Reducing salinity stress in Murcott mandarin orchards using different soil amendments

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ABSTRACT

Aims: The response of "Murcott" mandarin trees budded on Volkamer Lemon rootstock grown in salt-affected soil to different alleviating salinity stress additions was studied.
Study design: This research was designed to fit The complete randomize block design (CRBD)
Place and Duration of Study: The present study was carried out in a private "Murcott" mandarin orchard located in "El-Adlia Association", El-Sharqia Governorate, Egypt, during two successive seasons 2014/2015 and 2016/2017.
Methodology: Eight different treatments were used as follow: 1) Control, 2) Magnetite at 138 kg/ha (Mag, knowing that ha = 10000 m^2), 3) Effective microorganisms at the rate of 12 L/ha. (EM), 4) Biotic at the rate of 12 L/ha. (B), 5) Mag+B, 6) Mag+EM, 7) B+EM and 8) Mag+B+EM.
Results: These different treatments mitigated salinity stress, reduced leaves osmotic pressure, thus increased fruit set, fruit yield, fruit quality, root distribution, photosynthetic pigments and mineral concentrations in leaves of Murcott trees compared with the control. Proline accumulations in fresh leaves, as well as soil pH and EC at the end of the two seasons also were recorded.
Conclusion: The combination between B and EM in the presence or absence of Mag enhanced the ability of mandarin to alleviate salt stress and produced the highest yield and fruit quality.

Keywords: Abiotic stress, Magnetite, EM, Leaves osmotic pressure, Fruit set, Yield, Fruit quality

1. INTRODUCTION

In Egypt, citrus is the main fruit crop in production and exportation [1]. The total world area of tangerines, mandarins, clementines and satsumas harvested in 2014 was 2,333,825 ha with world production 30,418,767 tonnes. Asia, Americas, Europe, Africa and Oceania contribute by 66.1, 13.8, 13.0, 6.8 and 0.4% of the world production, respectively. The top producers of these varieties are China, Spain, Japan, Brazil, Turkey and Egypt in the 6th rank [2]. Global production for 2016/2017 is forecast at 28.5 million metric tons [3].

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Salinity is the major oldest serious environmental problems affecting about one-third of earth's irrigated soils. Thirty to fifty percent of arable land loss has been expected due to salinity by the year 2050 resulting in huge depletion of agricultural productivity worldwide [4]. There are several factors affecting the salinity-crop relationship, such as climate and farming practices and the physical and chemical soil properties [5].

The excessive and frequent use of chemicals (fertilizers, pesticides, and plant growth regulators) in conventional cultivation has often produced adverse environmental impacts, making plants even more susceptible to pests and disease, and disturbing the ecological balance of soils thus crop yield and quality have decreased [6]. Many efforts have been devoted to reduce agrochemicals application and replace them with environmentally friendly materials without affecting yield [7].

Using magnetite (magnetic iron) in alleviating salinity stress on plants is a new advantage has added to magnetite benefits. Magnetite is a raw rock that has 6 Mohs on the hardness scale, brownish-red or black color, very high iron content and magnetic naturally. It is one of the most useful factors affecting crop yield [8].

The plant's health is affected by various biotic and abiotic stresses. This unexplored part of plant science is of a considerable advantage because of using biotechnology branch in applying the valuable microorganisms for upgrading crop yield and protection, thus opened some remarkable doors for the business [9]. Effective microorganisms or EM is one of the most popular microbial technologies being used worldwide now. The environmentally friendly EM technology claims an enormous amount of advantages. Addition of EM to manure may raise the micro-fauna diversity of the rhizosphere and many benefits are derived from that increase [6]. Effective microorganisms have been used as a commercial biofertilizer since they contain a combination of co-existing useful microorganisms collected from natural environments [10].

Biotic is a commercial product containing lactic acid bacteria, yeast and photosynthetic bacteria. Lactic acid suppresses harmful microorganisms and increases rapid decay of organic matter. Therefore, lactic acid bacteria enhance the decomposition of organic matter (lignin and cellulose) and accelerate the fermentation process of these materials. Bioactive substances such as enzymes, amino acids, sugars and hormones created by yeasts encourage active cells and roots division. Photosynthetic bacteria are independent that synthesize nucleic acids, amino acids, sugars, and bioactive substances [6]. In a recent study, the addition of yeasts, photosynthetic bacteria, lactic acid bacteria, actinomycetes and fermenting fungi mixture enhanced soil fertility thus promote plant growth as well as mitigated salinity impact by protecting the photosynthesis apparatus [11].

