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Study on the Effect of Pre-harvest Treatments by Seaweed Extract and Amino Acids on Anna Apple Growth, Leaf Mineral Content, Yield, Fruit Quality at Harvest and Storability

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Abstract : Seaweed extract (*Ascophylum nodosum* L.) and amino acids were studied as a foliar applications on the growth, leaf mineral content, fruit set, yield, fruit quality and storability of apple cv. ''Anna'' under cold storage conditions.

Anna apple trees treated with mixture of seaweed extract (2 ml L^{-1}) plus amino acids (0.5 ml L^{-1}) exhibited significantly higher shoot length, leaf area, number of leaves per shoot, chlorophyll, N, K, Fe, Mn and Zn content of leaves with no significant change in P content. Seaweed extract (2 ml L^{-1}) either alone or combined with amino acids (0.5 ml L^{-1}) increased significantly fruit setting and yield. Anna fruits lightness, hue angle, fruit firmness, weight loss percentage and soluble solid content were significantly affected by different treatments, while titratable acidity % was not significantly influenced. At the end of storage period the mixture application of seaweed extract (2 ml L^{-1}) + amino acids (0.5 ml L^{-1}) gave the highest values of lightness , fruit firmness, soluble solid content and lowest value of hue angle (high density of red color), weight loss percentage and titratable acidity %.

In brief, pre-harvest sprays by mixture of seaweed extract (2 ml L^{-1}) + amino acids (0.5 ml L^{-1}) is highly effective to "Anna" apples for enhancing growth, leaf mineral content, fruit set, yield, fruit quality at harvest and storability under cold storage conditions.

Keywords: Apple, Anna, Seaweed extract, Amino acids, Cold storage, Yield, Fruit quality and Storability.

Introduction

Seaweed (*Ascophyllum nodosum* L.) is a known source of plant growth regulators such as cytokinins, auxins and auxin-like compounds, organic matter and fertilizer nutrients, amino acids and vitamins, complex polysaccharides are not present in land plants, betaines and betaine-like compounds, sterols and growth inhibitors ABA[1] which an important roles in metabolism and productivity of plants. Recently biological agriculture and horticulture are applied as a foliar spray increases uptake of plant nutrients, promotes growth and gives resistance to frost, fungal diseases and stress conditions. Moreover, it is effective for ripening of fruits, increasing post-harvest, shelf-life, improves the quality of the products and serves as an excellent soil conditioner[2,3,4].

Many investigations cleared out that, application of seaweed extract as a foliar spray was found to increase growth, productivity and fruit quality of some fruit crops including apple[5], Perlette grape[6] and both Valencia and Washington Navel orange[7].

Foliar applications of seaweed extract has been reported to peach trees generally resulted in greater shelf-life of treated fruits. After 21 days, more than 50 percent of untreated peaches were unmarketable, compared to less than 20 percent of the fruit receiving seaweed sprays. Earlier applications, beginning with full bloom gave better results. Seaweed applied to peach has no appreciable effect on pH, firmness, soluble solids or total titratble acidity[8]. Similar results were observed when peaches sprayed with seaweed extract before harvest kept longer. After 16 days from harvest, rotten fruits percentages were 14.7 and 32.2 for treated and untreated trees, respectively[9].

Also, foliar application of the amino acids is very necessary for plants. It's can directly or indirectly influence the physiological activities in plant growth and development due to they considered as precursors and constituents conducted of proteins[10] which are important for stimulation of cell growth. They contain both acid and basic groups and act as buffers, which help to maintain favorable pH value within the plant cell[11]. Moreover, amino acids with their ant oxidative properties play an important role in plant defense against oxidative stress induced by unfavorable conditions. They are responsible for enhancing the biosynthesis of proteins, plant pigments, vitamins and natural hormones such as IAA and ethylene and stimulating cell division[12]. Many investigations cleared out that, application of amino acids as a foliar spray was found to increase growth, leaf mineral content, fruit set, yield and quality of Momtaz and Ohadi pistachio[13], Manfalouty pomegranate[14].However, effects of foliar application of amino acids on the apple were not investigated.

The purpose of this study was to investigate the effects of seaweed extract and amino acids as pre-harvest treatments separately or in mixture to enhance growth, leaf mineral content, fruit set, yield and its role in improvement the quality of Anna apple fruits as well as storability under cold storage conditions.

