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SOME MICROORGANISMS AFFECTED BY VARIOUS ORGANIC AMENDMENTS AND ITS EFFECT ON VOLKAMERIANA ROOTSTOCKS

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ABSTRACT

This investigation aimed to study the effect of various organic additives on the beneficial microbial populations, *i.e. Pseudomonas*, *Azotobacter*, *Azospirillum*, fungi and yeast, vegetative growth of volkameriana rootstocks and soil parameters. CRBD experiments were carried over two seasons out in the nursery of the Horticulture Research Institute, ARC. Eight treatments of organic amendments were applied with volkameriana rootstock seedlings *i.e.* humic acid (H), poultran 19 (P), compost extract (C), humic acid + poultran 19 (H+P), humic acid + compost extract (H+C), poultran 19 + compost extract (P+C), humic acid + poultran 19 + compost extract (H+P+C) and without organic additives (control). The organic amendments reduced growth media pH and EC. However, these organic additives increased the seedling thickness and length. H+P+C treatment recorded high results for most studied parameters followed by compost extract compared with the other treatments. Generally, the obtained results showed positive effects of the organic applications on soil microbial population.

Key words: *compost, humic acid, microorganism, organic amendment, poultran, rootstock, volkameriana.*

1. INTRODUCTION

In Egypt, citrus receives a great attention due to its importance for local consumption or as an important source of foreign currencies by exportation to the world markets. The area from citrus cultivated in Egypt has increased rapidly with the reclamation of new desert lands reaching about 483 thousand feddans (Ministry of Agriculture and Land Reclamation, Egypt 2011).

Producing citrus seedlings needs suitable rootstocks, fertile soil media good agriculture management as well. Volkameriana lemon, *(Citrus volkameriana)* is used as rootstocks for citrus, due to its tolerance and its acceptable resistance to a wide varge of citrus diseases. Moreover, it induces the vigor of citrus seedlings and consequently reduces the time needed in the nursery for seedlings production (Russo, 1956 &1959).

Organic fertilizers are used to enhance the fertility of soil media in comparison to the chemical fertilizers. They have lower nutrient content and are slow release. However, they are as effective as chemical fertilizers over long periods of use (Naguib, 2011).

In this respect, humic substances have provided positive effect on plant growth by increasing soil aggregation, aeration and permeability (Tan and Nopamornbodi, 1979; Mylonas and McCants, 1980). These substances were found to stimulate plant growth since they increase the absorption of soil nutrients (Vaughan and McDonald, 1971). Moreover, humified organic matter is also known to increase microbial growth and its activity (Visser, 1985).

In addition, compost applied to the soil improves its quality by altering the chemical and physical properties, increasing organic matter content, raising water holding capacity and inducing overall diversity of microbes (Sylvia, 2004, Healther *et al*., 2006).

According to the producers of biological crops, the addition of poultran 19 manufactured entirely from natural raw materials stimulates the multiplication of natural flora in the soil (Seryn *et al*., 2006). These organisms proride“micro ploughing” of the earth, which creates intensified plant root growth.

This investigation aimed to study the differences between some organic additives on the beneficial microbial population and seedlings growth.

2. MATERIALS AND METHODS

2.1. Characterization of the experiment site

The present study was conducted under green house conditions of the Horticulture Research Institute, Agriculture Research Center, Giza, Egypt on volkameriana rootstocks during the two growing seasons, 2010/2011 and 2011/2012.

Seedlings were propagated by planting seeds manually in plastic bags with dimensions 15 x 35 cm and thickness 75 μ. One seed was sown per each plastic bag. Each plastic bag was filled with a mixture of (4:1) sand : compost. In addition, 5 ml of a composite inocula of *Pseudomonas*, *Azotobacter* and *Azospirillum* (1:1:1) were added at the top of the soil in each plastic bag for all treatments. The treatments studied were; addition of humic acid (H) (5ml/l), poultran 19 (P) (2ml/l), compost extraction (C)(20 ml/l), humic acid + poultran 19 (H+P), humic acid + compost extraction (H+C), poultran 19 + compost extraction (P+C), humic acid + poultran 19 + compost extraction (H+P+C) and without organic additives as a control treatment. The recommended fertilization program of NPK was applied to all treatments.

2.2. Measurements

2.2.1. Vegetative growth parameters

The various growth parameters such as seedling length (cm), stem thickness (mm), leaf number and shoot: root ratio were measured at each season for all treatments.

