statistically analyzed using MSTAT-C computer software version 2.10 (Michigan State University, East Lansing, Mc, USA). ANOVA (analysis of variance) were used to test the overall significance of data, while the LSD test (least significant difference) was used to compare between means of treatments by Duncan's multiple range tests at a significance level of 0.05%.

Results and Discussion

Fruit set percentage: Foliar application of moringa leaf aqueous extract at 4%, 5%, 6% significantly ($P=0.05$) increased the fruit set percentage compared to untreated 0% (Figure 1a). The highest fruit set percentage was obtained with spray 6% moringa leaf aqueous extract (8.39% and 8.05%) in the both seasons, respectively. Meanwhile, the lowest fruit set percentage was obtained with 0% moringa leaf aqueous extract (7.67% and 5.95%) during 2015 and 2016 seasons respectively.



Fruit drop percentage: Treated trees showed significant decrease in fruit drop percentage of "Hollywood" plum as compared with control trees during both seasons. Minimum fruit drop was recorded in trees treated with 6% moringa leaf aqueous extract (5.09%) in the first season and (7.34%) in the second season (Figure 1b), compared with maximum fruit drop (11.40% and 10.01%) in 0% *Moringa oleifera* leaf aqueous extract during first and second seasons, respectively.



The increase in fruit set and decrease fruit drop percentage of 'Hollywood' plum trees might be attributed to the content of moringa extract from proteins, vitamins such as A, B1, B2, B3, C and E, β carotene, phenolic, sugars, and minerals such as calcium, magnesium, sodium, iron, phosphorus and potassium and several hormones as auxin, gibberellins and cytokinins which regulate internal mechanism for controlling fruit set and abscission of ovaries [25]. These finding agreed with Nasira et al. [18], who found that foliar application of moringa leaf extract at fruit set stage increased the fruit set and reduced fruit drop.

Fruit yield: Fruit yield (Kg/tree) was significantly ($P=0.05$) increased with foliar application with different concentrations of moringa leaf aqueous extract in both the two seasons (Figure 1c). Highest yield was recorded with 6% followed by 5% then 4% moringa leaf aqueous extract (58, 50 and 47 Kg/tree) in the first season, respectively and (59, 55 and 49 Kg/tree) in the second season, respectively. Whereas, the lowest yield was recorded with 0% moringa leaf aqueous extract (35 and 40 Kg/tree) during 2015 and 2016 seasons.

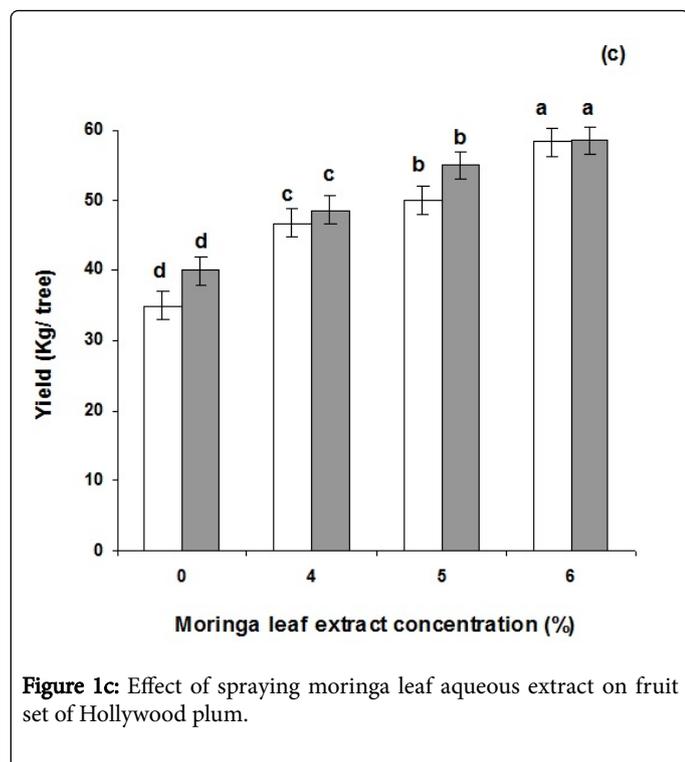


Figure 1c: Effect of spraying moringa leaf aqueous extract on fruit set of Hollywood plum.

