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Effect of Tryptophan and Some Nutrient Elements Foliar Application on Yield and Fruit Quality of Washington Navel Orange

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Abstract: The present study was carried out on fruitful Washington navel orange trees budded on sour orange rootstock planted on clay loamy soil with flood irrigation in El-Qalyobia governorate during two successive seasons 2013 and 2014 to study the effect of foliar application with tryptophan (25 &50 ppm), amino calcium (1%) and potassium nitrate (0.5%) on: fruit set percentage, size of navel-end opening, fruit yield and fruit quality of "Washington" navel orange. Generally, results revealed that, tryptophan at 25 and 50 ppm, were the highest value concerning fruit set percentage, yield (kg/ tree) and improved vegetative growth characteristics. Whereas, amino calcium treatment showed the highest percentage of closed navel- end opening fruits followed by tryptophan (25 & 50 ppm). While, potassium nitrate treatment significantly increased fruit total soluble solids.On the other hand, leaf chemical properties total sugars, tryptophan, indoles, N, P, K, Ca and B were determined and reflect varied response with varied treatments.

Key words: Navel end opining · Tryptophan · Amino calcium · Potassium nitrate

INTRODUCTION

Citrus is one of the most important world fruit crops. Citrus in Egypt is ranked as the first fruit crop. According to Central Administration of Horticulture and Agricultural Crops [1]. 2015 report, the total navel orange cultivated area is 179,876 fed. and the total production is 1,697,222 ton.

Navel orange [*Citrus sinensis* (L.) Osbeck] is characterized by, the presence of small secondary fruit, exist within the primary fruit and an opening at the stylar-end called, navel-end opening. The navel, extended as, a whorl of secondary-carpel primordial, within the primary-carpel, when the length of flower bud is 1.5 to 2.0 mm. In the navel oranges production , problems may be related with the presence of the navel and the size of the navel-end opening, as stylar-end decay, fruit splitting and secondary fruit yellowing that may cause fruit abscission before maturity [2].

In private citrus orchard located in El-Qalyobia Governorate, Egypt during navel orange harvesting time it was observed that, many fruits have abnormal navel "secondary fruit" appearance with different size, the navel may protrude outside the primary fruit at the styler-end, also more fruits have navel-end opening at the styler-end with diverse dimensions. The incidence of rind splitting which extended from the navel side could be observed in lots of fruits. high percentage of these deformed navel orange disorder reduced fruit class for exporting or local marketing and we may loss our superiority in the world marketing and demand, for Egyptian unique navel orange fruit. Therefore we did this trail to try to reduce the percentage of navel size and navel end opening in navel oranges in Egypt.

Dimensions of navel-end opening have important consideration for navel orange fruit quality. Reducing navel-end opening size is very important goal for navel oranges production, mainly for exportation and as well as local consumption [3]. Although, the navel-end size is a genetic trait it is affected by environmental conditions. As irregular water relations and Climate conditions after fruit set may influence the navel-end size, if a hot day occurs during the period when the fruit are more than 40 mm, the moisture will be withdrawn from the fruit by the wilting leaves and after irrigation the water may be returned faster than the ability of the rind to stretch causing cracks at the navel-end and as the fruit grows, the cracks expand and start to open up resulting in an open navel-end [3, 4].

IAA can be synthesized through the precursor amino acid, tryptophan. The conversion of tryptophan to indole acetic acid in lime fruit has been demonstrated by Rashad [5], he supplied lime fruits with radioactive

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tryptophan-3-14C in a special medium and incubation for six hours then analyse the lime fruit tissue and the medium for metabolites of tryptophan, analyses indicates existence of indolic products.

Recently the first complete pathway that convert tryptophan to IAA, have been well established. Tryptophan is first converted to indole-3-pyruvate (IPA) by the "tryptophan aminotransferase enzyme" and then (IPA) is converted to IAA, by the "flavin monooxygenases enzyme" [6].

Carol Lovatt [7] has approved the use of tryptophan and analogs as a plant growth regulator, he reported that leaves, flowers and fruits of 'Washington' navel orange take up, translocate and convert tryptophan to IAA. In addition, the application of tryptophan to 'Frost' navel orange and Fina Clementine trees at the end of fruit cell division stage, significantly increased the cumulative total yield with no negative effect on fruit quality [8]. In the same concern, foliar sprays of tryptophan improved tree yield and fruit quality of Valencia orange [9]. Thus tryptophan may be a safe and cost-effective, substitute for synthetic auxins as 2, 4-D, to be used in commercial citrus production.

