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EFFECT OF MICROBIAL BIOFERTILIZATION ON REDUCING CHEMICAL FERTILIZERS, VEGETATIVE GROWTH, NUTRITIONAL STATUS, YIELD AND FRUIT QUALITY OF APPLE

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ABSTRACT

Microbial inoculum contained main organisms (beat-based): Azotobacter spp. and Bacillus megatherium which found in 'Biogein', 'Rhizobacterein', 'Phosphorein' and arbuscular mycorrhizal (AM) fungi were used to reduce the recommended dose of nitrogenous and phosphoric fertilizers of "Anna" apple trees. The treated trees were seven years old budded on MM. 106 rootstock grown on sandy soil at Elbostan district under drip fertigation system during two successive seasons (2003 and 2004) to investigate the influence of the microbial biofertilization on tree growth, yield and fruit quality. The treatments were 100% of recommended mineral fertilizers dose/tree without microbial biofertilization (control) and 100% (F1), 50%(F2), 25% (F3) of recommended mineral fertilizers dose plus microbial fertilizers.

shoot length, leaf area and leaf dry weight of treated trees with microbial biofertilization were significantly increased. while, specific leaf weight (SLW) was not statistically affected with any treatment. also, chlorophyll a, chlorophyll b and total chlorophyll content of the leaf recorded significant increases compared with uninoculated control trees.

Microbial inoculum significantly increased 'Anna' apple leaf carbohydrate content (soluble sugars, starch and total carbohydrates) than uninoculated control ones. All microbial fertilization treatments markedly affected nutritional status of 'Anna' apple trees. Leaf concentrations of N, P, Ca, Fe and Zn were increased in a significance manner. While, Mg and Mn concentrations were consistently higher without significant difference in a comparison with control.

As for yield and fruit quality, yield parameters (number and weight) were significantly increased as microbial biofertilizers was applied. Fl and F2 treatments recorded the highest fruit weight and number per tree. At the same time, Fl and F3 recorded the highest average fruit weight, moreover, improved TSS % and acidity % of fruit juice at harvest time significantly in both seasons. So, the recommended treatment is F2 which contain microbial biofertilization + 50% of recommended mineral fertilizers.

Key words: Apple - Malus domestica - Microbial biofertilization -Mineral fertilizers - Nutritional status.

INTRODUCTION

Anna apple / (*Malus domestica*, Borkh) is the most cultivated apple cultivar in Egypt. Acreage reached 64135 feddans in 2003 (MLAR, 2003).

The advanced capability of chemical analysis enabled the detection of critical amounts of any compound. This led to identifying traces of nitrogenous, heavy metals and pesticides residues and such like of harmful compounds in fruits. Nitrates and nitrites are a hazard residues, normally originated from applications of nitrogenous fertilizers. Nitrates is usually reduced in the human body to nitrite and subsequent reaction of nitrite with amino acids leads to a formation of nitrose amines which are carcinogenic compounds (Hallberg, 1989). Otherwise, the effect of the form of N nutrition on soil stability is an important consideration for the management of sustainable agriculture systems (Bethlenfalvy *et. al.*, 1999). Moreover, phosphoric fertilizers contain cadmium and lead residues (Srikant *et. al.*, 1994) which could cause several health problems.

Accordingly, the biological fertilizers have a growing importance over the chemical fertilizers from the standpoint of environmental safety, quality of fruits and sustainable agriculture. Recently, several studies were carried out on the dual inoculation of different plants with nitrogen fixers and arbuscular mycorrhizal (AM) fungi (Rahman and Parsons, 1997; Amora-Lazcano *et. al.*, 1998 and Bethlenfalvy *et. al.*, 1999).