Materials and Methods

The present study was carried out during 2014 and 2015 seasons on "Anna" apple trees (*Malus domestica*, Borkh) six years old budded on Malus rootstock and planted at 3.5 X 3.5 m in a loamy clay soil under surface irrigation system in the Experimental Orchard of the Horticulture Research Station at El Kanater El Khayreia, Kalubia Governorate, Egypt. Trees were normal growth, uniform in vigour, trained on open vase training system and received uniform management practices. Selected "Anna" apple trees were sprayed twice at full bloom stage (March15 and 13 in the first and second season, respectively) and one month after fruit setting stage (May 20 and 18 in the first and second season, respectively) with aqueous solutions of 2 ml L^{-1} seaweed extract as ''ANA'' [seaweed 12%] and 0.5 ml L^{-1} amino acids as ''Mono Acid Powder'' [contains free amino acids 25% are present viz., aspartic 3.2%, glutamic 2.1%, alanine 3%, arginine 2%, glycine 3.6%, lysine 5.6%, valine + methionine 2%, serine 1.3%, tyrosine 0.5% and thronine1.7%], each alone or in mixture besides control (Unsprayed trees).Tween-20 at 0.01% was added as a surfactant. All trees were thoroughly sprayed till run off. The experiment followed complete randomized block design with three replicates for each treatment.

Vegetative growth measurements:

- Shoots length (cm.): The average lengths of five current shoots on the selected branch were measured.
- Numbers of leaves / shoots: The average numbers of leaves on them were counted.
- Leaf area (cm^2): Three fully expanded leaves were collected and their leaf area (cm^2) was measured by using portable leaf area meter [Model: YMJ-A 20110122-1]. Total leaf chlorophyll content was measured by using TYS-A chlorophyll meter portable.

Determination of leaf mineral content:

At mid-August during each season of study twenty five mature leaves were collected at random from the end of spur per shoot were taken. Leaves samples washed several times with tap water, rinsed three times in distilled water and then dried at 70˚C in an electric air-drying oven. The dried leaves of each sample were ground in a porcelain mortar to avoid contamination with any minerals; 0.5 g from the ground dried material of each sample was digested with H2O2 and H_2So_4 according to[15]. Suitable aliquots were then taken for mineral determinations.

- Leaf nitrogen (N) contents were determined by using micro Kjeldhal as described by[16],
- Phosphorus (P) was determined calorimetrically according to[17],
- Potassium (K) was determined by flame photometer as described by[18].
- For determination of leaf iron (Fe) contents, manganese (Mn) and zinc (Zn) were measured by atomic absorption according to[19]. The concentration of Fe, Mn and Zn were expressed as (ppm), on dry weight basis.

Fruit set percentage:

The total number of flowers on each tagged limb was counted at full bloom. The number of set fruits was counted on the same limbs after one month from full bloom. Fruit set percentage was calculated as follows:

Fruit set % = (Number of developing fruitlets / Total number of flowers) X 100

Determination of yield:

The total yield of each studied tree was determined in (Kg).

Storage Fruits:

Mature apple fruits (free from any pathogen infection, uniform in shape, weight and color) were picked separately from each treatments , then, transported to the laboratory and packed in perforated carton boxes in three replicates. Fruits stored at 0°C and 90% relative humidity (RH) for two months. Fruit quality characteristics were determined at 2 weeks intervals, a**s a follow:**

- **Fruit color:** Lightness and hue angle were estimated using Minolta Calorimeter (Minolta Co. Ltd.,Osaka, Japan) as described by[20].
- **Fruit firmness:** was determined as ($Lb/inch^2$) by using fruit pressure tester mod. FT 327 (3-27 Lbs).
- **Fruit weight loss percentage:**The fruits were weighed before cold storage to get the initial weight, and then weighed at each sampling date. Fruits weight was recorded, then percentages of weight loss were calculated according to the following equation: -

$FWL\% = [(W_i – W_s)/ W_i] x 100$

Where: W_i = fruit weight at initial period.

W_s = fruit weight at sampling period.

- **Soluble Solids content (SSC %):** Abbe refractometer was used to determine the percentage of total soluble solids in fruit juice according to[21].

- **Titratable Acidity %:** Titratable 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[21].

Statistical analysis:

A randomized complete block design was used to analysis of variance for comparison between the control and the other. All data were subjected to statistical analysis according to the procedures reported by[22] and means were compared by Duncan's multiple range tests at the 5 % level of probability.

