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Effect Of Some Biostimulant On Growth And fruiting Of Anna Apple Trees In Newly Reclaimed Areas

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Abstract: This study was conducted during 2005 and 2006 seasons using EM (a commercial Biostimulant) containing more Than 60 selected strains of effective microorganisms, applied in two forms, The first is a solid form, called the " Bokashi " and the second is a solution form, called the " Bokashi " and the second is a solution from the two forms were applied to soil from mature Anna apple tress, which are 15 years old, budded on MM 106 rootstock grown a calcareous Egyptian soil orchard at El–Nubaria region. The aim of this research was to investigate the effects of these two forms on the vegetative growth, leaf mineral content, yield and fruit quality at both harvest and the end of marketing period. The results indicated, in both seasons, That generally most the EM biostimulant treatments, has significantly increased the vegetative growth; the number curent shoots/main branch,shoot lenght,diameter the number and leaf area, as well as, leaf chlorophyll reading and leaf mineral values, N, P, K, Fe, Zn and Mn as compared with the untreated trees. The yield and fruit quality i.e.fruit weight, their dimensions, total sugars % and TSS % at harvest were improved as compared to the control. In addition, the fruit quality at the end of marketing period showed an increase in TSS % and total sugars % and a decreased acidity % while fruit firmness as slightly reduced as a result of using EM treatments. The results has also indicated that all the EM applications increased the number of the soil microflora, i.e total fungi (TF), total bacteria (TB) and total actinomycetes (TA) and some macro and micro elements (i.e. N, P, K, Fe, Zn, and Mn) in soil as compared to the control. The Bokashi form was more effective than the solution EM form and it is proved that using EM in Bokashi form at T3 (8 kg / tree / year) was superior to the most results gained compared to the other treatments in both seasons.

Key words: Biostimulant, EM, Anna, Apple, Growth, Fruiting,Calcareous Soil, Microorganisms.

INTRODUCTION

Anna apple is one of the most important deciduous fruits that shows great success and is widespread in the newly reclaimed areas in Egypt. Most of this soil is considered calcareous. This type of soil has its own problems as an excess of $CaCo_3$ and high ph value that cause a precipitation of mineral contents in an unavailable from for plants. Among of methods followed for improving the quantity and quality of Anna apple trees is the application of major fertilizers to satisfy the needs of plant from such elements since good growth is mostly associated with good yield.

Biofertilizers are the microbial inoculants having specific strains of bacteria and fungi, alone or in combination which is applied to soil for increasing crops productivity through their metabolic activities independently or in association with the plant root system[1]. In general, biofertilizers are environment friendly, decreased agricultural costs with maximum out put. These biofertilizers play an important role in enhancing crop productivity through nitrogen fixation, phosphate solubilization, plant hormone production ammonia excretion and controlling various plant diseases[2,3]. In addition,[4] mentioned that biofertilizers are carrier based on preparations containing beneficial microorganisms in a viable state intended for soil application and designed to improve soil fertility and help plant growth by increasing the number and biological activity of desired microorganisms in root environment.

Applying organic manures in calcareous soils are very important methods for providing the plants with their nutrition requirements without having an undesirable impact on the environment. The value of

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organic fertilizers as a source of humus, macro and micro nutrients, as well as, increase the activity of the useful microorganisms has been revealed by[5] for many years, soil microbiologists and microbial ecologists have tended to differentiate soil microorganisms as beneficial or harmful according to their functions and how they affect soil quality, plant growth and yield, and plant health, a more specific classification of beneficial microorganisms has been suggested by[6] which he refers to as effective microorganisms or EM. If used properly, EM can significantly enhance the growth, yield and quality of crops[7].

Many authors documented favorable effects of using EM biostimulant on growth yield of several corps.

