

Impact of Different Sources of Natural, Mineral and Bio-Fertilizers on Apple Trees Performance, Growth and Yield on Sandy Soil

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THIS study was carried out during two successive seasons 2014 and 2015 on 8-year-old “Anna” apple trees budded on Balady rootstock and planted at 4 x 4 m apart on sandy soil at South El-Tahrir in a private farm under drip irrigation system. Addition of natural elements compound (NEC) as soil application beside other biofertilizers extract was investigated as compared with the mineral fertilization.

Biofertilizers markedly affected soil organic matter, pH and E.C. as their values changed with Azolla, Cyanobacteria individually and/or in combination. The higher increases in nitrogen, phosphorous, potassium and magnesium content in apple leaves nutrients were observed with the treatment (Azolla + Cyanobacteria and natural elements).

Biofertilizer extracts alone or in combination with (NEC) increased leaf area, leaf fresh weight, total leaf chlorophyll content, fruit set %, and yield. Foliar application of Cyanobacteria reduced fruit drop than foliated by Azolla in the two seasons. Treating trees by a combination of biofertilizer and NEC resulted in the highest fruit firmness, TSS %, vitamin C and total fruit sugar as compared with the other treatments. During cold storage, results showed, that the least weight loss percentage was recorded by NEC + Cyanobacteria and Azolla treatment in the two seasons. While the highest acidity % was recorded by the control and NEC treatments.

Generally, application of biofertilizers extract (Azolla, Cyanobacteria and their mixture) with natural element compound (NEC) improved growth, yield and fruit quality at harvest and after storability process. In addition, the increase in microbial activity in the soil may reduce additions of inorganic fertilizers.

Keywords: Apple component, Azolla, Cyanobacteria extract, Sandy soil, Biological assay.

Introduction

Apple- king of all fruits have long been associated with the biblical story of Adam and Eve, the fruit was originated in the Middle East just about 4000 years ago! It is one of the most favorite and popular fruits ever known. Apart from health care and nutrition, it is also known for medicinal values. The total production of apples fruit amounts to 629613 tons, according to the 2013 statistics inventory of the Egyptian Ministry of Agriculture, the total acreage of apples was (72616^{1*}) Feddans in A.R.E.

For a long time, growers have been interested in improving production of deciduous fruit trees by using foliar spray

with biostimulants. Biostimulants were defined by Rao and Burns (1990) who stated that, they increase plants nutrient and water uptake and resistance to water stress. In addition, improving crop yield and fruit quality without adverse effect on the environment, a major goal of horticulturist.

Chemical fertilizers are expensive and cause pollution of the environment. The use of nitrogenous fertilizers causes acidification of soil and severe application reduces microbial activity in soil (Pablay *et al.*, 2000).

Pollution problem has focused the attention of researchers on the possibility of using biofertilizers as an alternative or complementary for mineral fertility. N₂-fixing Azolla and blue-green algae (Cyanobacteria) serve as an excellent source of utilizing solar energy efficiency for providing the soil with organic matter (Venkatarman, 1981).

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Azolla is a genus of small, fast growing aquatic ferns that has a symbiotic association with a nitrogen fixing cyanobacterium floats freely on the surface of water and has a global distribution (Peters *et al.*, 1982). In addition, Azolla improves soil physical properties such as bulk density beside its ability to improve soil chemical properties (organic matter, N, P, K, Cu, Mg and Na) (Awodun, 2008).

Abd El-Rasoul *et al.* (2004) indicated that inoculation with Cyanobacteria enhances NPK uptake, soil microbial activity in terms of increasing the number of soil actinomycetes, total count of bacteria, total Cyanobacteria, CO₂ evolution and dehydrogenase activity.

Strik and Staden (2003) stated that both Cyanobacteria and Azolla extracts are characterized by their cytokinins, gibberellins and auxins content that enhance plant growth and proved to overcome and reduce E.C. degree and adverse effects of salinity. Mandal *et al.* (1999) demonstrated the positive effect of N₂-fixing Cyanobacteria on plant growth and yield to the production of growth promoting substances, *i.e.* gibberellins, cytokinin, auxins, abacisic acid, vitamins, antibiotics and amino acids.

Natural Elements Compound (NEC) can be used as mineral fertilizer source to supply nutrient elements to the crops with a lower cost and safe product. NEC is well known as the main source of elements for a plant growing under natural conditions pertaining to the weathering products of the geological raw materials and organic matter-source. Some ions of these elements are not easily released; therefore they are not suitable for direct application to plants. The increase in the weathering process by microorganisms and their metabolism, respiration by plant roots and microbe degradation of organic matter can elevate carbonic acid concentration in the soil ground water leading to the release of minerals (Chapell *et al.*, 1987 and Keller & Woed, 1993).

Yousry (2011) studied the effect of the natural element compound soil application on "Navel" orange trees, found that tree canopy, total yield efficiency was not affected in the 1st season, while fruit drop was reduced, when compared with the control treatment of chemical fertilizer. During the 2nd season, positive effects were observed on leaf area, fruit-set and yield which significantly increased and fruit drop is reduced, but trees canopy were not affected.

Moreover, NEC application had no effects on fruit weight, volume, total soluble solids and acidity when compared with control treatment. NEC significantly increased the leaves elements content and improved yield compared to the control in both seasons.

Natural potassium fertilizer up to 360 Kg/ feddan can significantly increase the percentages of N and K contents, number of seeds and seeds weight/plant. (Ezzat *et al.*, 2005 in lentil plants, El-Hadi *et al.*, 2003 in legumes). Moreover, magnetite (magnetic iron) is one of the important factors affecting plant growth such as the production of dry matter of garlic (Eid *et al.*, 1991), increases ferrous content on bean (Sharma *et al.*, 2003), nitrogen on bean (Maurya *et al.*, 1993), phosphorus in spanish (Reddy and Malewar, 1992), potassium in french bean (Singh *et al.*, 1995), sulfur and yield of cauliflower (Singal and Saraf, 1995).