2.2.2. Chemical parameters

1 - Leaf mineral content

The wet digestion of 0.25 g plant leaves with sulphuric acid and perchloric acid was carried out as reported by (Piper, 1950).

Total Nitrogen (T.N.): Total nitrogen in leaf samples was determined by the modified micro–Kjeldahl method as described by (Van-Schouwenburg and Walinga, 1978).

Total Phosphorus (T.P.): Total content of phosphorus in plant leaves was determined colormetrically according to Jakson (1958).

Total Potassium (K): Total content of potassium in leaves was measured using the flame photometer method (Corning 410) according to (Piper, 1950).

Zink (Zn), Manganese (Mn) and Copper (Cu): The element concentrations were determined by the atomic absorption spectrophotometer according to the method described by Carter (1993).

Calcium (Ca): Calcium content was determined according to (Page *et al*., 1982).

2.2.3. Soil analysis

Physical and chemical analyses of the soil growth media were conducted according to (Bouyoucos, 1963). The total nitrogen was determined in 5 gm soil using micro – Kjeldahl mentioned by (Jakson, 1958), soluble P and K were determined in a 1:5 soil water, according to Jackson (1967) and Bradfield and Spincer (1965). The main physical and chemical properties of the soil used are found in Table (1).

2.2.4. Microbial determination

Total bacterial counts and the population of *Azotobacter*, *Azospirillum*, *Pseudomonas* , Fungi , as well as Yeast were counted in the soil media at the end of each season by using nutrient agar medium for total colony count (Difco, 1985).

The other media that were used for the

Table (1): Physical and chemical properties of the soil media.

<table>
<tr><td rowspan="3">Alluvial sand</td><td colspan="4">Size distribution of soil particles (%)</td><td rowspan="2">O.M (%)</td></tr>
<tr><td>Silt</td><td>Fine sand</td><td>Coarse sand</td><td>Clay</td></tr>
<tr><td>0.6</td><td>18.5</td><td>49.4</td><td>23.6</td><td>8.5</td></tr>
</table>

<table>
<tr><td colspan="8">Cations and anions (Meq/l)</td></tr>
<tr><td colspan="4">Cations</td><td colspan="4">Anions</td></tr>
<tr><td>Ca^{++}</td><td>Mg^{++}</td><td>Na^{+}</td><td>K^{+}</td><td>CO_3^{--}</td><td>HCO_3^{-}</td><td>Cr</td><td>SO_4^{--}</td></tr>
<tr><td>5.0</td><td>1.8</td><td>19.8</td><td>2.5</td><td>-</td><td>2.22</td><td>15.4</td><td>11.48</td></tr>
</table>

Table (2): The chemical analysis of soil saturated paste extract.

pH	**Ec (O/S/m)**	**Mg/kg soil**			**ppm**			
		N	**P**	**K**	**Fe**	**Zn**	**Mn**	**Cu**
7.77	1856	103.3	55.14	374.7	9.126	4.846	4.368	1.310

selective individual microorganisms are Modified Ashby Media (Abd el-Malek and Ishac,1968) for *Azotobacter*, Kings Media (Blazevic *et al*., 1973) for *Pseudomonas*, Martin's medium Rosbengal streptomycin agar (Allen,1959) for fungi + yeast and Synthetic malat medium (Reihold *et al*., 1985) for *Azospirillum* were used.

2.2.5. Experimental design and statistical analysis

The CRBD design of eight treatments with four replicates for each treatment was followed throughout the whole work. Each replicate was represented by four seedlings. The obtained data were statistically analyzed using the Statistical Analysis System (SASM-Agri). The effects were tested using the general Linear Model. Multiple comparisons of means were performed according to Duncan test (Snedecor and Cochran, 1972).

3. RESULTS AND DISCUSSION

3.1. Vegetative parameters

3.1.1. Seedling length (cm)

The obtained results of seedling lengths showed significant differences between treatments Fig. (1). The combination between humic acid, poultran 19 and the compost extract (H+P+C) gave the best results followed by the addition of compost extract (C) alone. The results of H, H+P, H+C and P+C treatments have no significant differences. However, the lowest values of seedling length resulted from the control and humic acid treatments (20.9 & 23.8 and 27.1 & 27.9 cm), respectively in both experimental seasons. This behavior agrees with the data reported by Robert (2011), who mentioned that the excessive concentrations of humic acids and/or fulvic acid can inhibit seed germination and can kill young seedlings at high concentrations.