In this respect[8] stated that EM, a commercial Japanese product, to mung bean improved nutrient uptake efficiency, enhanced root growth and brunching and increased yield, In another trial, EM + molasses increased onion yield by 29%,The proportion of the highest grade onions by 76% pea yields by 31% and sweet corn cob weights by 23%[9]. [10] Stated, also, that EM was able to improve the Kelsey plum yield. Thus, the present investigation was imposed to evaluate the effects of EM. commercial biostimulant which consists of several effective microorganisms with different forms on vegetative growth, yield and fruit quality at harvest, also at the end of marketing period of Anna apple trees grown under calcareous soil condition.

MATERIALS AND METHODS

The current study was imposed in the newly reclaimed calcareous soil a private Farm, El – Nubaria region, El-Behiera governorate, during 2005 and 2006 seasons under a flood irrigation system, using fifty six trees, 15 years old the Anna apple trees budded on mm 106 rootstock and planted at 3.5 × 3.5 m apart. These trees were similar in their vigor, as possible which were treated with common agricultural practices in both seasons.Leaf analysis for the mineral contents before the experiment is shown in table (1)

Prior to executing the experiment, the soil's physical and chemical properties of the experimental site which were determined according to the methods described by Black[11], and the results of this analysis are presented in table(2).

EM is a commercial biostimulant produced by EMRO corporation, Okinawa, Japan, marketed locally by the ministry of Agriculture and land reclamation, Egypt, and contains more than 60 selected strains of " effective microorganisms ", (photosynthetic bacteria, lactic acid bacteria, yeast, actinomycetes, and various fungi).

Table 1: leaf mineral content before starting the experiment

N %	P %	K %	Fe %	Zn %	Mn %
1.55	0.15	1.08	97	26	46

EM biostimulant was applied to the soil in two forms, the first one was a solid form called Bokashi which consisted of (EM + molasses + well water) adding (rice husk + pressed olive cake (olive kossab) + cattle organic manure). The second was a solution from which contain (EM + molasses + well water). These two forms were fermented one week before its usage. The chemical analysis of the several organic manure which was used in Bokashi form is shown in Table (3). The selected trees were subjected to EM biostimulant treatments during the two experiment seasons as follows:

- T1: 2 kg / tree / year of Bokashi.
- T2: 4 kg / tree / year of Bokashi.
- T3: 8 kg / tree / year of Bokashi.
- T4: 3 L / tree / year of EM solution.
- T5: 6 L / tree / year of EM solution.
- T6: 12 L / tree / year of EM solution.
- T7: control (untreated Trees).

These quantities were added at ten equal does and the treatments were started at the beginning of February at three weeks intervals till end of September, during both seasons. All EM treatments were applied in circle around the tree trunk beneath the canopy (50 cm away from the tree trunk) and were incorporated into the top 20 cm layer of soil. The experimental treatments were arranged in a randomized complete blocks design and the each replicate. The following parameters were replicated 4 times with a two tress for each replicate.

The following parameters were determined in the two successive seasons:

1-vegetative Growth Measurements: Number of current shoot / main branch, their lengths and diameters and leaf area were determined four main branches as similar as possible were chosen at the four cardinal points of each treated tree, tagged and the average of the current shoot per selected branch was counted, their lengths and diameters were measured with (cm) on mid October, in both seasons. Leaf area was determined using the formula.

Table 2: physical and chemical analysis of the experimental soil.

Soil depth	Texture	Ph	EC (ds/m)	Total $CaCO_3$	O.M (%)	Total N%	Soluble cation mg/l			Soluble anion (mg/l)		
							Ca^{++}	Mg^{+}	K^{+}	HCO_3^{-}	Cl^{-}	So_4^{-}
0-30	Sandy loam	8.2	1.13	33.14	0.35	0.012	7.4	5.4	2.2	5.7	4.0	5.9
30-60	Sandy loam	8.15	1.92	32.10	0.12	0.015	6.9	5.6	1.9	7.4	4.1	5.7

- La = 0.56 (0.79 × w^2) + 20.01, where LA = Leaf
- Area (cm), w = the maximum leaf width (cm), according to Ahmad[12].