In addition, Ca Mg(CO₃)₂ dolomite is essential for an economic citrus production in sandy reclaimed soils, where Ca and Mg content are positively related with citrus yield (Fidalski and Auler, 1997 and Fidalski, 1999). Recently, under Egyptian conditions a great attention is being devoted to reduce the inorganic fertilizers, the high cost of production and environmental pollution.

Generally this study aims to prove the possibility of using biofertilizers (Azola, Cyanobacteria) as an alternative of mineral fertilization, and the role of natural elements compound (NEC) derived from the geological raw materials as a plant nutrient instead of the chemical fertilizers in apple production in reclaimed sandy soil.

Materials and Methods

This study was carried out in a private farm located at south El-Tahrir sector for two successive seasons 2014 and 2015 on 8-years old "Anna" apple trees budded on Balady rootstock, and planted at 4×4m, apart, grown on sandy soil under drip irrigation system.

Studied treatments are as follows:

T₁, Control, the recommended mineral fertilizer doses of the farm supplements through fertigation system were as follows. Ammonium nitrate (33.5% N) 150 kg, Calcium mono phosphate (15.5% P₂O₅) 45 kg, Potassium sulphate (48 % K₂O) 100 kg/tree.

- T₂. Compound of natural elements (NEC) 0.5 kg/ tree soil application around tree. Chemical analysis of NEC is presented in Table 1:
- T₃. Azolla extract 5Kg / 20 L water: Azolla pinnata aquatic ferns was obtained from Agric. Microbiology Res. Dept., Soil, Water and Environment, Institute. Azolla was collected and incorporated into 0.01% mercuric chloride for 1 min and washed gently in running water for several times. Application of Azolla extract was applied as foliar sprays.
- T₄. Cyanobacteria (CB) extract: 5 L/20 L: It was grown in the lab and mixed to obtain a suspension used as foliar sprays.
- T₅. NEC + Azolla extract.
- T₆. NEC + CB extract
- T₇. Azolla + CB, the suspensions for both Azolla and CB were also mixed together.
- T₈. NEC + Azolla + CB.

TABLE 1. Chemical analysis for the natural elements compound (NEC)

Element	Concentration (%)	Element	Concentration (%)
SiO ₂	38.56-40.15	Na ₂ O	1.32-2.19
TiO ₂	0.76-0.85	K ₂ O	3.97-4.51
Al ₂ O ₃	7.80-7.85	P ₂ O ₅	6.14-8.52
Fe ₂ O ₃ (magnetic)	3.58-4.52	SO ₃	5.38-6.28
MnO	0.61-0.74	Cu	2.33ppm
MgO	2.47-3.92	Zn	3.67ppm
CaO natural rock	13.45-16.69	Mo	3.00ppm

Azolla and Cyanobacteria were applied as foliar application 4 times on trees and soil surface around trees during the two seasons: (a) at the 2nd week of January (swelling bud stage). (b) 1st week of February (flowering stage). (c) 1st week of March (fruit set). (d) (one month before harvesting, 1st week of May).

To evaluate the efficiency of the tested treatments on tree fruiting the following measurements were carried out.

1) Soil properties

Some chemical and biological properties of the soil were determined. Organic matter %, soil pH and EC were evaluated according to Page *et al.* (1982) Total bacterial count was performed on nutrient agar using the spread plate method according to (APHA 1992) and total CB counts were conducted in triplicate on the BG II medium according to Sardinha *et al.* (2003).

2) Leaf parameter

Leaves were taken at random from the middle of branch during mid-August in both seasons to determine leaf fresh weight (gm.), leaf area, leaf chlorophyll content using a chlorophyll meter (Model SPAD 502; Minolta Corporation, N.J., USA).

To determine leaf nutritional status, samples of twenty mature leaves were collected at random at mid. August, ground and digested with H₂O₂ and H₂SO₄.

Total nitrogen and phosphorus were determined colorimetrically, also potassium and magnesium contents were determined, using Flam photometer and the concentration of N, P, K and Mg were expressed as percent according to Page *et al.* (1982).

3) Vegetative growth: Leaf fresh weight, leaf area (cm²) and leaf chlorophyll content

4) Yield component and fruit properties

Determination of yield and fruit quality

Eight selected branches around tree were labeled for determination of different data during seasons fruit set % and fruit drop were calculated as follows:

$$\text{Fruit set \%} = \frac{\text{No. of developing fruitlets}}{\text{Total No. of flowers}} \times 100$$

$$\text{Fruit drop \%} = \frac{\text{No. of fruitlets at setting} - \text{No. of fruits at picking time}}{\text{No. of fruitlets}} \times 100$$

The analysis of mature fruit was carried out when fruits of the control attained maturity according to stands recorded by El-Azzouni *et al.*, (1975).

Yield was determined as Kg/tree.

Fruits physical properties

Fruits were picked for determining the following measurements:

- Fruit weight (gm), fruit shape index (fruit length/diameter), fruit size (cm³), fruit firmness (lb/inch²), peel color (Hue angle) determined after McGuire (1992).

Fruits chemical properties

1. (TSS%) Total soluble solids of fruit juice were determined by a hand refractometer according to A.O.A.C. (2016).
2. Acidity of fruit juice, was determined (as malic acid) according to A.O.A.C. (2016).
3. Total and reducing sugars %, was determined in pulp fruit samples according to A.O.A.C. (2016).
4. Vitamin C (Ascorbic acid) was assessed by the method of A.O.A.C. (2016) as mg/100g fruit.
5. The fruits contents of some macro-nutrients N, P, K, Ca and Mg as percent and micro-nutrients Na, Fe, Zn and Cu as ppm, using the methods explained by (Page *et al.*, 1982).