However, Abdel-Aziz *et al*. (2010) stated that, the use of compost, sheep manure resulted in positive results on lemon tree vegetative growth. In this respect, organic amendments generally improved plant growth (Nuria *et al*., 2012). It has been demonstrated that, the quantity and quality of the organic additives affect both the physicochemical properties of the soil and the biotic factors associated to the soil microbiota, such as microbial biomass, diversity, community structure and biochemical activities (Albiach *et al*., 2000 and Saison *et al*., 2006). In spite of this, some biotic factors, such as changes in pH or nitrogen content control some plant diseases (Tenuta and Lazarovits, 2002 and Wolstenholme, 2012). All of these changes have been related and can positively affect all plant growth parameters. Also, these results are in line with those obtained by Oburoni (2004), who showed that, organic fertilizers generally increase plant growth. Moreover, as it will be shown later, the microbial population was enhanced with the application of organic treatments, which stimulate plant growth through the production of plant hormones; the inhibition of plant pathogens; improving soil structure (Davison, 1988) and microbial leaching of inorganic pollutants (Ehrlich, 1990).

3.1.2. Stem thickness (mm)

Stem thickness of both experimental seasons are illustrated in Fig. (2). The highest values resulted from the addition of H+P+C, followed by the application with (C). The results of H+C, P+C and the (P) had no significant differences between them in both seasons. However, the lowest values resulted from the application of (H) and the control treatments without significant differences in both seasons. In this respect, it is noticed that, the organic fertilizer had a positive effect on seedling stem thickness.

Generally, seedling's stem gains thickness by growth of lateral meristems. Various factors affect the growth rates: of these, namely, water, organic fertilization, etc. Moreover, it is well known that in plants, there are two kinds of lateral meristems, the vascular cambium and the cork cambium. These lateral meristems are formed as rings within the plant body as the stem increases in thickness. Stem thickness usually increase by cell elongation and the production of new cells. One of the main factors that affect this process is fertilization particularly the organic type which gave the raw materials for all the growing energy cycles (Devlin and Witham, 1983). These results agree with those of Oburoni (2004), who stated that organic fertilizers increase stem girth.

3.1.3. Leaf number

The number of leaf for volkameriana seedlings was influenced by organic fertilizer as shown in Fig. (3). However, it is obvious that, the highest value of leaf number was achieved with the (H+P+C) treatment with no significant difference with the (C) treatment in both seasons. The addition of humic acid alone gave the lowest leaf number values. The applied concentration of humic acid inhibits germination and plant growth, and if added with a high concentration can damage or kill the young seedlings (Robert, 2011). However, the application of P, H+C and P+C resulted in reasonable values without significant differences.

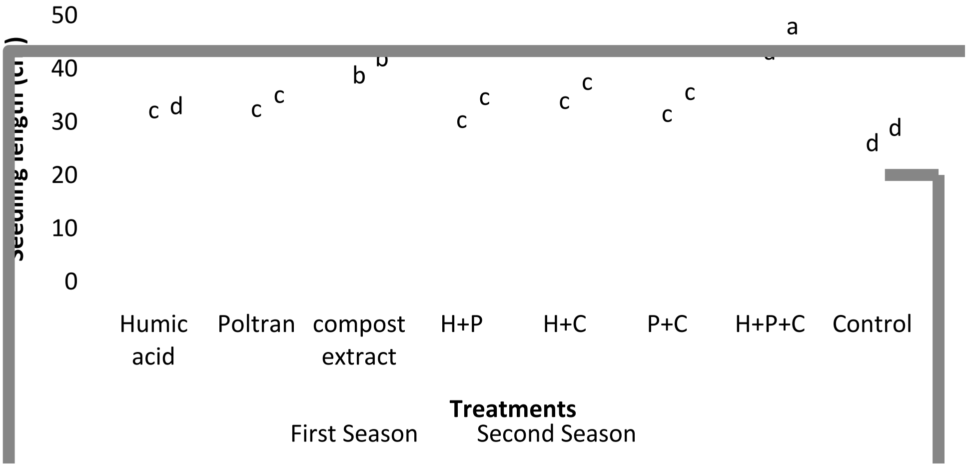

Fig. (1): Effect of different organic additives on seedling length (cm) of volkameriana seedlings.
*Means with the same letter have no significant difference

