USE OF SOME BIOSTIMULANTS IN ACTIVATION OF SOIL MICROFLORA FOR YIELD AND FRUIT QUALITY IMPROVEMENT OF 'CANINO' APRICOT

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ABSTRACT

The effect of soil application of biostimulants Phosphorin (Ph), Microbin (M), K-Humate (KH), and/or dry, active bread yeast (Y) were compared with no application (control) on soil rhizosphere microbial counts, foliage characters, fruit yield, and fruit quality attributes of 'Canino' apricot (*Prunus armeniaca* L.) grown in sandy soil during the 1999/2000 and 2000/2001 seasons. Biostimulants applied had a significant positive effect on rhizosphere count of various groups of microorganisms measured (viz, total bacteria, phosphate solubilizing bacteria, total fungi, yeasts, and actinomycetes), shoot length and diameter, leaf area, fruit yield, and fruit weight, size, total soluble solids (TSS) content, and TSS/acidity. Values obtained for each measurement decreased in the following order of biostimulants' application in both seasons: (Ph + M + KH +Y)> (Ph + M + KH) > (Ph + M) > (Ph) > control. Yield increase over the control treatment averaged, over the two studied years, 41.5%, 64.5%, 92.1% and 124.3% for the (Ph), (Ph + M), (Ph + M + KH), and (Ph + M + KH + Y) treatments, respectively. Meanwhile, application of biostimulants had no significant effect on leaf chlorophyll content, ratio of fruit equatorial diameter to polar diameter, and fruit titratable acidity.

Key words: Apricot, Biostimulants, Bread Yeast, Humic acid, K-Humate, Microbin, Phosphate Mobilizing Bacteria, Phosphorin, *Pruunus armeniaca*, *Sacchromyces cerevisiae*, Soil Microflora.

INTRODUCTION

Plant vigor, yield and quality improvement, acquired disease resistance, and tolerance to adverse environmental conditions are major goals of plant scientists and farmers alike. Though a great deal of progress has been achived in these directions through the use of various agricultural chemicals, including chemical fertilizers, environmental pollution has been a major drawback to their use. Biostimulants, including biofertilizers, offer a substitute or , at least, a partial one for the use of agricultural chemicals in maintaining proper plant growth and yield (Subba Rao, 1984).

Different biofertilizers, containing various bacterial species active in nonsymbiotic N_2 fixation and/or phosphate mobilization, have been used with various degrees of success on different plants including fruit crops. Phosphorin was used with guava (Haggag, *et al.*, 1995),'Anna' apple (Mansour, 1998), and sour orange (Boutros *et al.*, 1987b); Microbin was used with 'Anna' apple (Mansour, 1998); while various biofertilizers were used with citrus (Boutros *et al.*, 1987a); 'Red Roumy' grapevines (Ahmed *et al.*, 1997a; Akl *et al.*, 1997), and 'Nemaguard' peach (Mohamed and Mahmoud, 1999).

Improvement of yield, growth, and/or fruit quality attributes have been also obtained with foliar sprays of active bread yeast (*Sacchromyces cerevisiae*) on 'Anna' apple (Ahmed *et al.*, 1995; Mansour, 1998), 'Red Roumy' grapevines (Ahmed *et al.*, 1997b), and 'Valencia' orange (Hegab, *et al.*, 1997), and with soil and foliar application to 'Thomson Seedless'

grapevines (El-Mogy, *et al.*, 1998). Recently, many commercial products containing humic acid (HA), including K-humate (KH) have been promoted for use on various crops (Liu *et al.*, 1998). Benefits ascribed to the use of HA, particularly in low organic matter, alkaline soil, include increased nutrient uptake, tolerance to drought and temperature extremes, activity of beneficial soil microorganisms, and availability of soil nutrients (Senn and Kingman 1973; Russo and Berlyn, 1990). Humic materials may also increase root growth in a manner similar to auxins (Senn and Kingman 1973;O'Donnell, 1973; Titini *et al.*, 1991). Liquid fertilizer containing HA increased 'Starkrimson' apple fruit weight, yield, and soluble solids content (Li *et al.*, 1999).