5) *Storability studies*

Fruits were picked at maturity stage, uniform samples from different treatments in both seasons and stored at 5°C. and R.H. 90-95% to keep quality of apple fruit for one month of storage. The determination procedures were as follow:-

- a- Weight loss % was calculated as the difference between fruit weight at the start of storage and fruit weight at the inspection date as the

following equation:

$$\text{Weight loss \%} = \frac{A - B}{A} \times 100$$

Where:

- A = the initial weight. B = weight at inspection date.
 b. Fruit firmness was determined as lb/inch² using pressure tester of 5/16 inch plunger.
 c. TSS (%): total soluble solid was determined by a hand refractometer.
 d. Acidity of fruit juice, was determined (as malic acid) according to A.O.A.C. (2016).

7) *Statistical analysis*

The experimental treatments were used in a randomized complete block design with three replicates. The collected data were tabulated and undergone to prepare analysis of variance using SAS program (1994), which was followed by Duncan's new multiple range test (Steel and Torrie, 1980) to compare the differences among means of various treatments.

Results and Discussion

Soil properties

The effect of examined treatments on some chemical properties of the soil after harvest are shown in Table 2, data show that application of T₈ (Az. + CB + NEC) increased the soil organic matter percentage OM over all treatments. The highest levels of organic matter were obtained in treatment T₈ (0.80 O.M%) and followed by T₇ and T₅ which gave 0.63% O.M. Moreover, the application of Az. + CB + NEC (T₈) effectively decreased soil E.C and pH as compared with the other treatments. These results were in harmony with El-Shahat (2007) who reported that, incorporation of Azolla decreased soil pH.

TABLE 2. Effect of different treatments on some chemical properties of soil after harvest (average of two seasons)

Treatments	O.M. (%)	pH in suspension (1:2.5)	EC dSm ⁻¹
T1 Control	0.35	7.85	1.06
T2 NEC	0.51	7.73	1.03
T3 Azolla (Az.)	0.6	7.36	0.90
T4 Cyanobacteria CB	0.58	7.32	0.93
T5 NEC + Az	0.63	7.30	0.90
T6 NEC + CB	0.61	7.30	0.91
T7 Az + CB	0.63	7.31	0.88
T8 NEC + Az + CB	0.8	7.30	0.84

It is quite clear that the type of biofertilizer markedly affected soil O.M, pH and E.C. as their values changed with Azolla, CB individually and/or in mixture.

Changes in root rhizosphere could give an approximate vision to the ability of different applications to enhance crop production. However, all treatments affected biological activity of the soil. Table 3 obviously shows increasing of the total bacterial counts and CO₂ evolution compared to the control and natural elements compounds treatments in the two seasons. The maximum microbial activity was achieved by the combined effect of T₈ (Az + CB. + NEC) treatment application, the changes of biological activity in root rhizosphere were greatly fluctuated among the other treatments. Also, it could be noticed that, the Az. and CB as single application or in combination with natural compound increased microbial activity.

Leaf parameters

Leaf mineral content

Data in Table 4 revealed that, apple trees treated with mixture of Az., CB and NEC (T₈) exhibited the highest values of N, P, K and Mg than the other treatments and the control. Treatment (T₈) is considered the most suitable medium for most of beneficial microorganisms. Application of this mixture plays an important role in enhancing CB and other microorganisms and to fix atmospheric nitrogen which is reflected on the increase of N content in apple leaves. The same trend may be expected for the increase of P, K and Mg solubility where they led to relative increase of such elements in the leaves. Moreover application of Az. + CB was more effective than Azolla or CB, nutrient alone, compared with control (T₁) in the two seasons.

TABLE 3. Biological activity

Treatments	T.C. Bacteria Cfu x 10 ⁶ /(g ⁻¹ soil)		T.C. Cyanobacteria Cfu x 10 ⁴ (g ⁻¹ soil)		CO ₂ evolution	
	1 st season	2 nd season	1 st season	2 nd season	1 st season	2 nd season
T1 Control	21.00gh	73.20d	50.00h	63.50h	13.00h	17.00h
T2 NEC	39.00f	45.35g	96.25g	123.00f	35.00g	37.60g
T3 Azolla (Az)	23.00g	30.20h	112.76e	165.00e	39.80f	45.22f
T4 Cyanobacteria (CB)	49.70d	61.73e	101.18f	211.33d	43.91e	55.20e
T5 NEC + Az	33.22e	54.20f	135.40d	262.18b	55.13d	61.22d
T6 NEC + CB	54.88c	111.36c	161.60c	80.11g	59.81c	69.18c
T7 Az + CB	73.33b	154.21b	221.00b	293.17a	71.22b	91.36b
T8 NEC + Az + CB	111.25a	215.33a	239.40a	253.00c	114.11a	116.35a

Means within a column having the same letters are not significantly different according to Duncan's Multiple Range Test at 5% level.
T.C = Total count. Cfu = Colony forming unit.