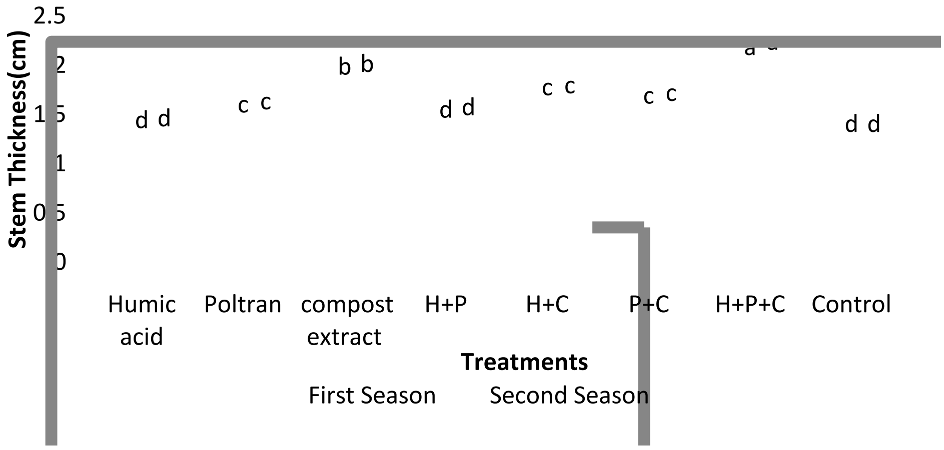

Fig. (2): Effect of different organic additives on stem thickness of volkameriana seedlings.
*Means with the same letter have no significant difference

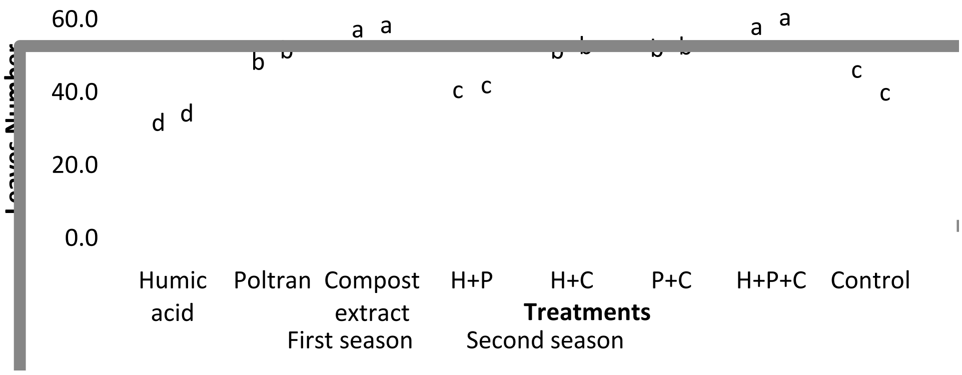

Fig.(3): Effect of different organic fertilizers on leaf number of volkameriana seedlings.
*Means with the same letter have no significant difference

3.1. 4. Shoot:Root Ratio (S/R)

The ratio between shoot length and root length are illustrated in Fig. (4). The obtained data showed a great positive effect associated with the combination of (H+P+C) treatment, *i.e.* the maximum significant value of this ratio was recorded in both seasons, while there were no significant differences among most of the organic treatments, particularly in the second season. However, the lowest values resulted from the humic acid and the control treatments in both experimental seasons. However, the results recorded by Oburoni (2004) working on citrus lemon showed that organic fertilizers increase root development and plant growth. Moreover, humic materials (humin, humate, fulvic acid) may increase root growth in a manner similar to auxins (Tattini *et al*., 1991). Generally, organic applications help in solubilization and mineralization of nutrients, particularly mineral phosphates which promote root development and consequently reflected on plant shoot growth (Dey *et al*., 2004). The obtained results are in line with the results on microorganisms particularly *Azospirillum* spp. which is considered one of the non-symbiotic N_2 fixers that increase root number (Okon *et al*., 1989) and enhance the enzyme activities in the root zone promoting the concentration of IAA and soil aeration (Fallik *et al*., 1988).