2-Chemical Components of the Leaves: Leaf chlorophyll reading was recorded using MINOLTA CHLOROPHYLL METER SPAD–502 at the field in mid July. The average of ten readings was taken on the middle of leaves form all over the tree circumference. Leaf mineral contents were determined in mid July of both seasons. Samples of 30 leaves / tree were taken at random from previously tagged shoots of each tree. Leaf samples were washed with tap water, rinsed twice in distilled water, oven dried at 70 C^0 to a constant weight and then ground. The ground samples were digested with sulphoric acid and hydrogen peroxide according to Evenhuis[13]. N and p calorimetrically determined according to Evenhuis[14] and Murphy[15] respectively K was determined against a standard by flame photometer according to[16], Fe, Zn and Mn were measured by Atomic absorption spectrophotometer The concentration of N, P, K, were expressed as (%) while the Fe,Zn, Mn were expressed as ppm, on dry weight basis.

3-Yield and Fruit Quality: Fruit were collected at maturity stage (mid July). From each tree of various replicates and yield was recorded as weight in kg.

Fruit quality described as physical and chemical Properties as follows:

Physical Properties: Fruit weight (g), fruit firmness was estimated by Magness [17] pressure tester which has a standard 5/16 of inch plunger and recorded as 1b / inch2 and F dimensions (cm).

Table 3: chemical analysis of the organic manure, cattle manure, pressed olive cake and rice husk which constitute the Bokashi

Organic manure source	N%	P%	K%	O.M%
Cattle manure	1.6	1.10	0.50	15
Pressed olive cake	0.6	0.30	0.20	30
Rice husk	0.5	0.10	0.40	35

Chemical Properties: Tss% was determined by hand refractometer, acidity % was determined (as malic acid) by titration with 0.1 normal sodium hydroxide with phenol phethalin as an indicator, according to A.O.A.C [18], and total sugars% content were determined according to Malik [19].

4-Shelf Life: The fruits were kept under the ambient atmospheric conditions to mimic the conditions of marking through the local markets at the retail level. As for the post harvest procedure, the fruits were sorted out, to exclude the unmarketable ones. Then, washed and left to dry up before being packed in a standard carton box. A sample of 10 fruits from each replicate was taken at harvest time and left at room temperature (25 – 28 C^0 and 85% RH). When 50% of the fruits were unmarketable, the experiment was terminated and the number of days were calculated and considered as the marketing period.

5-Soil Micro Flora, Macro and Micro Soil Elements Content: Soil microbial courts were made on samples collected on September from three sites around each tree. After composting soil samples, small portions were used for density estimation of colony forming units (CFU) of total bacteria (TB) using the soil extract agar medium according to Allen[20], Total fungi (TF) using the rose-bengal streptomycin agar medium according to Martin[21] and Total actinomycetes (TA) using Jensen's medium to Allen[20] this part of study was conducted at the agricultural microbiology department, soil, water and environment, institute, The same composted soil samples were used for determined N, P, K Fe, Zn and Mn according to the methods of Chapman[16]. All obtained data were statistically analyzed according to Snedecor[22] and LSD test at 0.05 levels was used for comparison between treatments

RESULTS AND DISCUSSIONS

1-Vegetative growth Characters: The date representing the effect of different EM biostimulent

treatments on vegetative growth parameters were listed in table (4). In general, the results indicated that, the two forms of EM biostimulant were predominant to the control, of all the vegetative parameters i.e., number of new shoots/ main branch. New shoot length, new shoot diameter and leaf area as well as, the chlorophyll reading in both seasons. It was obvious that T3 (8 kg of Bokashi/tree) was superior and associated with the highest values of all previously mentioned characters. The enhancement of plant growth by the EM biostimulant application may be attributed to the profound effect of plant growth regulation substances produced by the effective microorganisms (bacteria, yeast and fungi) or In improving the availability and acquisition of nutrients from the soil which promoted the vegetative growth, Martin[22] and Jagnow[22] indicated that the bacteria produced adequate amount of IAA and cytokines, which increase the surface area per unit root length and cytokines, which increase the surface area per unit root length and hence enhanced the root hair branching with an eventual increase in acquisition of nutrients from the soil. The present results are in harmony with those reported by El Gama[25]l, Barakat[26], Ghoneim[27], Eissa[28] and El-Araby[29].