Calcium ion plays a key role during cell wall formation in cross-linking acidic pectin residues and retard tissue senescence, through cross-linking pectates and cementing cell walls [10]. Also calcium provides structural integrity to cellular membranes, as calcium ion stabilizes lipid bilayers, by binding to phospholipids and so, calcium ion controls membrane permeability [11].

Amino acids plays very important and specific role in plant, it represent the building blocks of proteins and have central role in nitrogen metabolism providing molecules that shuttle organic nitrogen through the plant, besides that, amino acids restore the specific enzymes for protein synthesis and play fundamental roles during signalling processes , actually some amino acids act as signalling molecules themselves and others are precursors for the synthesis of phytohormones or other secondary metabolites with signalling function. [12-14].

Potassium is essential for vital physiological functions as starch formation, transport of sugars and proteins synthesis. Citrus fruits remove great amounts of potassium compared with other nutrients potassium has principal effects on external and internal citrus fruit qualities [15, 16].

The objective of this study was to evaluate the effect of tryptophan and some nutrient elements application on Washington navel orange fruit quality and specifically navel end abnormality.

MATERIALS AND METHODS

The present study was carried out during two successive seasons 2013 and 2014 in a private citrus orchard in (El-Qalyobia Governorate, Egypt). 40 years- old Washington navel orange trees (*Citrus Sinensis* (L.), Osbek), budded on sour orange rootstock (*Citrus aurantium*), grown on clay loamy soil at 5 × 5 m under flood irrigation system, were used to investigate the influence of foliar spray with treatments under study on fruit quality of Washington navel orange fruits and specifically navel-end opening fruit characteristics. Forty five trees (three replicates with three trees at each replicate) were subjected to five investigated treatments as follows: Control (Uniform fertilizer program). tryptophan at 25 ppm., tryptophan at 50 ppm., amino calcium (A commercial product contain: 9% amino acids and 6% calcium oxide) at1% and potassium nitrate (KNO_3, K_2O 40%) at 0.5%.

Tryptophan treatments (at 25 ppm or 50 ppm) and amino calcium 1% treatment were applied as foliar sprays at full bloom stage and fruitlets cell division stage. Whereas, potassium nitrate 0.5% treatment was applied as foliar sprays, twice a season, at fruit cell expansion stage and at color break (beginning of maturity) stage, respectively.

A complete randomized block design was used, each treatment was replicated three times one tree for each replicate, thus the total number of trees in this experiment was 45 (5 treatments X 3 replicate X 3 tree in each replicate).
The following parameters were investigated:

Vegetative Growth: About ten newly develop twigs from vegetative buds outgrowth of spring cycle shoots were used at the first week of September to study the following vegetative growth parameters: Shoot length (cm), shoot diameter (mm), number of leaves/shoot and leaf area (cm^2).

Fruit Set: Total number of flowers and number of fruitlets were counted then fruit set percentage (%) was calculated according to the equation:

Fruit set% = (number of fruitlets/ number of flowers) ×100

Yield per Tree: At harvest (first week of December under these experimental conditions) fruits of each tree were harvested to estimate yield as number and kg.

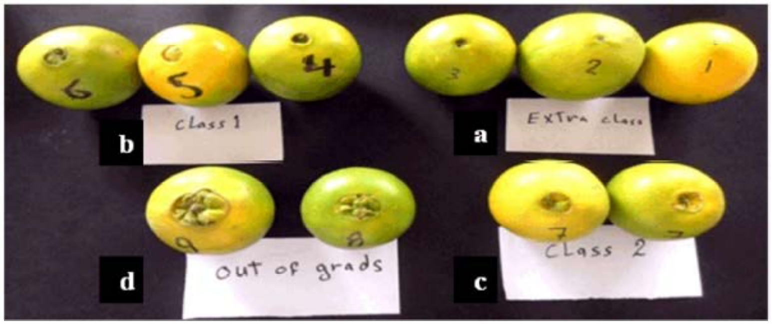

Fig. 1: Index for navel opening size: (a) extra class (b) class 1 (c) class2 (d) out of grade

Navel Size and Navel End Opening: At harvest time fruits samples were collected from different trees and navels opening were measured then fruits were graded and divided into four categories according to the size of navel opening as shown in Fig. (1): (a) extra class (b) class 1 (c) class 2 (d) out of grade.