3.2. Chemical analysis

3.2.1. Leaf mineral content

Fig.(5). Shows that the combination of (H+P+C) resulted in low level of nitrogen, phosphorus and cupper contents, whereas the level of potassium was high. This can be correlated with the high results of the vegetative growth. These results are in agreement with the data reported by Devlin and Witham (1983). They showed that plants consume more nitrogen and phosphorus producing more leaves and increase stem thickness while potassium functions as a main regulator for the physiological processes.

The other treatments such as P+C, H+P, H+C, C and P resulted in reasonable values that matched with the results of vegetative growth. However, it is known that nitrogen concentration in leaves is often not correlated with increased growth (Hanway 1963). Also, Thomas *et al*. (2005) found that nutrient levels are high early in the year when plants are small, but decrease steadily as the growth rate increases primarily due to the growth dilution effect. Moreover, the level of calcium did not significantly differ between all the tested treatments. Both zinc and manganese values were in the optimum levels as the results cited by (Oberza *et al*., 1992).

3.3. Soil growth media analysis

The addition of the combination of H+P+C, H+C, H+P and P+C slightly reduced soil pH. Generally, the values of soil pH ranged from slight acid, neutral to slightly alkaline (USDA, 1993). However, it was clear that all organic additives reduce soil pH which affect positively mineral availability and consequently seedling growth parameters. Moreover, Haby (1993) stated that, soil pH and organic matter strongly affect soil functions and plant nutrient availability. Specifically, pH influences chemical solubility and the availability of plant essential nutrients and organic matter decomposition.

On the other hand, the obtained results indicated that, the addition of humic acid slightly increased soil EC and pH followed by the control treatment, whereas the lowest values resulted from all the combinations of organic additives and they values ranged from 0.5 to 0.7 mlmose/cm (Table 2). These results are in line with the results of vegetative growth parameters. However, all treatments improved soil macro and micro-nutrients in the growth media.

3.4. Microbial Analysis

Results of total bacterial count, *Azotobacter*, *Azospirillum*, *Pseudomonas*, yeast and fungi estimated in soil growth media were graphed in Fig.(6). Most of the organic treatments enhanced the microbial counts. However, both the H+C+P and (C) treatments significantly increased the total bacterial count, *Azotobacter*, *Azospirillum*, *Pseudomonas*, yeast and fungi counts in the first season. This is true since the organic additives are considered as a basic media which enhance all the microbial activities and population. However, the least values were obtained with the control treatment. The same trend was obvious in the second season.

In this respect, Zvyagintsev *et al*.(2008) found that, the addition of 0.01 % solution of humic acid increased the population of both *Pseudomonas* and *Azotobacter*. Moreover, it is noticed that, humic substances increase the growth rate of many forms of beneficial microorganisms in part, by stimulating enzyme activities (Visser, 1985 and Pouneva, 2005). This is true for both aerobic and anaerobic microorganisms (Hartung, 1992). Rashedul *et al*. (2009) stated that, the amount of FAME "Fatty Acid Methyl Esters" was greater in compost amendments. However, long term

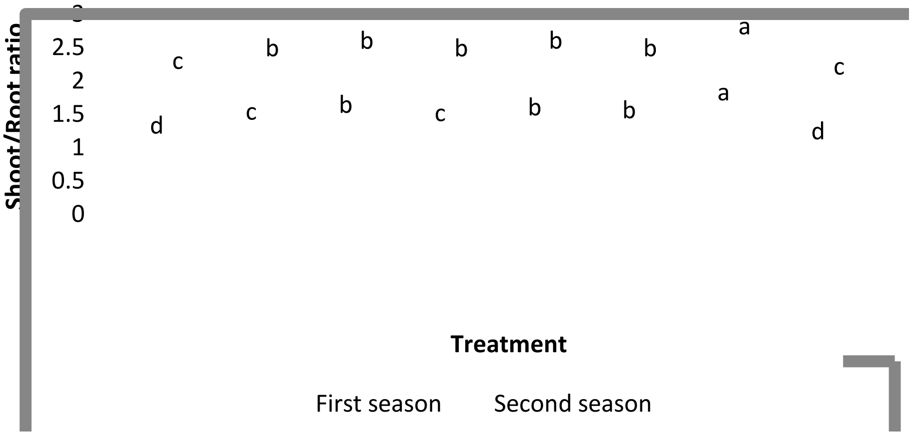

Fig. (4): Effect of different organic fertilizers on Shoot/Root ratio (R/S ratio) of volkameriana seedlings. *Means with the same letter have no significant difference