In alkaline soils as Egyptian ones, and particularly in poor P sandy soils, the soluble phosphate is derived from either organic matter or applied as mineralized phosphate fertilizers such as superphosphate and orthophosphoric acid. Graham and Timmer (1985) reported that no further P fertilizers may be necessary if rock phosphate and arbuscular mycorrhizal (AM) fungi inoculum are incorporated into media, whereas repeated application of soluble P would be required for slow-growing woody plants like citrus. Several studies reported that plant growth responses to mycorrhizal fungi were often due to significant increase in N and P uptake (Graham and Timmer, 1985; Rahman and Parsons, 1997 and Osundina, 1998). Habte and Aziz (1989) reported that the increased colonization of roots by AM fungi was accompanied by a significant increase in the concentrations of S, Cu, Zn while, K, Mn and Fe concentrations were consistently higher without statistical significance than the control. Johnson and Hummel (1985) reviewed large number of investigations indicating that infection of plant roots by arbuscular mycorrhizal (AM) fungi enhanced element uptake, water uptake and growth. Otherwise, AM fungi have come to be viewed not only as plant symbionts, but as symbionts of both plant and soil. Such a view does not replace the phytocentric evaluation of mycorrhizae with an agrocentric one, but considers

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AM fungi in a larger context: as a link between plant and soil in the plant-soil continuum (Bethlenfalvy *et. al.*, 1999).

The aim of this work is substituting a part of chemical nitrogenous and phosphoric fertilizers by a suitable microbial fertilizer for 'Anna' apple trees. The effect of these treatments on tree growth, yield and fruit quality were studied and suitable recommendation was given.

MATERIALS AND METHODS

This investigation has been carried out on seven years old "Anna" apple trees budded on MM.106 rootstock during three successive seasons of 2002, 2003 and 2004 (the recorded data were for 2003 & 2004 seasons only). Trees were grown at Elbostan district of Elbehira governorate where drip fertigation system was used.

Table (A):Some physical and chemical analytical properties of soil.

Sand %	Silt %	Clay %	Texture	O.M %	pH	EC ($ds.m^{-1}$)
93.44	6.56	-	Sandy	0.42	7.92	0.33

Cations (mg./L)			Anions (mg./L)				Macro-nutrient (ppm)		
Na^{+}	Ca^{+}	Mg^{++}	CO_3^{-}	HCO_3^{-}	Cl^{-}	SO_4^{-}	N	P	K
0.62	0.86	0.17	-	1.24	0.16	0.72	9	16	122

Single tree plot with 3 replicates for each treatment was arranged in random complete blocks design. Statistical analysis of data was computerized by "Irristat" package.

All trees were subjected to the common horticultural practices of the region, while treatments were applied as follows:

1. (C): Control trees received the recommended doses of chemical fertilizers (0.750 Kg ammonium sulphate + 1.0 Kg calcium super phosphate + 1.0 Kg potassium sulphate per tree / season) according to (Wassel *et. al.*, 1996) and Mansour,1998) without any microbial inoculation.
2. (F1): trees were microbial inoculated and received 100% of the recommended doses of chemical fertilizers.
3. (F2): trees were microbial inoculated and received 50% of the recommended doses of chemical fertilizers.
4. (F3): trees were microbial inoculated and received 25% of the recommended doses of chemical fertilizers.

Microbial inoculum was consisted of bacterial types of asymbiotic nitrogen fixers and phosphorus release bacteria (*Azotobacter* spp. and *Bacillus megatherium*) which found in three microbial biofertilizers: 'Phosphorein' 'Biogein' and 'Rhizobacterein' in addition to arbuscular mycorhizal (AM) fungi. *Azotobacter* spp. found in the microbial inoculum with density about 1 X 10^8 (CFU / g of inoculum); and *Bacillus megatherium* with cells density around 2 X 10^8 (CFU / g of inoculum). 'Biogein'; 'Rhizobacterein' and 'Phosphorein' are microbial biofertilizers produced and purchased from General Authority of Agricultural Equalization Fund, Giza, Egypt. The mycorhizae inoculum was prepared and provided from Soil Bacteriology Laboratory of Sakha Agricultural Research Station (A.R.C.). The AM inoculum, consisting of a mixture of soil; spores and root fragments, was produced in culture with onion.