- "Extra" Class (superior fruit quality): fruits must be free from any defects or protruding navels, with less than 5% exception of small sized navel opening.
- Class 1: fruits may tiny slight defects that do not affect the general appearance and quality of product such as slight navel opening size and protrusion. A total acceptance not more than10 %, of fruit that not agreeable requirements of the class I but meeting those of class II is allowed.
- Class 2: This class include unqualified fruits for insertion in higher classes but prove to minimum requirements specified for navel orange fruits. Some defects like small size navel opening and small navels protrusion fruits.
- Out of grade fruits: this category contains all fruits with more than 10 % of mentioned defects in previous three categories.

These grading scale based on the: International standards for fruit and vegetables by OECD (2010)– standard recommended by UN/ ECE under reference FFV-14(The Citrus fruit standard). [17]

Fruit Quality: After Navel size and navel end opening were examined in the field, 10 fruits were taken from each tree to determine fruit quality in laboratory.

Fruit Physical Properties: Average fruit weight (g), average fruit size (cm^3), fruit length and diameter (cm) were measured and fruit shape index (length/diameter) was calculated, fruit peel thickness (cm), fruit firmness (l.b/ $inches^2$) were measured.

Fruit Chemical Properties: T.S.S. %, acidity % (as mg citric acid/100 cm^3 juice), T.S.S./ acid ratio and vitamin C (ascorbic acid as mg/100ml juice) were determined according to A.O.A.C.[18]

Leaf Chemical Composition: Total indols was determined in fresh leaves three times (at April, June and August) according to Larsen [19] and total sugars was determined by 3, 5-dinitrosalicylic acid according to Miller [20]. Tryptophan was determined according to Spies and Chambers [21], as well as N, P, K, Ca and B were determinate in dry matter of leaves. Total N% was determined by semi-micro Kjeldahl method described by Plummer [22]. Phosphorus was estimated colorimeterically by using the chlorostannous reduced molybdophosphoric blue colour method as described by King [23]. Potassium and calcium concentrations were determined by using the flame photometer. Boron was determined by the atomic absorption spectrophotometer.

Statistical Analysis: Obtained data was statistically analyzed to determine the analysis of variance and significant differences between means according to Snedecor and Cochran [24], The multiple comparisons of means were performed according to Duncan's multiple test range [25] using MSTAT-C software [26].

RESULTS AND DISCUSSION

Vegetative Growth Characteristics: It is generally clear from the results in Table (1) that, at the two experimental seasons the highest values of vegetative growth characters (shoot length, shoot diameter, number of leaves and leaf area) tended to be recorded with tryptophan at 50 ppm treatment, followed by tryptophan at 25 ppm treatment when compared with control one, with some exceptions. The results are in agreement with those obtained by Hanafy Ahmed *et al*, [9] who found a gradual increase in most of the growth characters of Valencia

Table 1: Effect of treatments on vegetative growth characteristics of Washington navel orange trees

Treatments	Shoot length(cm)	Shoot diameter(mm)	Leaves No.	Leaf area (cm^2)
	First season			
Control	18.67 d	0.27 c	11.25 b	22.54 b
Tryptophan 25 ppm	22.67 a	0.31 b	12.95 b	29.07 a
Tryptophan 50 ppm	23.08 a	0.35 a	14.87 a	32.93 a
Amino calcium	20.34 c	0.29 b	12.25 b	24.49 b
Potassium nitrate	21.57 b	0.30 b	12.28 b	26. 68 b
	Second season			
Control	19.19 b	0.30 b	11.67 c	21.06 c
Tryptophan 25 ppm	24.09 a	0.32 ab	14.93 a	27.06 b
Tryptophan 50 ppm	23.96 a	0.33 a	15.33 a	31.64 a
Amino calcium	20.92 b	0.30 b	12.67 b	25.97 b
Potassium nitrate	21.83 b	0.31 b	12.44 b	24.03 b

Means in each colum fallowed by the same letter·s did not differ at $p<0.05$ according to Duncans multiple range tests

orange trees with increasing rate of tryptophan foliar applications (25, 50 and 100 ppm).

In this respect, the effect of tryptophan treatment may be due to the role of tryptophan affecting gene expression for producing the specific macromolecules required for permanent cell elongation [27].

Moreover, stem elongation which was generally observed on applying tryptophan to (Mentha viridis and Mentha longifolia) explained by the conversion of tryptophan to IAA [28]. It appears that, there is a relationship between IAA translocated basipetally and vigour of the different rootstocks, that basipetal IAA translocation used to predicate the vegetative vigour of citrus rootstocks [29] .There is a positive relationship between IAA and GA_3 and the growth of plant organs as well as the development of natural growth regulators in plants particularly in citrus fruits [30].

Fruit Set: Data represented in Table (2) demonstrated that, tryptophan at 25or 50ppm significantly increased fruit set percentage, followed by amino calcium treatment as compared with control for both seasons of study that, effect may be due to reduction in flower abscission and consequently increasing fruit set.