Results and Discussion

Vegetative growth and total leaf chlorophyll content:

Results in Table 1 showed that shoot length, number of leaves / shoot, leaf area and total leaf chlorophyll content of Anna apple trees were increased by the foliar application of seaweed extract + amino acids, either alone or in mixture. The mixture application of 2 ml L^{-1} seaweed extract + 0.5 ml L^{-1} amino acids gave the highest significant shoot length and leaf area followed by 2 ml L^{-1} seaweed extract treatment in both seasons. Meanwhile, there was no significant different between 0.5 ml L^{-1} amino acids treatment and control. Also, in this respect results revealed that the mixture application of 2 ml L^{-1} seaweed extract + 0.5 ml L^{-1}amino acids gave the highest significant number of leaves/ shoot followed by treatment of 0.5 ml L^{-1} amino acids in both seasons. Whereas, the different between 2 ml L^{-1} seaweed extract treatment and control was not significant. These results may be attributed to the effect of seaweed extract in increasing levels of endogenous hormones, i.e. IAA, GA_3 and active cytokinins in treated plants which promote cell division and cell elongation[23] as well as other compounds[24] in the seaweed extracts which affect cellular metabolism in treated plants leading to enhanced growth. Moreover, seaweed extracts improve nutrient uptake by roots[25], resulting in root systems with improved water and nutrient efficiency, thereby causing enhanced general plant growth and vigor. In addition, the regulatory effect of amino acids on growth could be explained by the motion that some amino acids e.g. phenylalanine, ornithine can affect plant growth and development through their influence on gibberellins biosynthesis[26]. Moreover, the physiological roles of amino acids which increased the metabolic processes rate and in the same time it is cells that each micronutrient in the amino green compound has a role in improving plant growth. Zn directly involved in the synthesis of the indole acetic acid (IAA). Mn is directly involved in the catalytic rates in plants being the enzyme activator on some respiratory enzymes and in reaction of nitrogen metabolism and photosynthesis[27]. These results are in parallel with the findings of[28,29] who stated that foliar application of seaweed extract increased significantly vegetative growth (i.e., shoot length, leaf area, leaf number and leaf weight). On the other hand, treated trees with mixture of 2 ml L^{-1} seaweed extract and 0.5 ml L^{-1} amino acids showed highest significant increase in total leaf chlorophyll content than untreated trees in the two seasons.

This increase in chlorophyll content was a result of reduction in chlorophyll degradation, which might be caused in part by betaines in the seaweed extract[30]. Similarly, exogenous application of seaweed extract resulted in 12 % increase in the chlorophyll contents in 'Fuji' apple leaves with a consequent increase in the photosynthesis and respiration rates[5,28]. Foliar application of 0.5 ml L^{-1} mixture of amino acids and seaweed extract significantly improved leaf size and chlorophyll contents in Perlette grapevines[6].

Table 1: Effect of spraying seaweed extract and amino acids on vegetative growth and total leaf chlorophyll content of Anna apple trees during 2014 and 2015 seasons.

Treatments	Shoot length (cm)		Number of leaves / shoot		Leaf area (cm^2)		Total leaf chlorophyll content	
	2014 season	2015 season	2014 season	2015 season	2014 season	2015 season	2014 season	2015 season
2 ml L^{-1} seaweed extract	44.90a	44.91a	36.33b	38.00b	37.62a	36.90a	34.63ab	36.37a
0.5 ml L^{-1} amino acid	37.00b	36.78b	44.33a	45.00a	33.31b	34.39b	33.81ab	34.98ab
2 ml L^{-1} seaweed extract + 0.5 ml L^{-1} amino acid	48.11a	49.12a	47.00a	48.33a	38.77a	38.97a	37.59a	36.44a
Control	35.22b	36.43b	36.33b	35.00b	32.30b	33.98b	31.48b	33.46b

Means within a column, following with the same letters are not significantly different at 0.05 levels.

Macro-Element Contents:

Results in Table 2 cleared that application of mixture 2 ml L^{-1} seaweed extract + 0.5 ml L^{-1}amino recorded the highest significant value of nitrogen leaf content in both seasons.Respecting phosphorus leaf content results showed no significant difference between the different treatments compared to the control. As for the potassium leaf content, application of mixture 2 ml L^{-1} seaweed extract and 0.5 ml L^{-1} amino recorded the highest significant value while untreated trees recorded the lowest value during two seasons of investigation.