TABLE 4. Effect of different treatments on nitrogen, phosphorus, potassium and magnesium contents in the apple leaves during two seasons

Treatments	N %		P %		K %		Mg %	
	1 st season	2 nd season	1 st season	2 nd season	1 st season	2 nd season	1 st season	2 nd season
T1 Control	1.25d	1.29d	0.08f	0.10d	1.00e	1.09e	0.22de	0.20e
T2 NEC	1.36d	1.41d	0.11e	0.12e	1.26d	1.22d	0.21e	0.24d
T3 Azolla (Az)	1.78c	1.83c	0.16d	0.15c	1.30cd	1.35c	0.26bc	0.21de
T4 Cyanobacteria (CB)	2.02b	2.10b	0.17d	0.21bc	1.41b	1.51b	0.23d	0.26c
T5 NEC + Az	2.00b	2.05b	0.20bc	0.17c	1.53a	1.59ab	0.26d	0.29b
T6 NEC + CB	2.36a	2.51a	0.19c	0.24b	1.42b	1.44b	0.25c	0.31ab
T7 Az + CB	2.39a	2.48a	0.21b	0.25b	1.39bc	1.47b	0.27ab	0.30ab
T8 NEC + Az + CB	2.50a	2.53a	0.26a	0.30a	1.59a	1.64a	0.28a	0.39a

Means within a column having the same letters are not significantly different according to Duncan's Multiple Range Test at 5% level.

*Vegetative growth**Leaf fresh weight*

Table 5 showed that, Azolla foliar applications treatment increased leaf fresh weight. Whereas, Azolla + natural element compound caused the highest weight when compared to other treatments in the two seasons.

In this respect, Venkatarman (1981) reported that Azolla is considered an excellent source for providing soil with organic matter and for improving N balance of many crops.

Leaf area (cm²)

Data in Table 5 revealed that, both Az. and NEC + Az. + CB treatments as foliar applications significantly increased leaf area in the two seasons. On the other hand, NEC alone and NEC + Az. resulted the lowest leaf area in the two seasons.

Our results are consistent with Mussa (2005) who reported that the use of both Azolla and CB extracts are characterized by its content of auxins, gibberellins and cytokinins that enhance the plant growth. Yousry (2011) in a study on the natural elements compound (NEC), soil application on “Navel orange” trees,

found positive effects on leaf area in the second season alone but tree canopy was not affected.

Leaf chlorophyll content

Data in Table 5 showed that, trees sprayed by CB alone or mixed with NEC, and CB + Azolla + NEC recorded the highest significant increase in the total leaf chlorophyll contents with no significant differences between the other treatments in the two seasons. It is well known that increasing chlorophyll contents in the plant cell depends on availability of nitrogen and Mg contents.

Eissa (2003) showed that, biostimulants spray plum tree, significantly enhanced shoot length, leaf area and chlorophyll content. Also, Abo-Hamda (2015) found that NEC increased leaf chlorophyll content of “Valencia” orange. Similar observations were attained by El-Shahat *et al.* (2014) who noticed that 75% recommended dose of NPK + Azolla of foliar + mixed bacteria enhanced the biological activity of the soil and availability of N, P, K, Mn, Zn and Cu.

TABLE 5. Effect of different treatments on leaf fresh weight, leaf area and leaf chlorophyll content in the apple leaves during two seasons.

Treatments	Leaf fresh weight (g)		Leaf area (cm ³)		Leaf chlorophyll content (SPAD)	
	1 st season	2 nd season	1 st season	2 nd season	1 st season	2 nd season
T1 Control	0.706e	0.583e	23.33b	17.50d	46.60b	43.33d
T2 NEC	0.740de	0.800c	16.00d	17.18d	46.23b	48.83bc
T3 Azolla (Az)	0.750c-e	0.897b	24.83a	27.47a	47.03b	50.93ab
T4 Cyanobacteria (CB)	0.737de	0.747cd	23.23b	24.00b	50.43a	50.87ab
T5 NEC + Az	1.033a	0.953a	18.93c	10.80f	44.57b	47.03c
T6 NEC + CB	0.777b-d	0.733d	23.30c	11.13e	52.97a	52.97a
T7 Az + CB	0.813b	0.733d	23.26b	21.80c	45.20b	47.13c
T8 NEC + Az + CB	0.797bc	0.620e	24.00ab	23.53b	52.03a	50.74a

Means within a column having the same letters are not significantly different according to Duncan's Multiple Range Test at 5 % level.

*Yield component and fruit properties.**Fruit set %.*

Data in Table 6 obviously indicated that the mixture of NEC + Az. + CB significantly increased fruit set percentage in the two seasons compared to the other treatments, followed in a descending order by mixture of Azolla plus CB, NEC + CB, NEC + Az. On the other hand, NEC alone significantly produced the lowest fruit set percentage in the two studied seasons. But if NEC was mixed with biofertilizers it had remarkable higher increases in fruit set percentage.

Yousry (2011) reported that soil application of NEC to “Navel oranges” had no effect on total yield in the 1st season, while in the 2nd season a positive effect on fruit set and yield was observed; this may be as a result to the slow realize of NEC elements.

Fruit drop %.

Data in Table 6 indicated that, the second season recorded the highest fruits drop percentage as compared to the first one depending on the high temperature waves during the second season. In

both seasons, applications with NEC, mineral fertility (control) and Az. or CB significantly indicated the highest number of fruit drop. Since, the lowest values of fruit drop were recorded with NEC + Az + CB, Az + CB, NEC + CB, respectively. It is evident that foliar trees by CB reduced fruit drop than trees sprayed with Az. in the two seasons. As a matter of fact, improving soil minerals content and trees nutrient balance will be reflected tree growth, yield and a decrease in fruit drop.

Yield

As for results of fruit yield (Table 6), it is evident that natural elements compound NEC + Az. + CB extract treatment significantly increased yield (82.87 Kg/tree) in the 1st season. While, bio-fertilizer product as Az. + CB has the highest significant increase of tree yield in the 2nd season, followed by NEC + Az. + CB which have no significance between them. So, we can notice that NEC + Az + CB treatment significantly induced the highest fruit set and yield but the least fruit drop.