Concerning the effect of tryptophan, it has been assumed that endogenous IAA block the ability of ethylene to induce abscission proceedings [31]. Since IAA may inhibit the action of ethylene on the expression of the, ethylene biosynthetic genes, as suggested by [32]. Additionally, auxins production prevents abscission in flowers and flower parts by mechanism, similar to what occur during leave abscission, since leaf abscission increased by removal of the distal leaf part, that effect was reduced by applying auxin at the cut end, there for, it has been hypothesized that , normal continuous auxin production by the distal leaf part prevents abscission [33]. Furthermore, it has been documented that auxins function as abscission inhibitor, during the initial phases of abscission, but once the process has been initiated auxins appear to stimulate abscission [34]. Also, foliar application of tryptophan to, navel orange trees, at end of cell division stage, at maximum peel thickness, significantly increased the yield due to increasing number of fruit per tree [35]. In the same concern our data are in harmony with findings by Nadia [36] who found that, applying 2, 4 D (at 20 pm) during April and June significantly increases in fruit set percentage of Washington navel orange trees.

The amino calcium treatment significantly registered second level in fruit set percent. In this respect, calcium is involved in reducing young fruits abscission as calcium ion maintains cellular integrity of cell walls and cell membranes. Since, the abscission of leaves, flowers and fruits is supposed to be through the weakening of the cell walls in the abscission zone and this weakening take place by solubilizing of the cementing substances in cell wall and hydrolysis of the structural components of the wall and as the cementing properties of walls is believed to be through the binding of Ca ions to pectic components by formation of salt bridges. Moreover it is thought that, calcium repress the sensitivity to ethylene, that depression of ethylene biosynthesis by calcium ion consequently may restraint normal senescence.

Collectively, it is suggest that perhaps the effect of calcium on abscission is more related to delaying senescence progress than cell walls cementing [10]. Moreover, it has been reported that there is relationship between polyamines metabolism and flowering and fruit set in citrus plant [37]. In conditions of, low calcium supply or disturbance in it is transport, local calcium deficiencies result, that lead to cell wall failure and membrane breakdown [38]. Recently it has been documented that, cell wall synthesis is coupled to cell wall extensibility by a chemical Ca^{2+}-exchange mechanism between calcium pectate complexes [39].

Table 2: Effect of treatments on yield and physical characters of Washington navel orange fruit

Treatments	Fruit set (%)	Yield(kg/ tree)	Fruit weight (g)	Fruit size(cm^3)	Fruit ship index	Fruit firmness(l.b/ $inches^2$)	Fruit peel thickness(cm)
	First season						
Control	4.80 c	37.44 c	198.23 c	208.33 d	1.07 a	12.87 b	0.31 c
Tryptophan 25 ppm	7.32 a	61.61 a	245.00 b	260.83b	1.00 c	12.17 bc	0.34 c
Tryptophan 50 ppm	7.41 a	64.83 a	258.33 a	279.86 a	1.00 c	12.56 bc	0.37 bc
Amino calcium	6.51 b	49.56 b	223.33 b	246.67 c	1.04 b	14.41 a	0.48 a
Potassium nitrate	5.11 c	51.22 b	260.00 a	280.00 a	1.03 b	11.43 c	0.41 b
	Second season						
Control	6.63 c	44.85 c	225.00 c	239.17 c	1.09 a	10.86 b	0.39 c
Tryptophan 25 ppm	7.95 a	73.91ab	266.67 b	288.25 b	1.03 c	12.25 a	0.41 bc
Tryptophan 50 ppm	9.20 a	77.31 b	263.23 b	286.80 b	1.03 c	12.70 a	0.44 abc
Amino calcium	7.21 b	56.65 b	298.34 a	320.58 a	1.06 b	13.77 a	0.49 a
Potassium nitrate	6.27 c	66.59ab	293.23 a	316.92 a	1.05 b	11.95 ab	0.47 ab

Means in each column followed by the same letter·s did not differ at $p<0.05$ according to Duncans multiple range tests

Yield per Tree: Data presented in Table (2) obviously reveal that, applied treatments showed significant increment in yield (kg/ tree) for both seasons, as compared with control treatment and the highest significant yield values were recorded for both tryptophan applications (50 or 25 ppm).

The effect of tryptophan treatments could be due to, it is role in increasing fruit set percentage by, the effect of IAA in reducing flower and fruitlet abscission, which was explained previously, besides the effect of tryptophan in increasing fruit size. As foliar application of tryptophan to navel orange at the end of the fruit cell division stage increase fruit size and cumulative total yield [8].