Table 2: Effect of spraying seaweed extract and amino acids on macro-element contents of Anna apple trees during 2014 and 2015 seasons

Treatments	N (%)		P (%)		K (%)	
	2014 season	2015 season	2014 season	2015 season	2014 season	2015 season
2 ml L^{-1} seaweed extract	1.81ab	1.87ab	0.17a	0.18a	1.44b	1.42b
0.5 ml L^{-1} amino acid	2.01ab	2.08a	0.18a	0.18a	1.38c	1.36c
2 ml L^{-1} seaweed extract + 0.5 ml L^{-1} amino acid	2.05a	2.11a	0.18a	0.19a	1.50a	1.54a
Control	1.73b	1.74b	0.17a	0.18a	1.34d	1.36c

Means within a column, following with the same letters are not significantly different at 0.05 levels.

Micro-Element Contents:

Table 3 showed that the mixture application of 2 ml L^{-1} seaweed extract and 0.5 ml L^{-1} amino acids display the highest significant Fe, Mn and Zn leaf content, followed by 2 ml L^{-1} seaweed extract, then 0.5 ml L^{-1} amino acids treatment. Meanwhile, untreated trees display the lowest micro-element contents during both seasons.

Our results could be explained in light of obtained results by[31,32] which showed that foliar application of amino acids promoted N uptake and accumulation of P, K, Ca, Mg, Fe, Zn and Mn in leaves. Moreover, seaweed is one of the bio-stimulants which enhanced the leaf mineral contents than the untreated trees. This is probably due to the impact of seaweed extracts in improving nutrient uptake by roots[25]. Similarly, [6]reported that 'Perlette' grapevines treated with spray applications of mixture amino acids and seaweed extract at flowering + fruit setting stages exhibited an increase in levels of leaf N, P, K, B, Fe and Zn contents as compared to untreated grapevines.

Table 3: Effect of spraying seaweed extract and amino acids on micro-element contents of Anna apple trees during 2014 and 2015 seasons.

Treatments	Fe (ppm)		Mn (ppm)		Zn (ppm)	
	2014 season	2015 season	2014 season	2015 season	2014 season	2015 season
2 ml L^{-1} seaweed extract	66.00b	65.67b	57.40ab	59.33ab	19.23ab	19.53ab
0.5 ml L^{-1} amino acid	62.00c	64.33bc	54.80b	56.60b	18.60b	19.27b
2 ml L^{-1} seaweed extract + 0.5 ml L^{-1} amino acid	87.00a	86.67a	59.33a	62.67a	19.43a	19.60a
Control	59.33c	61.67c	52.70c	50.90c	18.13c	18.43c

Means within a column, following with the same letters are not significantly different at 0.05 levels.

Fruit set percentage and yield:

From the results in Table 4, it can be noticed that, the highest fruit set percentage (14.05 and 14.31 %) was obtained by the mixture application of 2 ml L^{-1} seaweed extract + 0.5 ml L^{-1} amino acids, followed by application of 2 ml L^{-1} seaweed extract (12.66 and 12.73 %), then application of 0.5 ml L^{-1} amino acid (11.01 and 11.86 %) during 2014 and 2015 seasons, respectively. On the other hand, the lowest percentage of fruit set recorded in control trees (10.05 and 10.51%) in the two seasons, respectively. This increase in fruit set percentage resulting from the impact of the treatments led to increase the number of fruits per tree. The mixture application of 2 ml L^{-1} seaweed extract + 0.5 ml L^{-1} amino acids resulted in the highest increase in number of fruits per trees by 33% and 42% over the control in first and second seasons respectively. Increase number of fruits per trees that reflected on the yield. Yield of Anna apple trees were treated with 2 ml L^{-1} seaweed extract plus 0.5 ml L^{-1} amino acids achieved the highest increase by 35 % than the control during two seasons of this investigation.