In the newly reclaimed soil, apricot culture depends mainly on chemical fertilizers. Meanwhile, these soils are naturally poor in beneficial rhizosphere microflora which are considered vital to proper tree growth. Therefore, the objectives of this investigation were to study the effect of soil treatment with some biostimulants, including 2 biofertilizers, on soil microflora and growth, yield, and fruit quality attributes of 'Canino' apricot (Prunus armeniaca L.) which is currently the most important cultivar in Egypt.

MATERIAL AND METHODS

This Study was conducted during the 1999/2000 and 2000/2001 seasons at a private farm (El-Marwa farm Km 76, Cairo/Alexandria desert road) on apricot trees cv. 'Canino'. The eight-year-old trees, which were budded on 'El-Amar' apricot rootstock and planted 6 x 6 m apart, were growing in sandy soil under drip irrigation system and received the common cultural practices. Trees used in the experiment were selected to be healthy,

and as uniform as possible. Randomized complete block design with 3 replicates was used. Each experimental unit consisted of 1 tree.

Biostimulants used in the study were 2 biofertilizers, viz., Phosphorin (Ph) and Microbin (M), both being products of the MOA, Egypt), K-Humate (KH, 85% HA + 6% K; produced by Triad Energy Resources, Inc.; distributed by Lances Link SA, Geneve, Switzerland), and common, dry, active bread yeast, designated henceforth as 'yeast' (Y), obtained from local markets. According to the producer's label, Ph contains phosphate mobilizing bacteria (PMB) which are active in the release of the soluble calcium monophosphate from the unavailable calcium triphosphate, while M, contains various bacterial species that are active in both N_2 fixation and phosphate mobilization. All products used were in a granular form.

Treatments were applied to different groups of trees on Feb. 28, 2000 and Feb. 25, 2001 in the two seasons, respectively. Various products were added onto the ground close to drippers around trees at the following indicated rates per tree: (a) Ph at 5g, (b) Ph at 5 g + M at 5 g, (c) Ph at 5 g + M at 5 g + KH at 12.7 g, and (d) Ph at 5 g+ M at 5 g + KH at 12.7 g + Y at 9.6 g. Following application, products were mixed with the surface soil by shallow soil scratching. Soil of control plants were, likewise, surface-scratched but without biostimulants' application.

Measurements were recorded for soil microflora, vegetative growth characters, yield , and fruit quality attributes.

Soil Microflora:

Soil samples were collected from 3 sites around each tree, to 30 cm depth, using soil augers. Samples were collected in mid-June at the end of

the second harvest seasons. A thoroughly mixed composite sample was made from subsamples collected from soil of each experimental unit. Small portions of these composite samples were used for density estimation of colony forming units (CFU) of total bacteria (TB), phosphate mobilizing bacteria (PMB), total fungi (TF), total yeast (TY), and total actinomycetes (TA). This part of the study was conducted at the agricultural microbiology Department, Soil, Water and Environment Institute' ARC. Media used in culturing the various microorganisms were the soil extract agar medium (Allen, 1953) for TB, Bunt and Rovira's medium as modified by Abdel-Hafez (1966), for culturing the PMB, rose-bengal streptomycin agar medium (Martin, 1950), for plating both TF and TY, and Jensen's medium (Allen, 1953) for counting TA. The total CFU of various groups of microorganisms was determined by inoculating 1 ml of a suitable dilution per plate. Three plates were poured with selective media and incubated at 28 °C for 1 week for growing bacteria and 5 days for counting fungi and actinomycetes.

Foliage Measurements:

Foliage data were recorded in mid-August of both 2000 and 2001 seasons. Measurements included shoot length and shoot diameter as averages of 5 sampled shoots per tree and leaf area and leaf chlorophyll content as averages of 20 fully-expanded leaves per tree, sampled from the middle of 1-year-old shoots. Leaf area was recorded using a CL203 Area Meter (CID, Inc., USA), while a SPAD 502 chlorophyll meter (Minolta Corporation, Ramsey, N.J. USA) was used in recording chlorophyll readings.