In this respect, Hanafy Ahmed et al, [9] reported that, a significant increases in Valencia orange yield, by spraying rates of tryptophan (25, 50 or 100 ppm). Additionally, Agustí *et al.* [40] pointed out that synthetic auxins used at the end of phase II or at the beginning of phase III, prevent or delay preharvest fruit drop.

Navel Size and Navel-end Opening: It is clear from data presented in Figures (2 & 3) that amino calcium treatment produced the highest percentage of (extra class) fruits about 83.4% and lowest percentage of (out of grade class) fruits about 1% as compared with control treatment which gave the highest percentage of (out of grade class) fruits about 6.3% and about 12.4% (extra class) fruits. It means that amino calcium treatment effectively reduced both navel size and navel-end opening fruits in examined fruits. High percent of extra class fruits resulted with amino calcium treatment could be referable to, the role of calcium in preventing the abscission of fruit style through cementing cell walls cross-linking of pectates and also the suppression effect of calcium ion to tissue sensitivity for ethylene [10].

Moreover, the presence of amino acids may help in speeding up, the rate of rind tissue growth, by building up cells at the styler end during fruit growth as amino acids are considered the building blocks of proteins and they also restore the specific enzymes for protein synthesis [12, 13].

It was noticed that fruits treated by tryptophan at 25 and 50ppm were classified after amino calcium treatment with 63.5% & 60.3% of extra class grade, 31.4% &33.4% as class1 and about 1.8% and 2.2% out of grade fruits respectively. Taking into consideration that tryptophan is an auxin precursor and the inverse relationship between auxin and ethylene, we can understand that presence of tryptophan prevent ethylene to express itself in the styler end layer zone abscission, therefore the style did not fall in the early stage of fruit growth resulting in continued cells division and growth with a regular basis without causing increase in navel size or navel- end opening.

In this respect, Verreynne and Mupambi [41] reported that, managing the navel size and the navel end opening may be achieve by, delaying the abscission of the fruit style, that promote the development and growth of the fruit rind cells at the navel-end and that prevent, the occurrence of navel-end opening and, as the fruit style remained attached for a longer time, that lead to reduce the amount of fruits with "opened navel end".

It was documented that, styler abscission has been inhibited by, synthetic auxin 2, 4-dichlorophenoxyacetic acid in explants of Washington navel orange, Valencia orange and mandarin [42]. Foliar sprays of 'Newhall navel' and 'Washington navel' sweet orange, by 2, 4-D, synthetic auxins at full bloom , or after 100% petal drop, significantly increased the percentage of fruit with closed navel-ends and reduce the average size of the navel-end, as synthetic auxin imitate the role of endogenous IAA in

Fig. 2: Navel size and navel end opening as affected by applied treatments

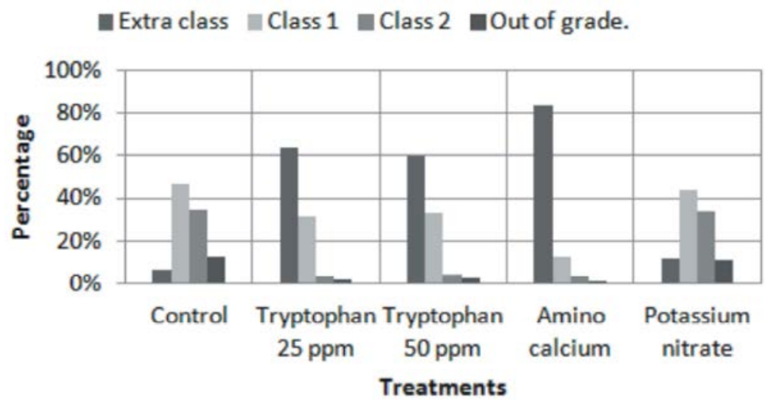

Fig. 3: Effect of treatments on Washington navel orange fruits classification

blocking the ability of ethylene to induce abscission events that lead to fruit style abscission [31]. But if 2, 4-D were applied 2 or 4 weeks after petal fall did not have significant effect [41]. These may related to that, during stage I of flower abscission lag phase, the presence of auxin decreases the zone sensitivity to ethylene and ethylene fails to stimulate abscission. In stage II capacity of auxin to retard abscission reduced, increasing sensitivity to ethylene [44].

However, the data in Figure (3) revealed that potassium nitrate had not clear effect on navel size or navel-end opening but it has a great effect on fruit quality.