Mussa (2005) stated that, the inclusion of such phytohormones in both CB and Azolla encourage the agronomists and agriculturists to use those biofertilizers that influence the yield especially in reclaimed soil. Similarly, Strick and Staden (2003) noted that both CB and Az. extracts are characterized by their cytokinins, gibberellins

and auxins content that enhance plant growth and yield. The positive effect of N₂-fixing CB on plant growth and yield to produce growth promoting substances, *i.e.* Gibberellins's, cytokinins, auxins, abscisic acid, vitamins, antibiotics and amino acids. Also, Eissa (2003) got better yield and fruit size by biostimulants spray. Aref *et al.* (2011) stated that Azolla and or CB as biofertilizer, increased significantly grains yield of Rice.

Fruit weight (g)

Results in Table 6 showed that the treatment NEC + Az + CB gave the highest significant average fruit weight during both seasons, followed by Az + CB. Control and NEC in the 1st season, control, NEC and CB in the 2nd season gave the least fruit weight.

Results are in line with Yousry (2011) who stated that soil application of NEC on "Navel orange" trees did not affect fruit weight, volume, TSS and acidity when compared with the control treatment (chemical fertilizer).

Fruit size

Results in Table 6 indicated that the mixture of natural element compound + Azolla + CB extracts treatment significantly increased the apple fruit volume in the two seasons, followed by Az + CB. While, NEC gave the lowest fruit volume in the two seasons if used alone but when NEC treated with Azolla or CB or Azolla + CB significantly increased fruit size.

TABLE 6. Effect of different treatments on fruit properties (fruit set %, fruit drop %, fruit size (cm³), fruit weight (g) and yield during two seasons

Treatments	Fruit set %	Fruit drop %	Yield kg/tree	Fruit weight (g)	Fruit size (cm ³)
First season					
T1 Control	15.80c	39.80a	53.63de	125.3d	124.4d
T2 NEC	10.53d	41.33a	53.14de	123.7d	120.67e
T3 Azolla (Az)	17.63bc	36.00ab	64.00c	132.0c	131.7c
T4 Cyanobacteria (CB)	14.33cd	33.87b	61.32cd	132.4c	126.0d
T5 NEC + Az	20.87b	30.00bc	61.90cd	138.5b	134.3c
T6 NEC + CB	21.00b	27.00c	63.40c	136.2b	138.5c
T7 Az + CB	23.00ab	25.00cd	72.80b	146.3a	167.8b
T8 NEC + Az + CB	25.00a	18.00d	82.87a	149.0a	178.9a
Second season					
T1 Control	11.50bc	42.57b	27.10d	97.20f	119.8f
T2 NEC	9.87c	51.67a	30.93d	104.7e	133.7e
T3 Azolla (Az)	13.17b	41.57bc	49.36b	108.0de	141.3d
T4 Cyanobacteria (CB)	12.17bc	40.00bc	51.03ab	106.0e	152.0c
T5 NEC + Az	13.97b	36.30cd	49.63b	111.3d	156.7b
T6 NEC + CB	14.23b	35.10cd	47.58b	157.3c	160.6b
T7 Az + CB	14.37b	30.00de	57.80a	169.7b	175.5a
T8 NEC + Az + CB	17.67a	28.33e	54.07ab	173.7a	176.7a

Means within a column having the same letters are not significantly different according to Duncan's Multiple Range Test at 5 % level.

Fruit dimensions

Data in Table 7 showed that a good correlation between fruit size and fruit dimensions, the mixture of NEC indicated the significant increase in fruit length and diameter through the two seasons, while each of the control, NEC, Az and CB induced the least fruit dimensions.

L/D ratio (fruit shape index)

Practically, “Anna” apple cv., fruit has a vertical shape, Table 7 show that treatments in this study fluctuated in their effect on fruit shape with a significant effect in the two seasons. The first season CB single or with NEC recorded the highest value of fruit shape index. In the second season, (Azolla + CB + NEC) and (CB + Az) treatments significantly recorded the highest value compared to the other treatments.

Fruit firmness lb/inch²

As shown in Table 7, data exhibited positive impact on, fruit firmness (lb/inch²) at harvest. Spraying trees by NEC + bio-fertilizer products Azolla or CB, produced the highest values of fruit firmness in the first season. Data also exhibited a significant relation between treatments. Spraying trees by NEC mixed with

bio fertilizer produced the highest values in the two seasons followed by a descending order by Azolla or CB alone or mixed with NEC. While NEC and the control recorded the lowest values. These results are in line with those obtained by Yousry (2011) who stated that a significant increase in peel thickness, firmness in “Navel orange” was observed during two seasons, when soil was applied with NEC.

TSS (%)

Data in Table 7 showed that the highest percentage of TSS % at harvest were obtained by mixed biofertilizers together (or/and) with NEC during the two seasons, while the control and NEC fruits exhibited the lowest values, with significant differences between treatments in the two seasons.

Hue angle

Hue angle is an actual measure of the fruit color. The decrease of hue angle in apple fruit represents a change from greenish yellow to red in both seasons depending on the effect of different treatments. Data in Table 7 showed that NEC + CB and CB alone recorded the lowest degree of Hue angle which were more ripening. That means more red color density.