Table 4: Effect of EM biostmulant treatments on some vegetative growth parameters and leaf chlorophyll reading of Anna apple trees during 2005 and 2006 seasons.

Em Treatments	No. of new shoots /main branch		Shoot length (cm)		Shoot diameter (cm)		Leaf area $(cm)^2$		Leaf chlorophyll reading (SPAD)	
Season	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006
T1	10.03	10.50	51.10	51.30	1.10	1.11	25.11	26.13	40.09	40.11
T2	10.0	11.10	51.10	51.10	1.12	1.12	25.12	26.14	40.12	40.14
T3	11.20	11.40	55.40	55.70	1.21	1.22	26.59	27.67	41.11	41.16
T4	9.78	9.85	47.16	47.30	0.95	0.94	24.16	24.17	39.0	39.12
T5	9.96	10.30	50.90	51.00	0.99	0.99	24.18	24.18	39.30	39.15
T6	10.75	10.60	52.20	52.50	1.16	1.16	25.12	25.14	40.36	40.38
T7 (control)	9.20	9.50	46.00	46.11	0.81	0.82	22.0	22.16	36.65	36.67
L.S.D at 0.05	0.407	0.240	0.865	1.048	0.046	0.038	0.013	1.047	1.152	1.081

Table 5: effect of biostimulant on leaf macro and micro elements contents (% and ppm dry weight respectively) of Anna apple trees during 2005 and 2006 seasons.

EM Treatments	N (%)		P (%)		K (%)		Fe (ppm)		Mn (ppm)		Zn (ppm)	
	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006
T1	1.91	2.00	0.18	0.20	1.17	1.18	118	120	57.0	59.0	40.0	40.0
T2	1.91	2.00	0.19	0.21	1.17	1.19	120	122	57.0	59.0	41.0	40.0
T3	1.92	2.01	0.19	0.21	1.19	1.21	120	122	59.0	59.0	44.0	45.0
T4	1.85	1.86	0.17	0.18	1.13	1.16	113	113	56.0	55.0	36.0	36.0
T5	1.86	1.88	0.17	0.18	1.14	1.16	114	114	56.0	55.0	38.0	39.0
T6	1.87	1.92	0.17	0.18	1.18	1.16	114	114	57.0	56.0	38.0	42.0
T7 (control)	1.65	1.72	0.15	0.15	1.10	1.12	108	108	52.6	54.05	33.0	33.0
L.S.D at 0.05	0.022	0.017	0.018	0.017	0.018	0.025	3.98	3.52	3.01	2.02	2.722	2.685

Leaf Mineral Content: The results presented in Table (5), clearly, indicated that all application of EM (commercial biostimulant) significantly increased all studied leaf mineral content, ic) N,P,K,Fe,Mn and Zn over those of the untreated trees in both season in general, it was noticed that all Bodashi treatments were more pronounced in enhancing n, p and Zn in both seasons. Meanwhile K, Fe and Mn were increased in the season only as compared to Solution EM treatments.

Table 6: Effect of EM biostimulation treatments on yield and fruit quality of Anna apple tree during 2005 and 2006 seasons.