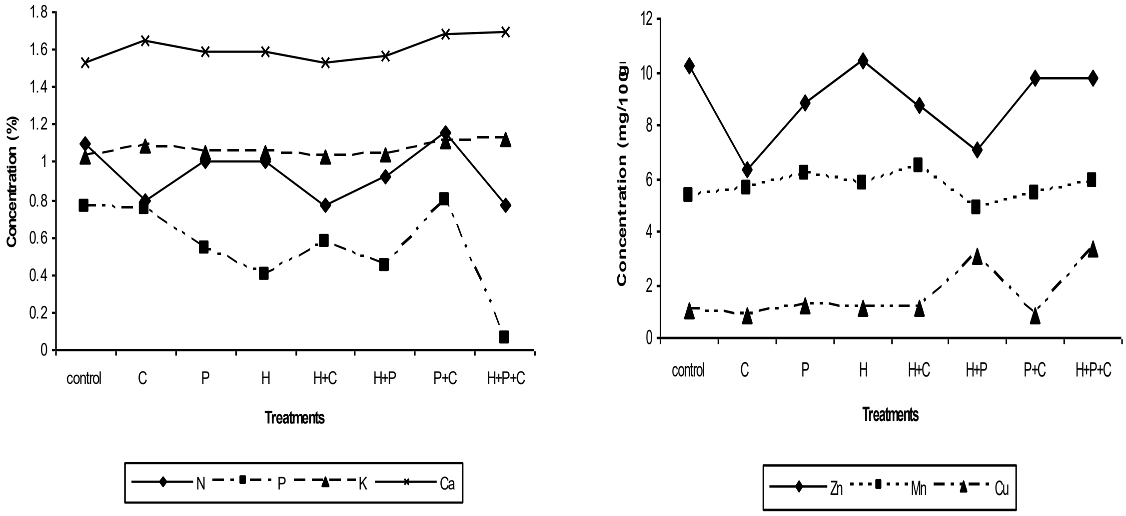

Fig. (5): Effect of different organic additives on leaf mineral contents of volkameriana seedlings.

Table (2): Effect of different organic additives on some chemical parameters of the soil cultivated with volkameriana seedlings.

Treatment	pH	EC	Mlmose/cm			ppm			
			N	P	K	Fe	Zn	Mn	Cu
Control	7.8	0.9	13.5	1.8	125	2.5	1.1	0.4	0.26
Compost extraction	6.6	0.6	37.3	5.1	150	4.1	2.4	0.7	0.30
Poultran 19	7	0.5	34.5	3.3	50	2.9	1.8	0.6	0.23
Humic Acid	7.3	1.5	36.6	4.5	65	3.8	1.9	0.7	0.27
H+C	6.2	0.6	34.1	3.5	100	2.9	1.7	0.5	0.29
H+P	6.1	0.6	32.9	4.5	75	3.3	1.5	0.6	0.20
P+C	6.4	0.7	35.7	5.3	90	4.5	2.7	0.5	0.47
H+P+C	6.2	0.5	35.0	5.4	255	2.9	2.0	0.5	0.41