TABLE 7. Effect of different treatments on fruit length, fruit diameter, fruit shape index, fruit firmness, TSS at harvest and Hue angle in apple fruits during two seasons

Treatments	Fruit length (cm)	Fruit diameter (cm)	F. shape index	Fruit firmness (lb/inch ²)	TSS at harvest	Hue angle
First season						
T1 Control	6.15d	5.87d	1.05d	11.33e-g	9.23c	92.20
T2 NEC	6.07d	6.20bc	0.98c	11.47ef	9.25c	103.60
T3 Azolla (Az)	6.53c	5.83d	1.12b	15.00bc	9.43bc	58.71
T4 Cyanobacteria (CB)	6.90b	5.90d	1.17a	15.50bc	9.30bc	35.80
T5 NEC + Az	6.93ab	6.33b	1.09bc	15.47bc	9.63bc	111.20
T6 NEC + CB	6.97ab	6.03b	1.16a	17.13a	9.57bc	12.90
T7 Az + CB	7.07ab	6.81a	1.04bc	15.60b	10.00b	90.00
T8 NEC + Az + CB	7.31a	6.93a	1.05bc	16.93a	11.07a	107.00
Second season						
T1 Control	6.73e	6.12c	1.10b	11.10c	11.73d	100.60
T2 NEC	6.73e	6.40b	1.05bc	11.89bc	12.80c	103.60
T3 Azolla (Az)	6.93d	6.57ab	1.05bc	13.47a	12.80c	43.10
T4 Cyanobacteria (CB)	7.17c	6.40b	1.12ab	13.73a	13.17bc	39.90
T5 NEC + Az	7.30c	6.47ab	1.13ab	13.87a	13.67bc	71.00
T6 NEC + CB	7.20c	6.53ab	1.10b	14.40a	14.23b	45.80
T7 Az + CB	7.63b	6.57ab	1.16a	11.05b-e	14.87b	41.50
T8 NEC + Az + CB	7.83a	6.70a	1.17a	14.13a	15.17a	78.00

Means within a column having the same letters are not significantly different according to Duncan's Multiple Range Test at 5 % level.

On the other hand, NEC, NEC + Azolla and control (mineral fertility) showed heigher degree of Hue Angle ranged from greenish-yellow to yellow to orange yellow returned to the chlorophyll pigments.

These results are in line with those of Sobieh *et al.* (2008) who noted that the hue angle (h) was a good factor to assess changes of the characteristic color on fruit.

Total acidity (%)

Data in Table 8 showed that fruit acidity was the highest in the NEC and control treatment in the two seasons. They were followed by other treatments with the least values being those of CB + NEC and Az + CB + NEC in the two seasons.

Fruit juice Vitamin C "ascorbic acid" content:

Data in Table 8 revealed that Az. combined with NEC + CB resulted in the highest increase in V.C. during the two seasons. On the other hand, CB combined with NEC or Az exhibited the lowest

value.

Total sugars (%)

Fruit total sugar content data presented in Table 8 indicated that the combination between NEC + Az + CB significantly enhanced fruit sugar as compared with the other treatments, followed in a descending order by Az. + CB in the two seasons. The control treatment led to the lowest percentage of the total sugars.

Reducing sugars (%)

Data in Table 8 indicated that NEC + biofertilizers (Az + CB) increased reducing sugars in fruits as compared with NEC and the control in the two seasons. However, fruit sugars content had a significant increase in persimmon fruits by the combination between organic compost and biofertilizers + NPK (Darwesh, 2012). Eissa (2003) indicated positive effects of various biostimulants on growth, yield and quality of some horticultural crops.

TABLE 8. Effect of different treatments on acidity, vitamin (C), total sugar and reducing sugar in the apple fruits during two seasons

Treatments	Acidity (%)		Vitamin C (mg/100 ml)		Total sugar (%)		Reducing sugar (%)	
	1 st season	2 nd season	1 st season	2 nd season	1 st season	2 nd season	1 st season	2 nd season
T1 Control	0.57bc	0.64bc	5.20c	3.37d	5.77c-e	6.10c	1.13bc	1.40b
T2 NEC	0.59a	0.67ab	7.03ab	4.60b	6.37cd	6.70c	1.02cd	0.89c
T3 Azolla (Az)	0.58ab	0.58de	4.10d	2.93e	5.57de	6.00c	0.75d	1.27b
T4 Cyanobacteria (CB)	0.54d	0.61cd	6.57b	4.17c	5.00de	5.70c	1.46b	2.13a
T5 NEC + Az	0.56c	0.63c	3.77de	3.07de	6.83bc	8.33b	1.45b	1.27b
T6 NEC + CB	0.52de	0.55e	3.30e	2.90e	7.57b	8.23b	1.43b	1.57b
T7 Az + CB	0.51e	0.69a	3.40e	3.10de	7.77b	8.40b	1.44b	2.40a
T8 NEC + Az + CB	0.47gh	0.59d	7.47a	5.00a	12.17a	11.90a	1.87a	2.40a

Means within a column having the same letters are not significantly different according to Duncan's Multiple Range Test at 5 % level.

Mineral fruit content

Data in Table 9 showed that, NEC + Az + CB treatment effectively caused an increase in all studied mineral content (N, P, K, Ca, Mg, Na, Fe, Zn and Cu). NEC + Az treatment induced higher P, Mg, Fe and Zn content. Also, CB + Az treatment get higher, P, Na, Fe, Zn and Cu fruit content. Moreover, NEC treatment showed more K, Mg, Na, Fe and Zn in apple fruits, while NEC + CB induced higher content of Mg, Fe and Zn elements. Besides, Azolla and CB increased both of Fe and Zn content. However, Zn fruit content did not show significant differences. Hence, the present treatments effectively enriched the nutrition status of apple fruits.

Storability studies

a. Weight loss %

Table 10 showed the effect of treatments as pre-harvest and during cold storage at 5°C till the end of the storage period (4 weeks). Results showed a gradual increase in weight loss towards the end of the storage period, the least weight loss percentage was recorded by the treatments mixed NEC + CB + Az and Az + CB at the 1st and 2nd seasons as well as NEC + Az treatment recorded the lowest weight loss % (3.7 %) in second season. While control and NEC gave the highest weight loss compared to the other treatments.

TABLE 9. Effect of different treatments on some macro and micro nutrients in apple fruits (average of the two seasons).