EM treatments	Average fruit Yield /tree (kg)		average fruit weight (g)		Fruit length (cm)		Fruit diameter (cm)		Fruit firmness (lb/inch)^2		T.S.S (%)		Acidity (%)		Total sugars (%)	
Season	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006
T1	45.21	45.32	158.21	167.11	5.61	5.90	5.10	5.27	11.99	11.97	14.36	15.10	0.36	0.37	10.20	10.45
T2	45.32	45.75	158.22	168.12	5.61	5.88	5.10	5.27	11.99	11.98	15.08	15.10	0.36	0.35	10.52	10.58
T3	46.28	46.65	160.18	169.21	5.65	5.95	5.12	5.33	11.98	11.97	15.11	15.12	0.36	0.34	10.55	10.65
T4	37.64	38.75	150.22	151.18	5.54	5.65	4.99	5.06	12.01	11.99	14.75	14.76	0.40	0.40	10.28	10.31
T5	38.26	39.22	152.14	152.15	5.55	5.55	5.01	5.02	11.99	11.98	14.77	14.78	0.40	0.40	10.31	10.33
T6	39.21	40.22	152.16	154.55	5.55	5.61	5.05	5.12	12.00	11.99	14.85	14.88	0.39	0.40	10.31	10.43
T7	33.25	34.62	138.63	147.17	5.40	5.36	4.88	4.89	12.00	12.00	13.06	13.08	0.41	0.40	10.01	10.02
L.S.D at 0.05	1.105	1.403	2.276	2.386	0.062	0.078	0.032	0.068	0.030	0.031	0.279	0.040	0.20	0.016	0.159	0.058

Table 7: Effect of EM biostimulont treatments on some chemical fruit quality of Anna apple trees at the end of marketing period during 2005 and 2006 seasous.

EM treatments	Fruit ferments		T.S.S %		Acidity %		Total sugar%	
Season	2005	2006	2005	2006	2005	2006	2005	2006
T1	10.44	11.83	14.95	14.90	0.33	0.33	10.50	10.54
T2	11.03	11.11	15.18	15.10	0.34	0.35	10.55	10.62
T3	10.70	10.97	15.0	15.22	0.34	0.32	10.85	10.82
T4	10.94	11.11	14.80	14.82	0.37	0.38	10.32	10.33
T5	11.31	10.94	14.85	14.80	0.38	0.35	10.52	10.35
T6	11.50	11.44	15.0	15.20	0.37	0.35	10.52	10.55
T7 (control)	10.55	10.89	13.50	13.80	0.40	0.40	10.10	10.11
L.S.D at 0.05	0.029	0.030	0.280	0.041	0.020	0.018	0.160	0.060

The above mentioned results revealed that the Bokashi treatments recorded high values because they contain the microorganisms and mixtre of several organic ie. Cattle manure rice husk and olive kossab. Which improved soil structure, aeration, retention of moisture, and consider a good source of essential nutrients and microelements these positive responses were acknowledged by numerous investigators such as El-Torky[30], using rise organic manures. In addition, the promoting effects of biofertilizers on the nutritional statues of the leaves could be related to the role of the effective microorganisms in improving the availability of nutrients and to the modifications of root growth, morphology and / or physiology through hormonal exudates of biofertilizers bacteria resulting in more efficient absorption of available nutrients the present result were, generally, in line with Jagnow[24]. Similar findings were recorded by El Gamal[25], Eissa[28] and Mohmoud[31].

3-Yields and Fruits Quality: The comparison among the EM biostimulant treatments for the yield and fruit quality of Anna apple trees are shown in Table (6). All the studied yield parameters were found to be significantly affected by two forms of EM treatments compared to the untreated trees, in both seasons. The highest values of yield were obtained due to the application of EM biostimulant as Bokashi treatments compared to solution EM in both seasons. In addition, the all applications of EM biostmulant generally, improved fruit quality (i.e. fruit weight, fruit dimensions, Tss % and Total sugars %) however firmness was not affected compared to the controlin both seasons.

These results reflect similar trends to those of plant growth and mineral content leaves as previously mentioned. Therefore, increasing Anna apple yield might be attributed to the increments on the amounts of metabolites synthesized by the plant which, in turn, accelerate plant growth and resulted in improving total yield. These results can be explained as the EM biostmulant contains more than 60 selected strains of microorganisms as bacteria, yeast, actinomycetes and various and various fungi. The high contents of minerals and vitamins as well as the cytokines contents in yeast might play a role in orientation and translocation of metabolites from leaves into the productive organs as recorded by Attala[32]. Also, similar results were recorded by Daly[32], Eissa[32], Sorial[32] and Dawa[32].