Inoculation of trees was applied at the end of February (beginning of bud burst) added in six holes (5 cm wide and 3 cm in depth) of soil per tree, under drippers, when trees were irrigated. Each tree received 5 gm. Biogein + 5 gm. Rhizobacterein + 5 gm. Phosphorein + 5 gm. of arbuscular mycorrhizal fungi.

Three branches, five years old, in different directions on each tree were selected and labeled to estimate growth parameters. All current shoots that developed on these branches were measured to get shoot length (cm). Li-Core-3100 Areameter was used to measure detached leaves of nine shoots (three shoots per branch) to get leaf area (cm^2). Leaves were dried at 70°C and weighed to get dry weight (mg.) and then specific leaf weight (SLW) was calculated as ($mg.cm^{-2}$) according to Ferree and Forshey (1988).

Thirty fully expanded mature leaves per branch on each tree based on mid current year growth were sampled at mid August (Reisenauer, 1978) to determine leaf constituents, starch, total soluble sugars and total carbohydrate according to Somogy (1952) and Nelson (1974). Spectrophotometer was used to estimate chlorophyll a and chlorophyll b extracted from fresh leaves with DMF as described by Rami and Porath (1980). The concentration of chlorophyll a and chlorophyll b and its total value were calculated by Rami' s formulas as (µg / ml) (Rami 1982). The results were presented as ($mg.cm^{-2}$) according to Zayan *et. al.* (2002).

Nitrogen was estimated by micro-Kjeldahl gunning method (A.O.A.C.,1990). Phosphorus was determined with a colorimetric method as described by Foster and Cornelia (1967). A flame photometer model E.E.L. was used to estimate potassium as reported by (Jackson, 1967). Calcium, magnesium, iron, zinc and manganese were determined by Perking – Elmer atomic absorption spectrophotometer model 2380 AL, according to Jackson and Ulrich (1959) and Yoshida *et. al.* (1972).

Fruits were picked at maturity stage and yield records were taken as fruit number, fruit weight per tree and average fruit weight. (TSS) was determined by using Abbe refractometer and total acidity percentage was estimated according to (A.O.A.C.,1990).

RESULTS AND DISCUSSION

Data of Table (1) demonstrated that all studied parameters of vegetative growth were influenced by microbial biofertilizer treatments. All microbial biofertilization treatments increased significantly shoot length of inoculated trees when compared with control trees. Trees received microbial fertilization + 100% of recommended mineral fertilization doses (F1) had the longest shoot length followed by those that received microbial biofertilization + 50% of recommended mineral fertilizers (F2) and then trees that received microbial fertilization + 25% of recommended mineral fertilizers (F3). Statistical analysis showed that differences were significant among all treatments in both seasons of study. Similar trend was also observed with area per leaf and leaf dry weight. On the other hand, statistical analysis showed that specific leaf weight (SLW) was not influenced by any treatment.

The increase in those parameters could be explained by the fact that asymbiotic nitrogen fixers (ANF) have the capability to fix atmospheric nitrogen (Nijjar, 1990) which could be used in tree growth and development of its structure.

The present results coincide with the findings of Mahmoud and Mahmoud (1999) that microbial fertilization led to improvement in plant vigor of peach seedlings. Moreover, they added that adding nitrogen fixing bacterium led to great promotion in all plant characters under study. It was also reported that inoculation with arbuscular mycorrhizal (AM) fungi significantly increased the growth of 'Carrizo' citrange and sour orange rootstocks (Graham and Timmer, 1985); leaf area of Troyer citrange (Vinayak and Bagyaraj, 1990); plant biomass, plant height, plant girth, leaf number, leaf area of acid lime (Reddy *et. al.*, 1996), shoot growth of Troyer citrange and 'Black Olympia' grape (Yamashita *et. al.*, 1998). Also, El-Sayed, (2002) contended that microbial biofertilizers increased shoot length and leaf area of Flame seedless grapevines, growth characteristics and total leaf area / vine (Ahmed *et. al.*, 2003 and Abd El-Hady, 2003), leaf area of 'Ettmani' guava (El-Sharkawy and Mehaisen, 2005) and all measured vegetative parameters of 'Canino' apricot (Ibrahim *et. al.*, 2005).