Yield Records:

Data were recorded for total yield per experimental unit, i.e., per tree, in both seasons, and results obtained were used in estimating yield per feddan and yield of various treatments applied as percentage of the control.

Fruit Quality Attributes:

Samples of 10 randomly picked fruits per experimental unit, were collected in mid May in both seasons, and used for measuring various fruit quality attributes in both seasons. Characters measured were: fruit equatorial and polar diameters, weight, size, TSS content using a hand refractometer, and titratable acidity. The later 2 criteria were used in calculating the TSS/acid ratio.

Data obtained were subjected to statistical analysis according to Gomez and Gomez (1984), and means were compared using the LSD test.

RESULTS AND DISCUSSION

Soil Microflora:

Data obtained on the number of CFU of various groups of rhizosphere microflora in response to soil amendment with various biostimulants are presented in Table 1. Generally, microbial counts of all groups of soil microorganisms were in the following descending order of soil amendments: Ph + M +KH +Y > Ph + M + KH > Ph + M > Ph > the control treatment which had the least significant microbial count of all groups of microorganisms, except for TF, whereby their count was not significantly different from that of the Ph treatment. While Ph + M treatment was significantly lower than Ph + M + HA treatment in all microbial counts, it was significantly higher than those of the Ph treatment, except for TA. Meanwhile, the Ph + M + KH + Y treatment was

Table (1): Effect of soil amendment with various biostimulants on the number of CFU of various groups of 'Canino' apricot rhizosphere microflora[z] (CFU x 10^6 g^{-1} dry soil).

Treatments [y]	Total bacterial counts	Phosphate Mobilizing bacteria	Total Fungi	Total yeasts	Total Actino-mycetes
Control	4.63	2.10	0.63	0.53	4.77
Ph	6.90	33.37	1.68	3.67	27.56
Ph + M	12.63	39.10	3.11	4.18	32.11
Ph + M + KH	15.31	48.23	4.32	7.13	39.65
Ph + M + KH + Y	15.01	63.83	5.79	10.36	43.01
LSD at 5%	1.92	4.07	1.06	1.56	4.78

[z] CFU = colony forming units. Soil sampling for microbial counts was made at the end of the 2nd harvesting season in mid-June 2001, following two seasons of soil biostimulants' application.

y: Ph: Phosphorin, M: Microbin, KH: K-Humate, Y: Dry, active bread yeast.

significantly the highest in all counts except those of TB and TA whereby their counts did not differ significantly from those in the Ph + M + KH treatment.

The least microbial counts were noted in the control treatment as expected in a sandy soil. However, all groups of soil microorganisms measured were present – a normal feature of agricultural soil (Alexander, 1977). Applying Ph alone resulted in the greatest increment in the counts of PMB and TA. The greatest single increment in TB resulted from the application of Microbin which containd, in addition to PMB, various species of nonsymbiotic N_2 fixing bacteria. The inclusion of KH with Ph and M resulted in relatively larger increments in the counts of various groups of microorganisms, probably due to the provision of a source of energy, nutrients, and growth regulators (Senn and Kingman, 1973; Russo and Berlyn, 1990) by the KH. Addition of yeast to the Ph + M + KH caused a relatively large increment in TY count as expected, but it also significantly increased the total fungal count, which includes yeasts, and PMB which was probably activated by the B vitamins provided by yeast (Barnett *et al.*, 1990). Thus, their count increase in the soil as a result of the applied treatments (Table 1), is of considerable importance.

Actinomycetes are microorganisms that share the properties of both fungi and bacteria. Some members of this family such as *Steptomyces* may secrete a range of vitamins, growth substances, and antibiotics (Whippes, 1997). Thus , their count increase in the soil as a result of the applied treatments (Table1), is of considerable importantce .

Foliage Measurements:

Table (2): Variation in foliage measurements of 'Canino' apricot in response to soil application of biostimulants (2000 and 2001 seasons).