Table 8: effect of EM biostimulant treatments on some macro and micro elements contents in soil of Anna apple trees during 2005 and 2006 seasons.

EM treatments	N (mg /100g soil)		P (mg /100g soil)		K(mg /100g soil)		Fe (ppm)		Mn (ppm)		Zn (ppm)	
Season	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006
T1	147	151	0.49	0.52	19.5	19.6	11.6	11.80	6.91	6.9	0.81	0.98
T2	152	156	0.51	0.59	20.02	21.3	13.2	13.8	8.1	8.0	0.88	1.15
T3	180	189	0.55	0.65	23.1	24.2	15.8	16.4	11.2	11.3	0.92	1.42
T4	138	142	0.48	0.99	19.11	19.1	10.8	11.2	7.2	7.2	0.79	0.91
T5	147	151	0.51	0.57	19.6	20.3	12.9	13.5	8.0	8.1	0.85	1.06
T6	161	169	0.52	0.60	21.02	21.4	15.1	15.4	10.53	10.80	0.91	1.38
T7 (control)	98	99	0.42	0.43	11.6	11.5	4.8	4.5	3.8	3.5	0.41	0.42
L.S.d at 0.05	5.9168	6.8983	0.0247	0.0373	0.6843	0.8084	0.6043	0.6848	0.5906	0.5590	0.398	0.0662

Table 9: Effect of EM biostimulant treatments on the number of various groups of rhizosphere microflora (CFU x 10^6 g^{-1}) of Anna apple trees during 2005 and 2006 seasons.

	Total Fungi /g dry soil		Total bacteria /g dry soil		Total actinomycetes /g dry soil	
Season	2005	2006	2005	2006	2005	2006
T1	18.4	21.7	12.9	15.60	10.3	10.9
T2	19.3	22.3	18.6	19.40	16.2	17.3
T3	24.8	27.6	22.3	26.80	19.8	20.9
T4	14.1	17.6	11.2	13.11	9.1	10.3
T5	16.8	21.11	17.11	18.40	15.6	16.4
T6	21.2	23.9	19.30	20.6	17.4	19.2
T7 (control)	6.90	6.8	7.80	8.20	7.1	7.4
L.S.D at 0.05	0.6584	0.6347	0.3806	0.388	0.4429	0.3356

4- Fruit Quality at the End of Shelf Life (Marketing Period): The results presented in table (7) show the values of firmness TSS%, acidity % and Total sugars % of apple fruits after being kept for 19 days under the ambient atmospheric conditions. The results clearly show that TSS % and Total sugars % highly increments which were recorded from both EM Treatments compared to the control at the end marketing period. The results may be explained that keeping the apple fruits under ambient atmospheric conditions enhanced the degradation of the more complicated insoluble compounds like starch into simples forms which are mostly sugars. However acidity % was reduced while fruit firmness slightly changed. The same trend was also observed by Eissa[28], Attala[32], Attia[35] and El-Seginy[36].

5-soil Macro and Micro Elements Content: The results presented in table (8), indicated that, the two forms of EM biostimulant increased the values of soil elements elements as compared with the untreated trees. It was obvious that T3 (high rate of Bokashi) treatment produced the highest significantly values of N, P, K, Fe, and Zn and while the control treatment was significantly the lowest values. Results were in the same trend study by Eissa[10] and Eissa[28]. On apricot and plum.

6-Soil Micro Flora Content: The results in table (9) indicated that all EM applications significantly affected the number of CFU of TB, TA, and TF/g of dry soil. In both seasons, high rate of Bokashi significantly resulted the highest count of all groups of microorganisms, followed by the highest rate of EM solution. Also, it was noticed that the control, was significantly the lowest in all soil micro flora counts. Similar trend was obtained by Eissa[28] and Barnett[38] which reported that, yeasts, which were provide in the soil EM treatment, produce B vitamins, therefore, they may enhance activity of other microorganisms.a actually, in this investigation soil applied EM, which contains more than 60 strains of microorganisms, has greatly increased of the number of various groups of microorganisms in the rhizosphere soil.

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