This can be attributed to the moringa leaf aqueous extract content of high minerals and hormones which are directly/indirectly involved in fruit growth and development process and consequently increase number of fruit/tree [26,27]. Moreover, enhanced fruit set and reduced fruit drop reflected increase yield. These results are in agreement with the results obtained by Sheren and El-Amary [17] and Nasira et al. [18]. They reported that foliar application of moringa leaf extract increased the yield (weight and total number of fruits, total number and percentage of marketable fruit; and decrease in number and percentage unmarketable fruits.

Physical fruit quality

Fruit weight: Fruit weight was significantly increased in “Hollywood” plum fruit harvested from the trees treated with moringa leaf aqueous extract at 4%, 5%, 6% in the two seasons of the study (Table 2). Fruit weight ranged (43.98-47.69 g) and (48.02-44.56 g) during 2015 and 2016 seasons, respectively. While, 0% moringa leaf aqueous extract (42.35 g and 41.39 g) in 2015 and 2016 seasons respectively.

Concentration of <i>Moringa oleifera</i> leaf aqueous extract (%)	Fruit weight (g)	Fruit length (cm)	Fruit diameter (cm)	Firmness (Lb/inch ²)	Fruit color	
					L* value	h° value
2015 season						
0	42.35 ^d	3.27 ^b	3.77 ^a	3.47 ^d	33.73 ^b	55.74 ^c
4	43.98 ^c	3.63 ^a	3.77 ^a	4.10 ^c	34.22 ^a	55.94 ^c
5	46.23 ^b	3.60 ^{ab}	4.07 ^a	4.90 ^b	34.41 ^b	59.74 ^b
6	47.69 ^a	3.80 ^a	4.37 ^a	5.27 ^a	39.26 ^a	61.14 ^a
2016 season						
0	41.39 ^d	3.30 ^b	3.79 ^c	3.61 ^b	37.06 ^d	56.40 ^d
4	44.56 ^c	3.71 ^a	3.92 ^{bc}	4.13 ^b	37.55 ^c	58.27 ^c
5	47.36 ^b	3.72 ^a	4.16 ^b	4.95 ^a	37.75 ^b	60.07 ^b
6	48.02 ^a	3.88 ^a	4.50 ^a	5.38 ^a	45.93 ^a	64.48 ^a
The difference between mean values shown on the same column with same letter is not significant according to Duncan's multiple range test at P<0.05						

Table 2: Effect of spraying moringa leaf aqueous extract on physical fruit quality of Hollywood plum.

Fruit length: Concerning fruit length Table 2, it was observed that there were no significant differences between concentration 4% and 6% in the first season and all concentrations of moringa leaf aqueous extract in second season.

Fruit diameter: Regarding fruit diameter, application of moringa leaf aqueous extract at 4, 5 or 6% to “Hollywood” plum had no significant (P=0.05) effect on fruit diameter compared to control trees 0% in the first season (Table 2). In second season, application of

moringa leaf aqueous extract at 6% gave the highest significant fruit diameter (4.50 cm) meanwhile 0% gave the lowest significant fruit diameter (3.79 cm).

This increase in fruit weight, length and diameter fruit weight, length and diameter are ascribed to the high level of potassium and zinc in moringa leaf aqueous extract. Potassium improves fruit quality by enhancing the formation and translocation of carbohydrates from the shoot to storage organs (fruits) and carbohydrate enzymes [28].

Zinc is precursor of tryptophan which is involved in synthesis of indole acetic acid that is required for fruit growth and development [29]. In addition to, the high content of cytokinin-like substances which plays a vital role in cell division and expansion, thus finally increased the fruit weight, length and diameter. Similarly, in 'Le Conte' pear fruit weight, size and volume increased by foliar application of moringa leaf aqueous extract [17]. Fruit weight, size, juice weight and pulp weight of 'Kinnow' mandarin was significantly increased with foliar application of moringa leaf extract at fruit set stage [18].

Fruit firmness: The results revealed that foliar application with moringa leaf aqueous extract significantly increased firmness of "Hollywood" plum (Table 2). Highest firmness was recorded in fruit harvested from trees treated with 6% moringa leaf aqueous extract (5.27 and 5.38 Lb/inch²) in first and second seasons respectively. Meanwhile, lowest firmness was recorded in fruit harvested from untreated trees 0% moringa leaf aqueous extract (3.47 and 3.61 Lb/inch²) during 2015 and 2016 seasons respectively.

Fruit firmness is the most important indicator for shelf life, preservation potential, consumer satisfaction and market value of the fruits [30]. The increase in fruit firmness might be due to the high calcium content in moringa leaf extract [31]. Since calcium play a very important role in structure of cell wall, thus contributing to the firmness of fruit tissue [32] prevents physiological disorders, reduce rate of respiration, maintaining firmness and slow down ripening process [33], thus prolonging shelf life of fruits.