The increase in fruit set percentage and yield of Anna apple trees treated may be due to the positive effect of seaweed extract on endogenous levels of growth promoters, macro and micro nutrients and carbohydrates[33]. Yield increases in seaweed-treated plants are thought to be associated with the hormonal substances present in the extracts, especially cytokinins[1]. Cytokinins in reproductive organs may be linked with nutrient mobilization. Fruit ripening generally causes an increase in transport of nutrient resources within the developing plant[34] and the fruits have the capacity to serve as strong sinks for nutrients. Photosynthate distribution could be shifted, perhaps markedly, moving from vegetative parts (roots, stem, and young leaves) to the developing fruit, to be utilized in fruit development[35]. Besides physiological role of amino acids within the plant and its impact on metabolism which reflected in increased yield.

Table 4: Effect of spraying seaweed extract and amino acids on fruit set percentage and yield of Anna apple trees during 2014 and 2015 seasons.

Treatments	Fruit set (%)		Number of fruits / tree		Yield (Kg/tree)	
	2014 season	2015 season	2014 season	2015 season	2014 season	2015 season
2 ml L^{-1} seaweed extract	12.66b	12.73b	134b	143b	47.00b	49.33b
0.5 ml L^{-1} amino acid	11.01bc	11.86bc	121c	137c	44.71c	46.57c
2 ml L^{-1} seaweed extract + 0.5 ml L^{-1} amino acid	14.05a	14.31a	151a	178a	58.63a	58.75a
Control	10.05c	10.51c	101d	103d	37.94d	38.00d

Means within a column, following with the same letters are not significantly different at 0.05 levels.

Fruit color:

Lightness (L* value):

Data in Table 5 showed that lightness (L*) gradually decrease towards the end of the storage period (8 weeks). Similarly, 36 noted that lightness of Anna apple gradually decreased during storage. Anna fruits lightness significantly affected by different treatments in both seasons. At the harvest, application of 2 ml L^{-1} seaweed extract alone and 0.5 ml L^{-1} amino acid treatment alone gave the highest values of L* in the first and second season, respectively, while the control treatment recorded the lowest values in the two seasons. At the end of storage period the mixture application of 2 ml L^{-1} seaweed extract + 0.5 ml L^{-1} amino acids gave the higher values of L* (62.28 and 56.67) in the two seasons, respectively. On the other hand, control treatment exhibited the lowest value of L*.

Table 5: Effect of spraying seaweed extract and amino acids on L * value of Anna apple fruits stored at 0°C and 90%RH during 2014 and 2015 seasons.

Treatments	Storage period per week 2014 season					Storage period per week 2015 season				
	0	2	4	6	8	0	2	4	6	8
2 ml L^{-1} seaweed extract	71.19a	67.34a	66.43a	63.49a	54.63b	67.67b	66.83a	65.99a	62.74a	56.19b
0.5 ml L^{-1} amino acid	68.07b	65.90b	63.73b	62.71a	52.64c	69.19a	66.83a	64.47a	62.68a	53.65c
2 ml L^{-1} seaweed extract + 0.5 ml L^{-1} amino acid	68.86b	66.29ab	64.74ab	63.71a	62.28a	67.86b	67.40a	62.27b	57.13b	56.67a
Control	60.61c	50.49c	56.69c	56.37b	52.58c	62.46c	61.59b	60.72b	56.01b	52.89c

Means within a column, following with the same letters are not significantly different at 0.05 levels.

Hue angle (h° value):

Data in Table 6 indicated that hue angle (h°) decreased (increase density of red color) with the advance in cold storage periods. This is in agreement with [37] recorded that hue angle (h°) in apricot stored was continued to decrease, reaching the lowest values at the end of storage periods. However, at the harvest spraying of 0.5 ml L^{-1} amino acid gave the lowest values of (h°) in the first season and 2 ml L^{-1} seaweed extract + 0.5 ml L^{-1} amino acids treatment in the second season, while control treatment recorded the highest values in the two seasons. At the end of storage period, the mixture application of 2 ml L^{-1} seaweed extract + 0.5 ml L^{-1} amino acids gave the lowest value of h° (high density of red color) in the two seasons, respectively. On the other hand, control treatment recorded the highest value of h° in the two seasons. These results are in line with finding of [38] found that seaweed extract improved the color and color distribution of Mondial Gala apple fruit.

Table 6: Effect of spraying seaweed extract and amino acids on h° value of Anna apple fruits stored at 0℃ and 90%RH during 2014 and 2015 seasons.