Treatments [z]	Shoot length (cm)		Shoot diameter (cm)		Leaf area $(cm)^2$		Chlorophyll Reading (SPAD)[y]	
	2000	2001	2000	2001	2000	2001	2000	2001
Control	26.0	28.1	0.45	0.46	34.2	33.1	36.0	42.1
Ph	39.8	41.2	0.48	0.49	36.4	35.4	37.1	44.5
Ph + M	45.2	48.3	0.49	0.50	37.1	37.2	37.7	46.3
Ph + M + KH	52.1	55.8	0.51	0.55	40.2	40.6	38.2	48.0
Ph + M + KH + Y	60.5	62.1	0.56	0.58	43.2	41.5	39.1	50.3
LSD at 5%	6.33	5.41	NS	0.09	5.96	7.18	NS	NS

z Ph : Phosphorin, M: Microbin, KH: K-Humate, Y: dry, active bread yeast.

y SPAD readings as measured by SPAD 502 Chlorophyll Meter.

As a general trend, all foliage measurements recorded were in the following descending order of soil amendment treatments with biostimulants: Ph + M + KH + Y > Ph + M +KH > Ph + M > Ph > control (Table 2). However, treatments applied had no significant effect on chlorophyll readings in both seasons and shoot diameter in the first season, In the second season, shoot diameter of the control treatment differed significantly only from the Ph + M + HA + Y treatment. The application of Ph had a significant stimulating effect only on shoot length. In both seasons, increments in shoot length were significant among all applied treatments. Meanwhile, values obtained for leaf area in the control treatment were significantly different from those in the Ph + M + HA +Y treatment, though values of both treatments were mostly not significantly different from the intermediate values obtained in the other treatments, viz, Ph, Ph + M, and Ph + M +KH.

The increasing stimulating effect of the various treatments in the order of their listing in Table 2 reflects their direct effect in addition to their complex effect on various groups of soil microorganisms (Table 1) as discussed above. Phosphorin and Microbin increased TB and PMB and probably resulted in greater N and P availability through nonsymbiotic nitrogen fixation and P mobilization, respectively. It is also known that some rhizosphere N_2 fixing bacteria such as *Azospirillum* may enhance plant growth by contributing growth hormones, such as cytokinins or auxins (Bouton *et al.*, 1979; Tien *et al.*, 1979). Growth regulators provided by KH (Senn and Kingman, 1973) and B vitamins contributed by yeasts (Barnett *et al.*, 1990) probably had a similar stimulating effect on growth.

The lack of significant effect of treatments on leaf chlorophyll content was probably due to the balanced nutrition program and excellent

care given to apricot trees used in this study, which had probably masked any treatment effect on leaf chlorophyll content, if there had been any. In a previous study (Liu et al., 1998), HA application had no significant effect on chlorophyll content of creeping bentgrass, *Agrotis stolonifera* L. Nevertheless, the increased vegetative growth as a result of the applied treatments in the present study in spite of the lack of any significant effect on leaf chlorophyll content (Table 1) is of paramount importance.

Yield:

Yield data are presented in Table 3. Treatments applied were mostly significantly different from each other in fruit yield which decreased in the following order of soil amendments: Ph + M + KH + Y > Ph + M + KH > Ph + M > Ph > control. The only insignificant yield differences obtained was that between the Ph and Ph + M treatments in the 2000 season. Yield in tons per feddan and Yield relative to the control followed a trend similar in significance to that of yield per tree from which they were calculated.

Yield response to the various biostimulants applied (Table 3) is understood in light of biostimulants' effect on both various groups of microorganisms (Table 1) and foliage measurements (Table 2). They all followed a similar response to biostimulant' application

Though biostimulants consistently increased yield of 'Canino' apricots, they had no significant effect on the chlorophyll reading, indicating that enhanced yield was due to some other mechanism other than enhancing the rate of photosynthesis per unit leaf area. Probably the positive treatment effect on shoot growth and leaf area contributed to higher photosynthetic rate per tree.