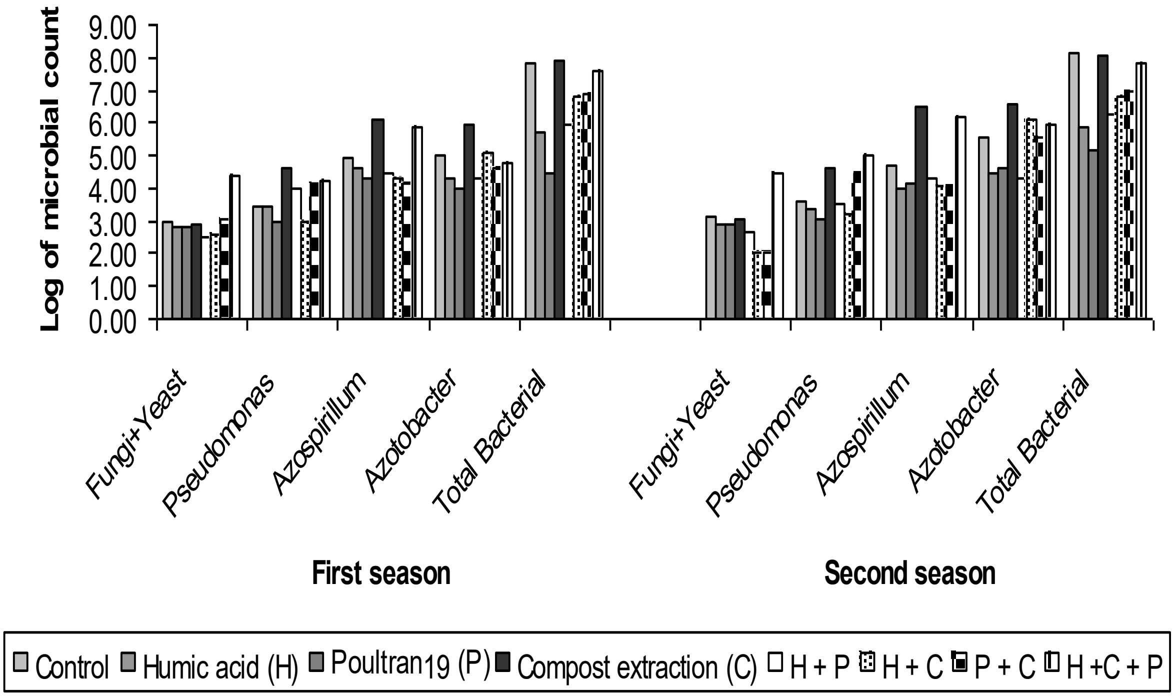

Fig. (6): Effect of different organic additives on bacterial populations in the soil.

experiments suggested positive effects of compost amendments on microbial diversity which could promote growth and productivity of the cropping system. In addition, a link between different fertilizer application treatments and soil microbial components was tentatively established, but it needs to be verified in further studies (Burkowska and Donderski, 2007).

In conclusion

The obtained results of the current experiments showed general positive effect of all organic fertilizer treatments particularly the combination of humic acid, poultran 19 and compost extract as well as the compost extract on the vegetative growth parameters mainly seedling stem thickness, which is considered as one of the important parameters of citrus seedling in terms of the ability to be ready for grafting, and the soil growth media microbial population. In addition, the results indicated a correlation between microorganism and the seedling root growth. However, the results of humic acid were not satisfied. This may be due to the high pH and Ec of the original materials. Generally, we can conclude that, the addition of organic amendments had a great effect on both the microorganisms and citrus rootstock seedlings.

Generally, we recommend repeating the same experiment with other organic substances to clarify the economic values of these results.

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تأثر بعض ميكروبات التربه ببعض الاضافات العضويه ومدى تأثيرها على أصل الفولكا ماريانا

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ملخص

تهدف هذه الدراسة الى إلقاء الضوء على مدى تأثير المواد العضوية المختلفة على المحتوى الميكروبي فى التربة خاصة كل من الميكروبات التالية (*Azospirillum Pseudomonas, Azotobacter* والفطريات والخمائر) وكذلك على النمو الخضري لشتلات أصل الفولكاماريانا فى المشتل وذلك لمدة عامين. أجريت هذه التجربة فى معهد بحوث البساتين، مركز البحوث الزراعية. حيث طبقت ثمانية معاملات مختلفة على شتلات الفولكاماريانا وهى كالتالى حمض الهيوميك (H)، حامض البولتران 19 (P)، مستخلص الكمبوست (C)، حمض الهيوميك + حمض البولتران 19 (H + P)، وحمض الهيوميك + مستخلص الكمبوست (H + C)، حمض البولتران 19 + مستخلص الكمبوست (P + C)، حمض الهيوميك +حمض البولتران 19+ مستخلص الكمبوست (H + P + C) ودون إضافات عضوية (المقارنه).

أوضحت النتائج المتحصل عليها إنخفاض كل من درجة حموضة ودرجة التوصيل الكهربائي للتربة .إلا أنها سببت زيادة سمك وطول الشتلة. كما أثبتت النتائج أن المعاملة بكل من حمض الهيوميك مع البولتران 19 ومستخلص الكمبوست قد أعطت أفضل النتائج لكل القياسات تحت الدراسة تلاها في ذلك المعاملة بمستخلص الكمبوست منفرداً.

وبصفة عامة أظهرت النتائج حدوث تأثير إيجابي لكل الإضافات العضوية المختبرة علي أعداد الميكروبات في التربة.

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