Table (1): Effect of microbial biofertilization on vegetative growth of 'Anna' apple trees.

Treatments *	Shoot length (cm.)		Area per leaf ($cm.^2$)		Leaf dry wt. (gm.)		SLW ($mg.\ cm.^{-2}$)	
	2003	2004	2003	2004	2003	2004	2003	2004
C	37.33	40.00	23.93	23.28	0.224	0.211	9.4	9.0
F1	72.33	77.66	35.21	34.97	0.308	0.296	8.8	8.4
F2	57.33	63.33	30.64	31.58	0.279	0.294	9.1	9.3
F3	45.66	50.66	25.52	25.30	0.251	0.206	9.9	8.2
L.S.D	7.13	8.64	4.22	4.06	0.043	0.037	1.21	1.26

***:- C: Control - F1: microbial biofertilization + 100% of recommended mineral fertilization doses – F2: microbial biofertilization + 50% of recommended mineral fertilization doses – F3: microbial biofertilization + 25% of recommended mineral fertilization doses**

Data concerning 'Anna' apple leaf content of chlorophyll were arranged in Table (2). Data showed that mixed inoculum surpassed 'Anna' apple leaf content of all determined chlorophyll phases (chlorophyll a; chlorophyll b and total chlorophyll) in a significant manner than traditional control trees. Trees treated with microbial biofertilizer and received 100% of recommended mineral fertilization doses (F1) had the biggest leaf content ($mg.cm^{-2}$) of chlorophyll a followed with those received microbial biofertilization + 50% of recommended mineral fertilizers (F2) and then trees received microbial biofertilization + 25% of recommended mineral fertilizers (F3). Statistical analysis showed that differences were significant among treatments and between treatments and control in both seasons. The same trend of influence was observed with chlorophyll b and total chlorophyll, except chlorophyll b under F3 treatment did not reach the level of significance when compared with the control in the second season.

These results are in harmony with report of Ezz and Nawar (1994) which concluded that inoculation of sour orange seedlings with mycorrhizal fungi increased total chlorophyll and chlorophyll b concentrations in the first season of the trial, and chlorophyll a concentrations in the second. Also, Ibrahim *et. al.* (2005) contended that biofertilization increased leaf chlorophyll content of 'Canino' apricot.

So, we can attribute the superiority of microbial biofertilization treatments in increasing 'Anna' chlorophyll leaf content to the influence of nitrogen fixers bacteria. These strains of rhizobacteria have capability to fix atmospheric nitrogen in a continuous release manner. This nitrogen was utilized by the tree to synthesize chlorophyll molecules of leaves. Also, phosphate dissolving bacteria combined with arbuscular mycorrhizal (AM) fungi may have released phosphorus ions that effectively acquired and taken up by the tree.

Table (2): Effect of microbial biofertilization on chlorophyll content of 'Anna' apple leaves.

Treatments *	Chlorophyll a ($mg.cm^{-2}$)		Chlorophyll b ($mg.cm^{-2}$)		Total Chlorophyll ($mg.cm^{-2}$)	
	2003	2004	2003	2004	2003	2004
C	4.09	4.33	2.31	2.21	6.41	6.52
F1	4.95	4.81	3.02	2.98	7.96	7.79
F2	4.51	4.64	2.81	2.59	7.33	7.24
F3	4.12	4.38	2.84	2.52	6.96	6.90
L.S.D	0.33	0.24	0.41	0.29	0.43	0.38

***:- C: Control - F1: microbial biofertilization + 100% of recommended mineral fertilization doses – F2: microbial biofertilization + 50% of recommended mineral fertilization doses – F3: microbial biofertilization + 25% of recommended mineral fertilization doses**

Data recorded in Table (3) exhibited influences of microorganisms addition on carbohydrates content of 'Anna' apple leaves. Microbial inoculum significantly increased 'Anna' apple leaf content of all studied carbohydrate determinations than non-inoculated control ones. The highest leaf content of total sugars was attained from trees treated with microbial biofertilizer and received 100% of recommended mineral fertilization doses (F1) followed by (F2), then (F3) and later the control ones. The same trend of influence was observed with starch and total carbohydrates with one exception, whereas F2 (microbial biofertilization and 50% of recommended mineral fertilizers doses) was the superior treatment followed by (F1) and (F2), respectively. Differences among treatments and between treatments and control were significant in both seasons.