It was noticed that control treatment contained a high percentage about 46.6% and 34.7% of fruits in (class1 and class 2), respectively but these grads is more suitable for local consumption only not for export. This means that Egyptian citrus industry can lose nearly 40% of income from navel orange exports.

Fruit Quality

Fruit Physical Properties: Concerning to fruit weight and fruit size, data in Table (2) illustrated that, applied treatments significantly increased fruit weight and fruit size when compared with control treatment, with some exceptions. The highest values were recorded with potassium nitrate follow by tryptophan at 50 ppm at the first season, while the highest values were recorded with amino calcium follow by potassium nitrate at the second season when compared with control treatment.

As regard to the effect of potassium nitrate, it is well documented that, nitrogen and potassium are essential in adequate amounts particularly, during fruit initiation and development stages, as they are, considered most important nutrients for citrus tree growth, yield and fruit quality [44]. Also, it has been reported that maintaining optimal potassium level in citrus trees, resulted in increasing yield, fruit size and fruit weight, as potassium is involved in the movement of sugars from the site of photosynthesis to storage sites and stimulates the synthesis of protein [45]. Moreover, foliar potassium can be used, during both cell division and rapid cell enlargement stages, to achieve maximum effect on fruit yield and quality [46].

For the effect of amino calcium on both fruit weight and fruit size it may be explained by the fact that at low soil temperatures, thus low root activity, less nitrogen and other nutrient are absorbed. Early pre-bloom and post-bloom sprays with nitrogen containing fertilizer

Table 3: Effect of treatments on fruit quality of Washington navel orange fruit.

Treatments	V.C(mg/100ml)	T.SS (%)	Acidity (%)	TSS/acid ratio
		First season		
Control	50.40 a	10.33 bc	0.94 a	11.05 c
Tryptophan 25 ppm	53.07 a	11.08 a	0.97 a	11.46 bc
Tryptophan 50 ppm	52.77 a	10.00 c	0.80 b	12.48 a
Amino calcium	51.31 a	10.92 ab	0.92 a	11.90 abc
Potassium nitrate	51.31 a	11. 29 a	0.91 a	12.40 a
		Second season		
Control	52.10 a	10.67 b	0.92 a	11.63 c
Tryptophan 25 ppm	56.52 a	11.67 a	0.89 ab	13.07 b
Tryptophan 50 ppm	53.46 a	11.67 a	0.73 c	15.90 a
Amino calcium	52.73 a	11.17 ab	0.83 b	13.40 b
Potassium nitrate	52.20 a	11.88 a	0.85 ab	13.98 a

Means in each column followed by the same letters did not differ at $p<0.05$ according to Duncans multiple range tests.

source (which in our study is the amino calcium compound), can balance this restricted root absorption, as foliar absorbed nitrogen will be broken down to ammonia, which will be metabolically changed to arginine then polyamines that, help cell division promoting growth resulting in larger fruit. As 70% of final fruit size is related to the number of cells in the fruit. Cell division size change throughout the rest of the year comes from cell enlargement [47].

Concerning to fruit shape index, the data revealed that, at the two experimental seasons the highest value was obtained by control treatment followed by amino calcium treatment.

As regarding fruit peel thickness and fruit firmness, data in Table (3) demonstrated that, highest values were recorded by spraying amino calcium as compared with control treatment at two experimental seasons.

The effect of amino calcium may be due to the known role of calcium ion in maintaining cell wall structure and membrane integrity by interacting with pectic acid forming calcium pectate that give rigidity to middle lamella and cell wall [48]. Moreover, Mignani *et al*. [49] reported that, calcium increases the fruit firmness by maintenance of cell trugor potentials. That preservation of firmness in calcium treated fruits might be due its accumulation in the cell walls, leading to facilitation in the cross liking of the pectic polymers which increases wall strength and cell cohesion [50].

Fruit Chemical Properties: Data represented in Table (3) revealed that, non significant increases in vitamin C were recorded by all treatments at the two experimental seasons when compared with control treatment.

Moreover, the highest values of total soluble solids and total soluble solids acid ratio were detected by potassium nitrate and tryptophan 50 ppm treatments at both seasons when compared with control treatment. Whereas, the lowest values of fruit acidity were obtained by the trees sprayed with tryptophan at 50 ppm for both experimental seasons.

In this respect, Ladaniya [45] reported that, excess potassium results in fruit with high acid content. Also, Spiegel –Roy and Goldschmidt [51] mentioned that, K acts as an osmotic agent in the opening and closing of stomata and it plays an important role in controlling the acidity of the citrus fruit juice. Potassium deficiency resulted in decreases juice soluble solids, acid and vitamin C content [44].