Treatments	N %	P %	K %	Ca %	Mg %	Na %	Fe ppm	Zn ppm	Cu ppm
T1 Control	0.39ef	0.19d	1.00d	3.65c	3.12c	0.70ab	1.43b	0.23a	6.03e
T2 NEC	0.40de	0.21bc	1.33a	4.36b	3.70ab	0.69ab	1.80ab	0.25a	7.22d
T3 Azolla (Az)	0.41d	0.22bc	1.03d	4.11bc	3.64b	0.53c	1.60ab	0.27a	7.18d
T4 Cyanobacteria (CB)	0.39ef	0.20c	1.00d	3.96c	3.60b	0.60bc	1.55ab	0.24a	7.50cd
T5 NEC + Az	0.46b	0.24ab	1.21cd	4.41b	3.76a	0.66b	1.89ab	0.28a	9.16b
T6 NEC + CB	0.43c	0.23b	1.21cd	4.30b	3.69ab	0.68b	1.86ab	0.26a	8.11bc
T7 Az + CB	0.44c	0.24ab	1.25b	5.11ab	3.71ab	0.70ab	1.91a	0.30a	9.80ab
T8 NEC + Az + CB	0.50a	0.26a	1.27a	5.40a	3.86a	0.71a	2.05a	0.32a	10.06a

Means within a column having the same letters are not significantly different according to Duncan's Multiple Range Test at 5 % level.

TABLE 10. Effect of treatments on fruit weight loss during storage for 0, 2 and 4 weeks.

Treatments	Weight loss (%)							
	At Harvest	After 2 weeks	After 4 weeks	Mean	At harvest	After 2 weeks	After 4 weeks	Mean
	First season				Second season			
T1 Control	0.00g	4.37e	13.23a	8.80A	0.00h	4.25f	7.67bc	5.96B
T2 NEC	0.00g	4.40e	12.03b	8.22AB	0.00h	6.02de	10.27a	8.14A
T3 Azolla (Az)	0.00g	4.60e	11.67b	8.13AB	0.00h	4.60f	8.30b	6.45B
T4 Cyanobacteria (CB)	0.00g	6.50d	9.13c	7.82B	0.00h	2.63g	6.30de	4.67CD
T5 NEC + Az	0.00g	5.07e	8.13c	6.60C	0.00h	1.83g	5.68e	3.76D
T6 NEC + CB	0.00g	2.63f	8.73c	5.68D	0.00h	2.78g	7.02cd	4.90C
T7 Az + CB	0.00g	1.97f	8.13c	5.05D	0.00h	4.20f	4.40f	4.30CD
T8 NEC + Az + CB	0.00g	2.42f	8.47c	5.45D	0.00h	2.35g	5.93de	4.14CD
Mean	0.00C	4.00B	9.94A		0.00C	3.58B	6.95A	

Means within a column having the same letters are not significantly different according to Duncan's Multiple Range Test at 5 % level.

b. Fruit firmness (lb/inch²):

Data tabulated in Table 11. As for the specific effect of treatments and storage at 5°C during the two seasons, results indicated a gradual decrease in the rate of firmness. However, fruit firmness decline towards the end of storage period. At harvest, the highest firmness value were obtained by mixture of NEC plus CB and NEC + Azolla + CB extract, while control and NEC exhibited the lowest firmness value in the first season.

The same pattern was observed with the means of treatments up to the end of storage period (4 week at 5°C) it is evident that treatment (CB + NEC) still the highest firmness during the storage process in the first season. In the second season, (Azolla + CB) and NEC treatments recorded the lowest firmness but Az. + CB + NEC and CB were the highest firmness.

c. TSS%

Data presented in Table 11 Total soluble solids

percentage increased gradually with the advance in cold storage up to 4 weeks in all treatments. The change in TSS is a result of, respiration rate and moisture loss by evaporation. The tendency of increasing TSS initial up to 2 weeks of storage period may be attributed to the high rate of moisture loss.

At harvest date, the highest percentage of TSS was obtained by NEC plus Az. and CB tell the end of storage period in the two seasons. While control and NEC + Az presented the lowest degree.

d- Acidity (%)

Data in Table 12 show that acidity was decreased with the progress in storage period tell to 4 weeks, then the amounts of malic acid exhibited a decrease trend with the advance storage period throughout the two seasons that means changes in fruit quality related to the increase in the TSS with less acidity.

TABLE 11. Effect of treatments on fruit firmness and TSS % during storage for 0, 2 and 4 weeks

Treatments	Firmness (lb/inch ²)				TSS (%)			
	At Harvest	After 2 weeks	After 4 weeks	Mean	At harvest	After 2 weeks	After 4 weeks	Mean
First season								
T1 Control	11.33e-g	9.63hi	7.97jk	9.64E	9.23f	9.37ef	9.53d-f	9.38C
T2 NEC	11.47ef	11.13e-g	10.80f-h	11.13D	9.25f	10.33bc	11.50a	10.36B
T3 Azolla (Az)	15.00bc	11.50ef	8.03jk	11.51D	9.43ef	10.30bc	11.27a	10.33B
T4 Cyanobacteria (CB)	15.50bc	11.30e-g	7.10k	11.30D	9.30ef	10.13bc	10.97a	10.13B
T5 NEC + Az	15.47bc	12.28e	9.10ij	12.28C	9.23f	9.50d-f	9.80c-e	9.51C
T6 NEC + CB	17.13a	14.33cd	11.60ef	14.36A	9.57d-f	10.40b	11.30a	10.42B
T7 Az + CB	15.60b	12.27e	9.77hi	12.54C	10.00b-d	10.13bc	10.33bc	10.16B
T8 NEC + Az + CB	16.93a	13.70d	10.13g-i	13.56B	11.07a	11.13a	11.27a	11.16A
Mean	14.80A	12.02B	10.13C		9.63C	10.16B	10.75A	
Second season								
T1 Control	11.10b-e	10.60c-g	10.13e-g	11.25BC	11.73l	12.63k	13.57ij	12.64E
T2 NEC	11.89bc	10.14e-g	8.32h-j	10.11D	12.80k	14.00g-i	15.27de	14.02D
T3 Azolla (Az)	13.47a	10.73c-f	8.10ij	10.77CD	12.80k	14.83ef	18.00a	15.62A
T4 Cyanobacteria (CB)	13.73a	11.53b-d	9.43f-h	11.57AB	13.17jk	14.37fg	16.50b	14.83B
T5 NEC + Az	13.87a	10.47d-g	7.42j	10.58CD	13.67h-j	15.08de	15.10de	14.38C
T6 NEC + CB	14.40a	11.23b-c	8.12ij	10.61CD	14.23gh	15.00e	15.97bc	15.09B
T7 Az + CB	11.05b-e	10.20d-g	9.30g-i	10.18D	14.87ef	15.67cd	15.17de	15.01B
T8 NEC + Az + CB	14.13a	12.14b	10.14e-g	12.14A	15.17de	16.07bc	16.17bc	15.80A
Mean	12.95A	10.88B	8.87C		13.55C	14.71B	15.72A	