Table (3): Yield response of 'Canino' apricot to soil application of biostimulants (2000 and 2001 seasons).

Treatments [z]	Fruit Yield				Yield relative to control (%)	
	(Kg/tree)		(Ton/Fed.)			
	2000	2001	2000	2001	2000	2001
Control	48.3	41.0	5.64	4.78	100	100
Ph	66.9	59.2	7.81	6.91	138.5	144.4
Ph + M	71.4	74.3	8.33	8.67	147.8	181.2
Ph + M + KH	86.5	84.1	10.09	9.81	179.1	205.1
Ph + M + KH + Y	100.8	98.3	11.76	11.47	208.7	239.8
LSD at 5%	6.96	5.50	0.81	0.64	10.66	19.19

[z] Ph: Phosphorin, M: Microbin, KH: K-Humate, Y: dry, active bread yeast.

Due to their high content of cytokinins and B vitamin (Barnett et al., 1990), yeasts might had played a vital role in photosynthate translocation into fruits (Skoog and Miller, 1957) and in the synthesis of protein and malic acid and minimizing their degradation (Natio et al., 1981).

Fruit Quality Attributes:

With the exception of 2 fruit characters, viz., ratio of equatorial diameter to polar diameter and titratable acidity, which showed no significant response to soil treatment with biostimulants, all other characters measured, viz., fruit weight, size, TSS, and TSS/acidity ratio, responded positively and significantly to the various soil amendments applied and their values were in the following descending order: Ph + M + KH + Y > Ph + M + KH > Ph + M > Ph > control (Table 4). However, much overlapping was noted among treatments in their effect on various characters. Though the control treatment resulted always in the least values, it did not differ significantly from the Ph treatment in all characters measured in, at least, one season, and from Ph + M treatment in fruit weight and size in the second season. On the other hand, the Ph + M + KH + Y treatment resulted always in the highest fruit values measured, though fruit weight and size values did not differ significantly from those of the Ph + M + KH and Ph + M treatments in both seasons and from values obtained for the Ph treatment in the first season. Values obtained for TSS and TSS/acid ratio were not significantly different between the Ph + M + KH + Y and Ph + M + KH treatments in the second season.

Table (4): Effect of soil amendment with biostimulants on fruit quality attributes of 'Canino' apricot (2000 and 2001 seasons).

Treatments [z]	Weight (g)		Size (cm^3)		Eq.diam./polar diam.[y]		TSS (%)		Titratable acidity (%)		TSS/acidity	
	2000	2001	2000	2001	2000	2001	2000	2001	2000	2001	2000	2001
Control	27.7	25.6	29.4	27.1	1.05	0.98	7.1	7.5	1.51	1.36	4.90	5.51
Ph	34.8	31.2	36.2	33.1	0.95	1.01	8.3	8.6	1.36	1.25	6.10	6.88
Ph + M	37.9	35.1	39.5	37.0	0.93	1.05	9.4	9.6	1.23	1.16	7.64	8.28
Ph + M + KH	40.5	39.8	42.1	41.1	0.97	0.93	10.3	10.1	1.16	1.08	8.88	9.35
Ph + M + KH + Y	43.3	44.5	45.3	45.9	1.05	0.97	11.7	10.9	1.01	1.02	11.58	10.69
LSD at 5%	10.0	10.5	9.5	9.9	NS	NS	1.09	1.10	NS	NS	1.74	1.82

z Ph : Phosphorin, M: Microbin, KH: K-Humate, Y: dry, active bread yeast.

y Ratio of fruit equatorial diameter to polar diameter.

These results also coincide with results obtained and discussed above concerning the response of various groups of soil microorganisms (Table 1) and foliage measurements (Table 2) to biostimulants' treatments.