The obtained herein results are in agreement with those concluded by Ezz and Nawar (1994) that mycorrhizal inoculation of sour orange seedlings increased leaf and root sugars and carbohydrates concentrations in comparison with non infected control.

Emphasis, findings of Eissenstat *et. al.* (1993) could illustrate the influences of biofertilizers on carbohydrates content of 'Anna' apple leaves where they reported that mycorrhizal colonization increased rate of photosynthesis of sour orange. The present explanation is strengthened by data of increased leaf chlorophyll phases determinations of Table (2) and findings of Shrestha *et. al.* (1995) which contended that photosynthesis and transpiration rate of AM-inoculated trees were greater than those of non-AM ones. The leaves of AM and non-AM trees assimilated $^{13}CO_2$ equally fast per unit leaf area and the distribution of ^{13}C into various parts of the bearing trees did not differ. AM trees, however, had 3 times more photosynthates per tree than uninfected ones because the former had a leaf area 3 times larger than the latter and grew more vigorously.

Data of microbial biofertilization influences on macro elements content of 'Anna' apple leaves were arranged in Table (4). Data of Table (4) exhibited that microbial inoculum significantly increased N and P leaf content than uninoculated control ones. The highest N and P concentrations were recorded from trees treated with microbial biofertilizer and received 50% of recommended mineral fertilizers doses (F2) followed by F1, F3 and C, respectively. Statistical analysis illustrated that differences among treatments and between treatments and control were significant in both seasons of the study.

Table (3): Effect of microbial biofertilization on carbohydrates content of 'Anna' apple leaves.

Treatments *	Total Sugars %		Starch %		Total Carbohydrates %	
	2003	2004	2003	2004	2003	2004
C	5.96	6.03	5.53	5.63	11.22	11.65
F1	6.71	6.86	6.06	6.14	12.77	13.01
F2	6.43	6.57	6.23	6.19	12.67	12.76
F3	6.29	6.33	5.83	5.96	12.11	12.12
L.S.D	0.28	0.26	0.24	0.23	0.36	0.39

***:- C: Control - F1: microbial biofertilization + 100% of recommended mineral fertilization doses – F2: microbial biofertilization + 50% of recommended mineral fertilization doses – F3: microbial biofertilization + 25% of recommended mineral fertilization doses**

The present results are in harmony with El-Sawy *et. al.* (1998) and Badr-Eldin *et. al.* (2001) who reported that nitrogen fixers rhizobacteria and arbuscular mycorrhizal (AM) fungi had a vital role in improving N_2 fixation processes. Also, Ahmed *et. al.* (2003); El-Sayed (2002) and Abd El-Hady (2003) concluded that microbial biofertilizers increased N content of Flame seedless grapevines, 'Ettmani' guava (El-Sharkawy and Mehaisen, 2005) and 'Canino' apricot (Ibrahim *et. al.*, 2005).

Vinayak and Bagyaraj (1990) demonstrated that mycorrhizae inoculum gave the best improvement in growth and nutrition, resulting in higher shoot P contents of Troyer citrange as well as stimulated the root of trees to release acid phosphatase into the soil which led to increased P soil content (Tang and He, 1991), and leaf P concentration of acid lime (Reddy *et. al.*, 1996), trifoliate orange and 'Black Olympia' grape seedlings (Yamashita *et. al.*, 1998), flame seedless grapevines (El-Sayed, 2002 and Abd El-Hady, 2003), 'Ettmani' guava (El-Sharkawy and Mehaisen, 2005) and 'Canino' apricot (Ibrahim *et. al.*, 2005).