Leaf Chemical Composition

Total Indoles: The data in Fig. (4) revealed that, tryptophan treatment at 25 and 50 ppm showed a great increase in the amount of indoles level at the second and third date compared with other treatment. This increment is due to the conversion of tryptophan amino acid to indole containing compound [5, 6]. Moreover it could be noticed that total indoles increased throughout, the period of cell elongation, that is the instance of exogenous auxins application, to be effective in increasing fruit size [52]. It could be concluded that the enlargement of the auxin-treated fruits is actually due to cell expansion rather than to cell division [54].

Total Sugars: It is obvious from data in Table (4) that, in the two experimental seasons the highest values of total sugar concentration were obtained by leaves of trees sprayed with tryptophan 50 ppm, potassium nitrate, or amino calcium treatments when compared with control treatment.

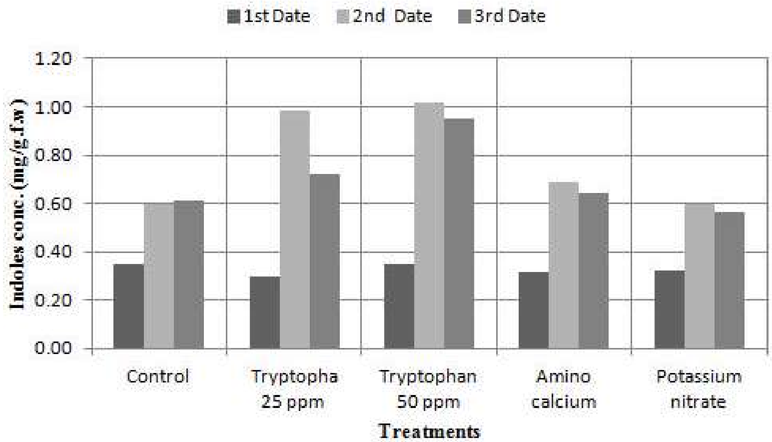

Fig. 4: Effect of treatments on indoles concentration at three date samples.

Table 4: Effect of treatments on chemical composition of Washington navel orange leaves.

Treatments	Total Sugar(mg/100 g. fw.)	Tryptophan(mg/g. d w.)	N (%)	P (%)	K (%)	Ca (%)	B (ppm)
			First season				
Control	27.63 b	0.039 c	2.00 c	0.223ab	1.47 b	3.39 b	26.99 a
Tryptophan 25 ppm	30.62 b	0.071 a	2.25 bc	0.215 b	1.62 a	3.31 b	27.18 b
Tryptophan 50 ppm	33.91 a	0.075 a	2.27 bc	0.214 b	1.65 a	3.40 b	26.99 b
Amino calcium	28.10 b	0.054 b	2.48 ab	0.235 a	1.34 b	4.09 a	31.61 a
Potassium nitrate	35.12 a	0.049 b	2.67 a	0.245a	1.69 a	3.13 b	25.85 b
			Second season				
Control	26.62 c	0.041 c	2.06 c	0.254 b	1.54 b	3.33 c	27.36 bc
Tryptophan 25 ppm	27.15c	0.073 a	2.21 bc	0.277b	1.72 a	3.59 bc	26.60 c
Tryptophan 50 ppm	34.73 a	0.080 a	2.19 bc	0.256 b	1.77 a	3.70 bc	28.46 b
Amino calcium	29.96 b	0.061 b	2.53 ab	0.267 b	1.61b	4.09 a	31.52 a
Potassium nitrate	30.46 b	0.054 b	2.72 a	0.315a	1.81 a	3.86 ab	30.20 a

Means in each column followed by the same letters did not differ at $p<0.05$ according to Duncans multiple range tests.

Concerning the effect of tryptophan treatments, Refaat and Naguib [55] found that, the increased in total carbohydrates percentage in peppermint leaves by application of L-tyrosine. As the promotive effect of the amino acids on the total carbohydrates content may be due to their important role on the biosynthesis of chlorophyll molecules which in turn affected carbohydrate content. Attoa *et al.* [56] working on *Iheris umara* L. plant, found that foliar application of tryptophan at 75 ppm increased the total carbohydrates.

For the effect of potassium, it is documented that promotes the translocation of photosynthates (sugars) for plant growth or storage in fruits [15].

For the role of calcium it has been documented that calcium is needed for activation of some enzymes and carbohydrate transport [57]. Furthermore, the accumulation of total soluble sugars may be due to the higher activity of acid invertase, in the presence of calcium [59].