Treatments	Storage period per week					Storage period per week				
	2014 season					2015 season				
	0	2	4	6	8	0	2	4	6	8
2 ml L^{-1} seaweed extract	54.63a	52.04b	50.78a	43.86bc	39.86c	56.94a	53.63a	52.31a	42.84b	39.84b
0.5 ml L^{-1} amino acid	47.82b	47.64c	46.12b	42.61c	38.61c	56.66a	52.55a	50.81ab	43.54b	40.54b
2 ml L^{-1} seaweed extract + 0.5 ml L^{-1} amino acid	54.47a	54.23a	51.49a	45.65b	42.65b	48.74b	47.31b	45.91c	43.09b	41.09b
Control	55.41a	55.29a	51.15a	50.87a	45.87a	56.22a	54.27a	49.98ab	49.70a	46.70a

Means within a column, following with the same letters are not significantly different at 0.05 levels.

Fruit Firmness:

As shown in Table 7, it is clear that the mixture application of 2 ml L^{-1} seaweed extract + 0.5 ml L^{-1} amino acids had the highest fruit firmness (12.83 and13.77 Lb/inch^2) during the 1st and 2nd seasons, respectively compared with remaining treatments. At the end of storage period application with all treatments did not significantly influence on the fruit firmness. However, Anna apple trees treated with spray applications of mixture 2 ml L^{-1} seaweed extract + 0.5 ml L^{-1} amino acids showed higher firmness value as compared to all other treatments during both seasons.

These results agreed with[39] on Valencia orange who mentioned that applying of seaweed extract led to significant increase in hardness of flesh fruits at harvest. While, the retention of firmness which occurred during storage could be explain by retarded degradation of insoluble protopectins to the more soluble pectic acid and pectin. During fruit ripening depolymerization or shortening of chain length of pectin substances occurs with an increase in pectinesterase and polygalactronase activities[40]. This is agreement with[41] noted that firmness significant decreased with storage period in both treated and untreated Anna apples.

Table 7: Effect of spraying seaweed extract and amino acids on firmness (Lb/inch^2) of Anna apple fruits stored at 0℃ and 90%RH during 2014 and 2015 seasons.

Treatments	Storage period per week					Storage period per week				
	2014 season					2015 season				
	0	2	4	6	8	0	2	4	6	8
2 ml L^{-1} seaweed extract	12.03b	9.90a	8.90a	8.73a	7.13a	13.07a	12.27a	10.13a	7.77a	7.07a
0.5 ml L^{-1} amino acid	12.80a	9.90a	8.90a	7.40b	7.17a	13.60a	11.80ab	10.07a	7.20b	7.13a
2 ml L^{-1} seaweed extract + 0.5 ml L^{-1} amino acid	12.83a	9.93a	8.93a	7.70b	7.43a	13.77a	11.57b	9.67b	7.67a	7.40a
Control	10.83c	9.67b	8.67b	7.60b	7.13a	11.53b	10.03c	9.73b	7.27b	7.20a

Means within a column, following with the same letters are not significantly different at 0.05 levels.

Weight loss percentage:

Table 8 cleared that a gradual increase in weight loss was shown towards the end of the storage period (8 weeks). Significant differences between regardless of all treatments. The lowest weight loss percentage was recorded by the mixture application of 2 ml L^{-1} seaweed extract + 0.5 ml L^{-1} amino acids (5.41 and 4.51%) in the first and second seasons, respectively. On the other hand, amino acid treatment exhibited the highest weight loss value (7.28 and 6.31%) in two seasons, respectively.

Table (8) Effect of spraying seaweed extract and amino acids on weight loss % of Anna apple fruits stored at 0°C and 90%RH during 2014 and 2015 seasons .

Treatments	Storage period per week				Storage period per week			
	2014 season				2015 season			
	2	4	6	8	2	4	6	8
2 ml L^{-1} seaweed extract	1.54c	1.82d	3.80d	5.62c	1.81b	2.03d	2.94d	4.97b
0.5 ml L^{-1} amino acid	2.62a	3.87c	4.47c	7.28a	2.72a	4.68a	5.87a	6.31a
2 ml L^{-1} seaweed extract + 0.5 ml L^{-1} amino acid	1.76b	3.99b	5.20b	5.41d	1.23c	3.44c	3.98c	4.51b
Control	1.82b	4.14a	5.49a	6.85ab	1.24c	4.08b	5.35b	6.62a

Means within a column, following with the same letters are not significantly different at 0.05 levels.