Means within a column having the same letters are not significantly different according to Duncan's Multiple Range Test at 5 % level.

TABLE 12. Effect of treatments on acidity % during storage for 0, 2 and 4 weeks

Treatments	Acidity %							
	At Harvest	After 2 weeks	After 4 weeks	Mean	At harvest	After 2 weeks	After 4 weeks	Mean
1 st season				2 nd season				
T1 Control	0.57bc	0.51e	0.49f	0.52A	0.64bc	0.55e-h	0.47ij	0.55AB
T2 NEC	0.59a	0.48fg	0.37l	0.48C	0.67ab	0.53f-h	0.41k	0.54B
T3 Azolla (Az)	0.58ab	0.46hi	0.40k	0.48C	0.58de	0.54f-h	0.46jk	0.53B
T4 Cyanobacteria (CB)	0.54d	0.49f	0.43j	0.49B	0.61cd	0.53f-h	0.53f-i	0.56A
T5 NEC + Az	0.56c	0.46hi	0.39k	0.47D	0.63c	0.54f-h	0.55f-h	0.57A
T6 NEC + CB	0.52de	0.47gh	0.36l	0.48C	0.55e	0.57e-h	0.51h-j	0.54B
T7 Az + CB	0.51e	0.48fg	0.46hi	0.48C	0.69a	0.52g-i	0.52g-i	0.58A
T8 NEC + Az + CB	0.47gh	0.40k	0.45i	0.41E	0.59d	0.54f-h	0.53f-i	0.55AB
Mean	0.54A	0.47B	0.42C		0.62A	0.54B	0.50C	

Means within a column having the same letters are not significantly different according to Duncan's Multiple Range Test at 5 % level.

The least acidity was exhibited by NEC + Az + CB treatment followed by NEC + Az in the first season while control treatment caused the highest acidity. The second season showed pronounced reduction in fruit juice acidity by using the biofertilizer Az and NEC.

To conclude, the application of biofertilizers (Azolla, Cyanobacteria) + NEC in a mixture to apple trees had positive impact on fruit set and fruit yield. Yet with more better fruit quality as evidenced by better physical and chemical attributes. Such application has extended to ensure better fruit characteristics during storage as compared to the control (mineral fertilizer).

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أثر استخدام مصادر مختلفة من التسميد (المعدني، الطبيعي والحيوي) على النمو والمحصول لأشجار التفاح في الارض الرملية

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تمت هذه الدراسة خلال موسمين متتاليين على أشجار التفاح عمر ثماني سنوات مطعوم على الأصل البلدي المنزرع على مسافة 4×4 متر بجنوب التحرير بمزرعة خاصة تحت نظام الري بالتنقيط عن طريق التربة بجانب مستخلصات حيوية من (الازولا (NEC) تمت أخافه مخلوط العناصر الطبيعية والسيانوبكتريا) استخدمت رشا ومقارنتها بالتسميد المعدني.

أظهر التسميد الحيوي تأثير إيجابي على محتوى التربة من المادة العضوية، EC, PH، كما تباينت النتائج عند استخدام الازولا والسيانو بكتريا منفردا أو مختلطين.

بالنسبة لمستوى العناصر بالأوراق N, P, K, Mg كانت أعلى المتوسطات للمعاملة (أزولا + سيانو بكتريا + NEC).

أظهرت معاملة (الازولا + سيانو بكتريا + مخلوط العناصر الطبيعية) تفوق واضح في النمو الخضري ممتثلا في مساحة الأوراق، الوزن الطازج ومحتواها من الكلوروفيل بالإضافة إلى زيادة العقد والمحصول.

الرش بالسيانو بكتريا أدى الى تناقص كبير في نسبة التساقط . رش الأشجار بالمخلوط الحيوي أزولا + السيانو بكتريا مع مخلوط العناصر الطبيعية أعطى زيادة في الصلابة، TSS، V.C، السكريات الكلية بالمقارنة بباقي المعاملات.

أظهرت المعاملات المختلفة استجابة في تحسين الصفات الثمرية عند التخزين البارد على 5 درجة مئوية بالمقارنة بالتسميد المعدني ومعاملة (NEC) مخلوط العناصر الطبيعية.

من البحث أمكن استنتاج أن التسميد الحيوي بمخلوط من مستخلص السيانو بكتريا والازولا مع مخلوط العناصر الطبيعية يؤدي إلى زيادة في النمو، المحصول، وتحسين الصفات الثمرية مع زيادة القدرة التخزينية للثمار بالإضافة إلى زيادة النشاط الميكروبي في التربة مما يؤدي إلى تقليل التسميد المعدني.