Tryptophan: Data in Table (4) illustrated the effect of treatments on tryptophan concentration, the highest values of tryptophan were recorded by leaves of Washington navel orange trees treated with tryptophan 50, 25 ppm and amino calcium treatments respectively at the two successive seasons when compared with control treatment.

In this respect, Mohamed *et al*. [59] working on periwinkle, found that total and soluble protein nitrogen were increased gradually by the application of tryptophan especially at the rate of 100 ppm which produced maximum values.

Minerals: The data in Table (4) indicated that, the highest values of nitrogen concentration were recorded by potassium nitrate application followed by amino calcium treatment respectively at two successive seasons.

In this respect, Erney *et al*.. [16] reported that, potassium nitrate spray (10-15%) on mature citrus trees,

Table 5: Feasibility study for the applied treatments on yield of Washington navel orange.

Treatments	Yield /Feddan (Ton)			*Yield return/Feddan (Egyptian pound)			Treatment return/F (Egyptian pound)			**Treat. Cost (pound)	Treatment profit (Egyptian pound)		
	2014	2015	AVG.	2014	2015	AVG.	2014	2015	AVG.		2014	2015	AVG.
Control	6.29	7.53	6.91	8491	10165	9328.5	0.0	0.0	0.0	0.0	0	0	0
Tryptophan 25 ppm	9.35	12.42	10.89	12622	16767	14694	4131	6601	5366	225	3906	6377	5141
Tryptophan 50 ppm	9.89	12.99	11.44	13351	17536	15444	4860	7371	6115	300	4560	7071	5816
Amino calcium	8.33	9.52	8.93	11245	12852	12048	2754	2686	2720	690	2064	1997	2030
Potassium nitrate	8.60	11.19	9.90	11610	15106	13358	3118	4941	4029	540	2579	4401	3490

*Washington navel orange/Ton:1350 Egyptian pounds

**Treatment Cost: included: material, spraying and labour cost where,

Tryptophan Kg.:2500 Egyptian pounds

Amino calcium compound liter.45Egyptian pounds

Potassium nitrate Kg: 65 Egyptian pounds

increased significantly the nitrate as well as the total nitrogen content .Moreover, phosphorus, magnesium and potassium considered as nutrients supporting nitrogen uptake by plants and its further transformation into plant biomass [60].

Concerning potassium concentration results indicated that, in the two experimental seasons significant increase were detected by leaves of Washington navel orange trees sprayed with potassium nitrate, tryptophan 50 or 25 ppm treatments when compared with control treatment, the highest values were recorded by the leaves of the tree treated with potassium nitrate.

In this respect, it is documented that, leaf N, P, K and Zn content significantly increased with increasing foliar potassium applied to Washington navel orange trees [61].

Furthermore, it is clear from the results that, in the second season significant increases in Ca concentration were recorded by all treatments when compared with control treatment. While, no constant trend could be detected at the first season. In addition, the highest values of Ca and B concentrations were obtained by trees sprayed with the amino calcium treatment at two experimental seasons.

In this respect, Lee *et al*. [62] found that, foliar applications of 0.5% Ca ($CaCl_2$) during the early stages of pear fruit development resulted in an increase of the Ca content of leaves and fruit (peel and flesh). Dong *et al*. [63] reported that, spraying "Cara Cara" navel orange trees (*Citrus sinensis* L. Osbeck) with Ca increase significantly Ca content in the pulp by and peel.

Feasibility Study: A feasibility study was prepared for estimating the economic return for the examined treatments, to settle on the treatment which had best profit. Data illustrated in Table (5) showed that, both tryptophan treatments at 50 or 25ppm, achieved the highest profit followed by, potassium nitrate treatment, then amino calcium treatment. However, concerning navel end size, which is considered as, an important quality parameter, amino calcium treatment showed the highest percentage of fruits in the extra class about 80%while tryptophan treatments recorded about 60 %. Despite the high costs of amino-calcium treatment, the effect of this treatment on the size of the navel and success in increasing the number of fruits with closed navel and increase the number of fruits of the extra class, considered a very vital point in the case of the exporting of navel orange fruit, since, the size of the navel opening is considered one of the most important quality parameter.

Recommendation: In conclusion, it could be reported that, tryptophan treatment at 25 or 50 ppm resulted in, increasing fruit set percentage and tree yield (kg/ tree), where as amino calcium treatment showed highest percentage of closed navel “extra class “followed by tryptophan treatment. With regarding to tryptophan effect on the navel size and navel-end opening in our experiment and previous results from one hand and tryptophan as natural growth regulator from the other we can recommend using tryptophan instead of synthetic auxins because it seem to be more safety compounds on human health.

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