Effect on chemical characteristics

Soluble Solids Content (SSC %):

Data in Table 9 cleared that soluble solid content (SSC %) of fruits gradually increased with the advance in cold storage. Significant differences between the treatments were obtained during storage periods at the most cases in the two seasons. The highest percentages of SSC were obtained by the mixture application of 2 ml L^{-1} seaweed extract + 0.5 ml L^{-1} amino acids (13.03 and 12.30%) in the two seasons, respectively. On the other hand, control exhibited the lowest value of SSC % (12.87and11.53%) in the first and second season. These results agreed with [6] described that foliar application of mixture amino acids and seaweed extract increased the SSC and SSC: TA ratio of grapes cv. Perlette. Furthermore, using foliar and/or soil application of amino acids on Florida Prince Peach which significantly improved TSS % [42].

Increase in SSC may be related with enzymes which are present in seaweed extract that enhanced the synthesis of different proteins, acids and sugars. Additionally, increase in SSC% may be due the hydrolysis of fruit starch is completed, no further increase in TSS could be detected and subsequently a decline in this parameter predictable since sugars along with other organic acids is primary substrates used for respiration[43]. Moreover, [44] recorded that the increase in SSC during fruit development is normally linked to changes in fruit color and ethylene production.

Table 9: Effect of spraying seaweed extract and amino acids on SSC % of Anna apple fruits stored at 0°C and 90%RH during 2014 and 2015 seasons.

Treatments	Storage period per week					Storage period per week				
	2014 season					2015 season				
	0	2	4	6	8	0	2	4	6	8
2 ml L^{-1} seaweed extract	10.07ab	10.13b	10.30a	10.93a	12.93a	10.20a	10.23a	10.30a	10.50a	11.93b
0.5 ml L^{-1} amino acid	9.93bc	10.17b	10.20a	10.93a	12.93a	9.83bc	10.20a	10.20b	10.23b	11.83b
2 ml L^{-1} seaweed extract + 0.5 ml L^{-1} amino acid	10.13a	10.23a	10.23a	11.03a	13.03a	9.93ab	10.17ab	10.20b	10.23b	12.30a
Control	9.87c	10.07c	10.20a	10.87a	12.87a	9.53c	10.03b	10.20b	10.27b	11.53c

Means within a column, following with the same letters are not significantly different at 0.05 levels.

Titratable acidity percentage in juice:

Data in Table 10 revealed that titratable acidity % decreased with the progress in storage period up to 8 weeks, without significant differences between the treatments was obtained during storage periods at the most cases in the two seasons. After 8 weeks, the highest values (0.22 and 0.21 %) were recorded by 0.5 ml L^{-1} amino acids treatment in both seasons, respectively. While the least percentage (0.19 %) of acidity was noticed by the mixture application of 2 ml L^{-1} seaweed extract + 0.5 ml L^{-1} amino acids in two seasons. This is agreement with [45] reported that treating "Hindy Bisinnara" mango trees with seaweed extract at 0.1 to 0.4 % twice, thrice or four times decreasing total acidity %.

Table 10: Effect of spraying seaweed extract and amino acids on acidity % of Anna apple fruits stored at 0℃ and 90%RH during 2014 and 2015 seasons.

Treatments	Storage period per week					Storage period per week				
	2014 season					2015 season				
	0	2	4	6	8	0	2	4	6	8
2 ml L^{-1} seaweed extract	0.36a	0.34a	0.32b	0.29a	0.20a	0.35a	0.34a	0.32a	0.28a	0.20a
0.5 ml L^{-1} amino acid	0.35a	0.32b	0.31b	0.29a	0.22a	0.34a	0.34a	0.31a	0.28a	0.21a
2 ml L^{-1} seaweed extract + 0.5 ml L^{-1} amino acid	0.35a	0.33ab	0.34a	0.28a	0.19a	0.35a	0.35a	0.31a	0.27a	0.19a
Control	0.37a	0.34a	0.34a	0.29a	0.21a	0.36a	0.35a	0.32a	0.28a	0.20a

Means within a column, following with the same letters are not significantly different at 0.05 levels.

Conclusions

From the aforementioned results, it can be concluded that spraying mixture 2 ml L^{-1} seaweed extract plus 0.5 ml L^{-1} amino acids at full bloom stage and one month after fruit setting stage led to improving growth, leaf mineral content, fruit set, yield, fruit quality and storability of Anna apple under cold storage at 0°C and (RH) 90% for two months.

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