Fruit color: Data in Table 2 showed that lightness (L*) and hue angle (h°) of "Hollywood" fruits were significantly increased with foliar moringa leaf aqueous extract. Application of 6% moringa leaf aqueous extract gave the highest values of L* and h° (39.26 and 61.14 in the first

and 45.93 and 64.48 in the second season respectively), while untreated trees 0% moringa leaf aqueous extract recorded the lowest values of L* and h° (33.73 & 55.74 and 37.06 & 56.40 in the two seasons respectively). Fruit color plays an important role towards consumer attraction. Plum fruit color is associated with the accumulation of carotenoids and anthocyanins. Both groups of pigments are more abundant in the peel but anthocyanins are mainly responsible for the surface color of the fruit. The main anthocyanins present in plums are cyanidin 3-rutinoside, cyanidin 3-glucoside and peonidin 3-rutinoside [2-3]. Applying moringa leaf aqueous extract significantly increased lightness (L*) and hue angle (h°) of "Hollywood" fruits. This increase may be to increase anthocyanin content (Table 3) as a result of application with moringa leaf aqueous extract to be due to the extract is rich in minerals which enhanced the activity of enzymes hence appearance of colored pigments.

Biochemical fruit quality

Table 3 shows the effects of moringa leaf aqueous extract on biochemical fruit quality of "Hollywood" plum in the 2015 and 2016 seasons.

Soluble Solids Content (SSC%): Application of different concentrations of moringa leaf aqueous extract significantly increased the soluble solids content in both seasons of the study. Highest soluble solids content were observed from foliar application 6% moringa leaf aqueous extract (14.87 and 14.95%) in first and second seasons respectively, whereas lowest soluble solids content were noted in control 0% moringa leaf aqueous extract (13.67 and 13.66%) during 2015 and 2016 seasons respectively (Table 3).

Concentration of <i>Moringa oleifera</i> leaf aqueous extract (%)	SSC (%)	TA (%)	SSC/TA Ratio	Ascorbic Acid (mg/100 g FW)	Anthocyanin (mg/100 g FW)
2015 season					
0	13.67 ^c	0.50 ^a	29.09 ^c	6.70 ^d	6.37 ^d
4	13.83 ^{bc}	0.47 ^{ab}	41.91 ^{bc}	7.06 ^c	7.13 ^c
5	14.10 ^b	0.33 ^b	42.73 ^b	7.60 ^b	8.05 ^b
6	14.87 ^a	0.33 ^b	45.06 ^a	8.77 ^a	9.42 ^a
2016 season					
0	13.66 ^d	0.43 ^a	34.15 ^c	7.02 ^b	6.45 ^d
4	14.15 ^c	0.40 ^{ab}	47.17 ^b	7.49 ^{ab}	7.23 ^a
5	14.50 ^b	0.30 ^b	48.33 ^{ab}	7.93 ^{ab}	8.10 ^b
6	14.95 ^a	0.30 ^b	49.83 ^a	9.11 ^a	9.50 ^a
The difference between mean values shown on the same column with same letter is not significant according to Duncan's multiple range test at P<0.05					

Table 3: Effect of spraying moringa leaf aqueous extract on biochemical fruit quality of Hollywood plum

Titrateable Acidity% (TA): Titrateable acidity percentage was decreased with increase moringa leaf aqueous extract concentration. There was no significant difference among applying the extract at 5% and 6% in both seasons.

SSC/TA Ratio: Soluble solids content/titrateable acidity ratio significantly increased with increase moringa leaf aqueous extract

concentration. Highest SSC/TA ratio were observed from foliar application 6% moringa leaf aqueous extract (45.06 and 49.83) in first and second seasons, whereas lowest SSC/TA ratio were recorded in 0% moringa leaf aqueous extract (29.09 and 34.15) during 2015 and 2016 seasons (Table 3).

The enhance of soluble solids content, titratable acidity and ratio of soluble solids content/titratable in "Hollywood" plum as a response to applications with moringa leaf aqueous extract was due to the high sugar and starch content of *Moringa oleifera* leaves (Table 1). Besides that, the leaves extract have high levels of cytokinins. Cytokinins promote carbohydrate metabolism and create new source-sink relationships leading to increase fruit soluble solids content [34].