The present explanation is strengthened by findings on rhizobacterial influences of Toro *et. al.* (1997) which concluded that rhizobacteria works as 'mycorrhizae helper bacteria' promoting establishment of both the indigenous and introduced arbuscular mycorrhizae endophytes. They stated that used rhizobacteria may have released phosphate ions which may be effectively taken up through the external arbuscular mycorrhizae mycelium.

Concerning data of 'Anna' apple leaf content of K, Ca and Mg elements as affected by biomicrobial fertilizer, it is obvious that K and Ca concentrations of inoculated trees were significantly increased in comparison with non-inoculated control, although differences among treatments were not significant. At the same time, Mg determination did not record any significant difference among treatments or between treatments and the control.

The present data support findings of Aziz and Habte (1989) which concluded that the increased colonization of roots by AM fungi was accompanied by increment in the concentration of K than the control. Also, El-Sayed (2002) and Abd El-Hady (2003) concluded that microbial biofertilizers increased K content of Flame seedless grapevines, 'Ettmani' guava (El-Sharkawy and Mehaisen, 2005) and 'Canino' apricot (Ibrahim *et. al.*, 2005).. While, Tang *et. al.* (1989) reported that autoradiograms indicated that mycorrhizal infection of trifoliate orange and 'Goutou' sour orange enhanced ^{45}Ca absorption and transport to the upper part of the plant as a result, total calcium content was significantly higher in mycorrhizal infected seedlings.

How any, inoculation with microbial biofertilizer in sandy soil of the present study increased soil microbial diversity, density, and activity resulted in more suitable condition for nutrients release in the rhizosphere and nutrients uptake by tree roots. This is the direct reason of increasing leaf content of nutrient elements in the present study as a result of microorganisms addition.

Table (4): Effect of microbial biofertilization on macro-elements content of 'Anna' apple leaves.

Treatments *	Macro-elements % on dry weight									
	N		P		K		Ca		Mg	
	2003	2004	2003	2004	2003	2004	2003	2004	2003	2004
C	1.97	1.85	0.19	0.21	1.23	1.28	1.14	1.14	0.34	0.34
F1	2.36	2.39	0.26	0.27	1.58	1.61	1.38	1.35	0.35	0.36
F2	2.57	2.65	0.28	0.29	1.58	1.63	1.39	1.35	0.36	0.38
F3	2.25	2.29	0.25	0.29	1.57	1.57	1.39	1.36	0.35	0.38
L.S.D	0.20	0.18	0.05	0.04	0.32	0.29	0.22	0.20	0.14	0.16

***:- C: Control - F1: microbial biofertilization + 100% of recommended mineral fertilization doses – F2: microbial biofertilization + 50% of recommended mineral fertilization doses – F3: microbial biofertilization + 25% of recommended mineral fertilization doses**

Data recorded in Table (5) illustrating influences of microbial biofertilizer addition on micro nutrients content of 'Anna' apple leaves showed that Fe and Zn concentrations of inoculated trees were significantly increased in comparison with non-inoculated controls. Statistical analysis exhibited that differences among treatments were not significant, wherever between treatments and control turned to a significance manner. At the same time, Mn determinations did not record any significant difference among treatments or between treatments and control.

The present results coincide with the findings of Aziz and Habte (1989) who reported that the increased colonization of roots by AM fungi was accompanied by a significant increase in the concentrations of S, Cu and Zn. Although, Mn and Fe concentrations were consistently higher without statistical significance than the control. Vinayak and Bagyaraj (1990) contended that mycorrhizae inoculum gave the best improvement in growth and nutrition, resulting in higher shoot Zn and Cu contents of Troyer citrange; and leaf Zn concentrations of acid lime (Reddy *et. al.*, 1996). Also, Ahmed *et. al.* (2003) reported that microbial biofertilizers increased Fe and Zn leaf content of Flame seedless grapevines.