Biostimulants probably had a direct stimulating effect on foliage measurements (Table 2) and another indirect effect through their positive effect on microbial counts (Table 1). These positive effects of biostimulants were also reflected on fruit weight and size (Table 4) which were, in turn, reflected on fruit yield (Table 3). However, the effect of biostimulants on yield probably encountered other unforeseen effects such as lesser fruit drop. Also, it is interesting to note that the positive effect of biostimulants on yield (Table 3) and fruit weight and size (Table 4) was not at the expense of fruit TSS content, which showed a clear-cut positive response to biostimulants' application (Table 4), probably due to the increased leaf area (Table 1).

Results obtained in this study are in line with and confirm previous findings concerning the beneficial and growth promoting effects of biofertilizers on various fruit crops (Boutros et al., 1987a, 1987b; Haggag et al., 1995; Ahmed et al., 1997a; Akl et al., 1997; Mansour, 1998; Mohamed and Mahmoud, 1999), the stimulating and beneficial effects of the application of active bread yeast whether used as foliar spray (Ahmed et al., 1995; Ahmed et al., 1997b; Hegab et al., 1997; El-Mogy et al., 1998; Mansour, 1998) or as a soil amendment (El-Mogy et al.,1998); and the benefits ascribed to HA application, particularly activating soil microorganisms and improving plant growth and fruit quality (Senn, 1973; O'Donnell, 1973; Russo and Berlyn, 1990; Tatini et al., 1991; Li et al., 1999), in addition to it's lack of effect on leaf chlorophyll content (Liu et al., 1998). Henceforth, the use of these biostimulants, particularly the combined

treatment of Ph + M + KH + Y is recommended in commercial production of Canino apricot in sandy soils for both yield and quality improvement

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الملخص العربى

استعمال بعض المنشطات الحيوية فى تنشيط كائنات التربة الدقيقة لأجل تحسين محصول ونوعية ثمار المشمش كاتينو

فوزية محمد عيسى

معهد بحوث البساتين –مركز البحوث الزراعية

قورن تأثير معاملات إضافة المنشطات الحيوية: الفوسفورين، والميكروبين، وكى هيوميت،وخميرة الخبز الجافة النشطة إلى التربة وعدم إضافتها فى بستان مثمر من صنف المشمش كاتينو على أعداد ميكروبات التربة فى محيط الجذور، وصفات النمو الخضرى، ومحصول الثمار ، وصفات جودة الثمار خلال موسمى ١٩٩٩/٢٠٠٠ ، و ٢٠٠٠/٢٠٠١. كان للمنشطات الحيوية المستعملة تأثيراً إيجابياً معنوياً على أعداد مختلف المجاميع الميكروبية بالتربة (وهى: البكتريا الكلية، وبكتريا تحويل الفوسفات إلى الصورة الميسرة لاستعمال النبات، والفطريات الكلية ، والخمائر، والأكتينوميسيتات)، وطول الأفرع وقطرها، ومساحة الورقة، ومحصول الثمار ، ووزن الثمار، وحجمها، ومحتواها من المواد الصلبة الذائبة الكلية، ونسبة محتواها من المواد الصلبة إلى الحموضة المعايرة. وقد تناقصت القيم المتحصل عليها من كل قياس حسب الترتيب التالى لمعاملات المنشطات الحيوية فى موسمى الدراسة: الفوسفورين + الميكروبين + كى هيوميت > الخميرة > الفوسفورين + الميكروبين + كى هيوميت > الفوسفورين + الميكروبين > الفوسفورين > الكنترول (بدون إضافات). وقد كان متوسط نسبة الزيادة فى محصول مختلف المعاملات خلال موسمى الدراسة مقارنة بمحصول الكنترول كما يلى: ٤١,٥% لمعاملة الفوسفورين، و ٦٤,٥% لمعاملة الفوسفورين مع الميكروبين، ٩٢,١% لمعاملة الفوسفورين مع الميكروبين والكى هيوميت،و ١٢٤,٣% لمعاملة الفوسفورين مع الميكروبين والكى هيوميت والخميرة. هذا بينما لم تؤثر معاملات المنشطات الحيوية على كل من محتوى الأوراق من الكلوروفيل، ونسبة قطر الثمرة القطبى إلى قطرها المدارى، وحموضتها المعايرة.