Ascorbic acid contents (vitamin C): All the moringa leaf aqueous extracts concentrations significantly ($p=0.05$) increased the ascorbic acid content of "Hollywood" fruits (Table 3). Highest content of ascorbic acid was observed with foliar application 6% moringa leaf aqueous extract (8.77 and 9.11 mg/100 ml) during 2015 and 2016 seasons respectively. While, lowest value was observed with 0% moringa leaf aqueous extract (6.70 and 7.02 mg/100 ml) during 2015 and 2016 seasons respectively. Since moringa has ascorbate so its exogenous application might be triggered the endogenous production of ascorbate [35] and was involved in sugar metabolism which are directly related to production of vitamin C [36]. These results are in agreement with Sheren and El-Amary [17] on 'Le Conte' pear and Nasira et al. [18] on 'Kinnow' mandarin finds that foliar application of moringa leaf aqueous extract increased vitamin C.

Anthocyanin content: There was highly significant effect of moringa leaf aqueous extract on anthocyanin of plum peel (Table 3). Aqueous extract of moringa at 6% gave highest value (9.42 and 9.50 mg/100 ml) in the first and second season respectively. Meanwhile, 0% moringa leaf aqueous extract gave lowest value (6.37 and 6.45 mg/100 ml) during 2015 and 2016 seasons respectively.

Antioxidant activity: The data indicated that the antioxidant activity increased significantly during both seasons as a result of application moringa leaf aqueous extract (Figure 2). There was a significant difference ($p<0.05$) between 0% and different concentrations of moringa leaf aqueous extract in terms of their effects on level of antioxidant activity. During both seasons, foliar application 6% moringa leaf aqueous extract had the highest antioxidant activity (38.11 and 39.35%) and 0% moringa leaf aqueous extract had the lowest antioxidant activity (35 and 36.11%) respectively.

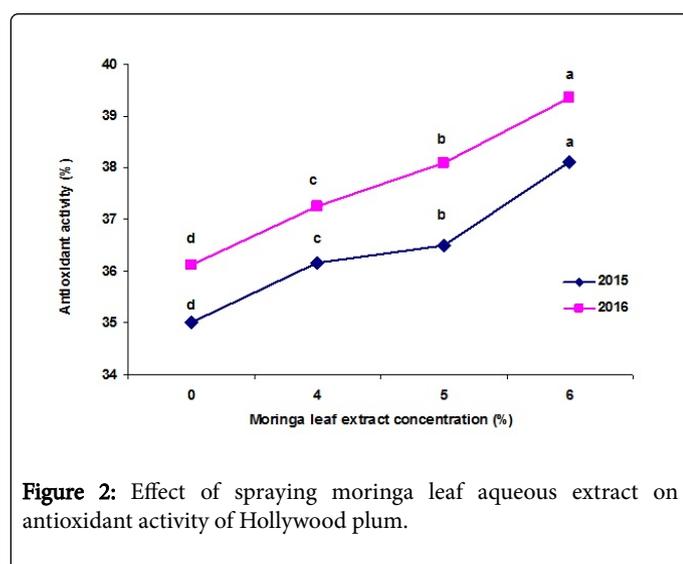


Figure 2: Effect of spraying moringa leaf aqueous extract on antioxidant activity of Hollywood plum.

The increase in antioxidant activity might be due to the extract of moringa leaf high antioxidants content (tocopherols, carotenoids, ascorbic acid, flavonoids and different other phenolic compounds)

[37]; when applied to the tree effect on the metabolic process and subsequently increased the endogenous level of antioxidants. Additionally, foliar application of moringa leaf aqueous extract increased anthocyanidin content (Table 3) which considered natural antioxidants [38]. Moreover, increase ascorbic acid (Table 3) which closely correlated with antioxidant activity [39]. These results are in line with Nasira et al. [18] who reported that aqueous extract of moringa increased the antioxidants of 'Kinnow' mandarin.

Conclusion

Foliar application of moringa leaf aqueous extract decreased the fruit drop, increased fruit set, yield, fruit weight, firmness, color, soluble solids content, vitamin C, anthocyanin content and antioxidant activity of Hollywood plum. Therefore, it may be concluded that foliar application with moringa leaf aqueous extract 6% at full bloom stage +fruit setting stage+two weeks after fruit setting stage as a bio-stimulants cheap source of plant growth hormones and minerals especially with the trend of organic farming for improved yield and quality of Hollywood plum and other horticulture crops.

Acknowledgment

This study has been supported by the project (ID, 5979) "Recent approaches in the utilization of *Moringa oleifera* and Moringa Peregrine as a good nutritional, medicinal and industrial plant in Egypt" financed by STDF.

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