Suggesting a role for mycorrhiza in host nutrient allocation, the influences of biomicrobial fertilizers in increasing leaf content of nutrient elements could be discussed in light of findings of Singh and Kapoor (1999) who concluded that release of organic and inorganic acids and increasing O_2 evolution due to phosphate dissolving microorganisms and other microbial types reduce soil pH, leading to change of nutrients to available forms ready for uptake by plants. Moreover, Rodelas *et. al.* (1999) and Singh and Kapoor (1999) suggested that plant hormones release by microorganisms increasing plant root growth causing in turn increasing plant root surface which improves nutrient absorption. While, Bhardwi, *et. al.* (2000) contended that production of antimicrobial substances for reducing plant root infection with pathogens which make the plants more healthy and consequently increase their nutrient uptake.

Table (5): Effect of microbial biofertilization on micro-elements content of 'Anna' apple leaves.

Treatments *	Micro-elements (ppm on dry weight)					
	Fe		Zn		Mn	
	2003	2004	2003	2004	2003	2004
C	101.33	100.17	49.00	45.67	25.45	25.91
F1	112.00	108.66	68.25	66.69	26.02	26.76
F2	110.66	111.00	69.93	63.46	27.76	25.18
F3	114.66	110.66	69.51	64.22	26.48	26.08
L.S.D	9.13	9.11	7.56	8.19	3.11	2.89

***:- C: Control - F1: microbial biofertilization + 100% of recommended mineral fertilization doses – F2: microbial biofertilization + 50% of recommended mineral fertilization doses – F3: microbial biofertilization + 25% of recommended mineral fertilization doses**

Response of 'Anna' apples yield to microorganisms biofertilizer is shown in Table (6). It is clear that all studied parameters of yield were significantly increased as biofertilizer was applied to trees when compared with uninoculated controls. Data also showed that F1, F2 and F3 treatments had similar effect on fruit weight per tree and fruit number per tree in both seasons.

Concerning fruit quality characteristics it was clear that F3 treatment recorded the highest average fruit weight followed by F2, then F1, in the first season. While, in the second one, treatments were ordered: F1, F2 then F3. At the same time, chemical fruit quality determinations of 'Anna' apple fruits were improved by microbial inoculation. Treatment of F1 increased significantly TSS % of fruit juice at first season and F3 showed similar trend at second one. While, acidity % of juice gave inconstant trend in first season and decreased rapidly by microbial biofertilization treatments in second one.

The obtained data are confirmed with report of Shrestha *et. al.* (1996). They found that inoculated Satsuma mandarin trees with AM fungi had better fruit quality than non inoculated control trees. The fruits of inoculated trees were larger, had higher juice sugar contents and better peel color than the controls. Also, El-Sayed (2002); Ahmed *et. al.* (2003) and Abd El-Hady (2003) concluded that microbial biofertilizers increased yield and improved physical and chemical quality characteristics of Flame seedless grape, 'Ettmani' guava (El-Sharkawy and Mehaisen, 2005) and 'Canino' apricot (Ibrahim *et. al.*, 2005).

Table (6): Effect of microbial biofertilization on yield and some fruit characteristics of 'Anna' apple trees.

Treatments *	Fruit No. / tree		Fruit weight / tree (Kg)		Ave. fruit weight (gm)		TSS %		Acidity %	
	2003	2004	2003	2004	2003	2004	2003	2004	2003	2004
C	207.3	224.0	25.3	28.6	122.3	127.3	11.9	13.1	0.75	0.96
F1	264.3	276.3	34.5	39.3	130.4	142.3	12.4	12.8	0.76	0.69
F2	271.9	257.3	36.3	35.5	133.0	138.0	11.9	12.6	0.73	0.70
F3	251.1	263.0	33.8	36.1	134.9	137.7	12.0	13.3	0.75	0.74
L.S.D	37.1	31.2	4.8	4.6	10.0	11.2	0.17	0.09	0.02	0.02

***:- C: Control - F1: microbial biofertilization + 100% of recommended mineral fertilization doses – F2: microbial biofertilization + 50% of recommended mineral fertilization doses – F3: microbial biofertilization + 25% of recommended mineral fertilization doses**

So, we can attribute the superiority of microbial fertilization treatments of enhancing fruit yield and quality of 'Anna' apple trees to improved and activated physiological status of whole tree by microorganisms. Related obtained data were found in Table (1) exhibited influences on vegetative growth especially leaf area, chlorophyll concentrations (Table 2), which in turn increased carbohydrates and such like photosynthates (Table 3), leaf nutrient elements concentrations (Tables 4 & 5). This explanation found support through findings of Dixon (1990) which disclosed that AM colonization increased cytokinin activity of citrus roots and leaves, photosynthesis and transpiration rate (Shrestha *et. al.*, 1995), increased enzymes (polyphenol oxidase) activity (Ezz and Nawar 1994), which in turn positively affected fruit yield and quality.

Finally, it could be concluded that , the most effective treatment is F2 which contain microbial biofertilization + 50% of recommended mineral fertilizers.

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الملخص العربي

تأثير بعض المخصبات الميكروبية علي خفض استخدام الأسمدة المعدنية و النمو الخضري و الحالة الغذائية والمحصول وجودة ثمار التفاح

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تم استخدام لقاح مكون من ثلاثة مخصبات حيوية (بيوجين – ريزوباكتيرين – فوسفورين) و التي تحتوي على ميكروبات أزوتوباكتر و الباسيلس ميجاثريوم ، مع إضافة الميكورهيزا الداخلية بغرض تخفيض الجرعة الموصى بها من الأسمدة المعدنية الأزوتية و الفوسفورية و المستخدمة في تسميد أشجار التفاح "آنا" عمر ٧ سنوات و المطعومة على أصل م. م ١٠٦ و المزروعة في التربة الرملية و التي تروى بالتنقيط.

أدى استخدام المخصبات الحيوية إلى زيادة معنوية في طول الفرع و مساحة الورقة و الوزن الجاف للورقة في حين لم يتأثر الوزن النوعي للورقة بأي من المعاملات ، كذلك حدثت زيادة معنوية في محتوى الورقة من الكلوروفيل أ و الكلوروفيل ب و المحتوى الكلي من الكلوروفيل عند المقارنة مع الأشجار التي لم يتم تخصيبها حيويا (الكونترول).

كما أدى التخصيب الحيوي للأشجار إلى تحسين محتوى الأوراق من السكريات الذائبة و النشا و الكربوهيدرات الكلية ، في حين ازداد تركيز عناصر النيتروجين و الفوسفور و الكالسيوم و الحديد والزنك في الورقة بصورة معنوية ، و كانت الزيادة غير معنوية لعنصري الماغنسيوم و المنجنيز.

كما أوضحت النتائج زيادة معنوية في محصول الأشجار (وزن و عدد) التي تم تخصيبها حيويا حيث سجلت المعاملتين (تسميد حيوي + ١٠٠% من الجرعة الموصى بها من الأسمدة المعدنية) و (تسميد حيوي + ٥٠% من الجرعة الموصى بها من الأسمدة المعدنية) أعلى وزن وعدد ثمار ، و في نفس الوقت سجلت المعاملتين (تسميد حيوي + ١٠٠% من الجرعة الموصى بها من الأسمدة المعدنية) و (تسميد حيوي + ٢٥% من الجرعة الموصى بها من الأسمدة المعدنية) أعلي متوسط وزن للثمرة و حسنت معنويا من القياسات الكيماوية لجودة الثمار (انخفض محتوي العصير من الحموضة و ازدادت النسبة المئوية للمواد الصلبة الذائبة).

بوجه عام يتضح أن المعاملة بالتسميد الحيوي + ٥٠% من الجرعة الموصى بها من الأسمدة المعدنية أعطت أفضل النتائج من ناحية الحصول على نمو خضري جيد و صحي وازدادت الكفاءة التمثيلية للورقة بزيادة محتواها من الكلوروفيل وتحسنت الحالة الغذائية للأشجار، كما أدت إلى زيادة المحصول مع تحسن جودة الثمار، بالإضافة إلى توفير تكلفة استخدام ٥٠% من الأسمدة المعدنية.