The aim of this study is to investigate the response of Murcott trees grown under salinity stress to magnetite, EM, biotic and their combinations in terms of production and fruit quality as well as some physiological parameters.

2. MATERIAL AND METHODS

The current study was established during two successive seasons of 2014-2015 and 2016-2017 (on season) in a private citrus orchard "El-Adlia Association", El-Sharqia Governorate, Egypt. Murcott mandarin trees (*Citrus sinensis* (L.) Osbeck × Citrus reticulate Blanco) about 5 years- old budded on Volkamer Lemon rootstock (*Citrus volkmeriana Tan & Pasq.*) grown on sandy soil at 3 x 6 m were used.

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The initial soil samples were collected from three depths (0-20, 20-40 and 40-60 cm) and analyzed for physical and chemical characteristics (Table1). The irrigation water is characterized by pH value equal to 8.39, EC 0.49 (dSm^{-1}), the soluble cations values were 2.0, 0.2, 1.5 and 1.2 meq/L for Na^+, K^+, Ca^{2+} and Mg^{2+}, respectively, 1.4, 2.6 and 0.9 meq/L for HCO_3^-, Cl^- and SO_4^{--}, respectively [12, 13].

Table (1): Some physical and chemical properties of the studied soils

Sample depth cm	pH (1:2.5)	EC dSm^{-1} (1:5)	Cations and anions meq/L								Physical properties (%)			Textural class
			Na^+	K^+	Ca^{++}	Mg^{++}	CO_3^{--}	HCO_3^-	Cl^-	SO_4^{--}	Sand	Silt	Clay	
0-20	7.81	6.1	37.4	0.4	16	7.2	-	1.9	54.7	4.4	86.0	11.6	2.4	Sandy
20-40	7.97	3.7	18.4	0.4	12.5	5.7	-	2.7	28.2	6.1	85.1	10.7	4.2	Sandy
40-60	8.03	2.7	16.3	0.4	6.3	4.0	-	2.8	15.5	8.7	85.2	12.3	2.5	Sandy

In the two experimental seasons, eight different treatments were used as soil applications as follows: 1) Control (Ministry of Agriculture recommendations without any extra additions), 2) Magnetite (Mag) at a rate of 138 kg/ha (biennial application), 3) Effective microorganisms (a commercial product contains some microorganisms occurring in nature, mainly including bacteria and fungi) at the rate of 12 L/ha (EM), 4) Biotic (a commercial product contains lactic acid bacteria, yeast and photosynthetic bacteria) at the rate of 12 L/ha (B), 5) Magnetite at 138 kg/ha plus biotic at the rate of 12 L/ha (Mag+B), 6) Magnetite at 138 kg/ha plus EM at the rate of 12 L/ha (Mag+EM), 7) Biotic at the rate of 12 L/ha plus EM at the rate of 12 L/ha (B+EM) and 8) Magnetite at 138 kg/ha plus biotic and EM at the rate of 12 L/ha (Mag+B+EM). All treatments received the recommendations of the Ministry of Agriculture.

The treatments were applied five times per year at the second week of February (flower initiation), March (full bloom stage), the second week of May (cell division of fruitlets stage), August and September. Except magnetite that was biennial application. These treatments were applied at the three years during 2014, 2015 and 2016 but the results were recorded at 2014- 2015 and 2016- 2017 (on seasons). The following parameters were recorded:

2.1. Flowering and fruit set

The leafy and woody inflorescences were counted at the last week of March then the percent of each were calculated. The fruitlets from both leafy and woody inflorescences were counted at the third week of June and then fruit set percentage was calculated according to the equation:

Fruit set% = (number of fruitlets/ number of inflorescences) ×100

2.2 Average yield per tree

Yield was determined at harvest stage (the second week of February) under the experimental condition by " ton/ha" and number of fruits per tree was counted.

2.3 Fruit quality

At harvest stage, representative samples of 10 fruits were taken from each tree and the following characters were determined:

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2.3.1 Physical characteristics

Average fruit number/tree, average fruit weight (g), fruit size (cm^3) as well as fruit length and diameter (cm) were measured and fruit shape index (length/diameter ratio) was calculated and fruit peel thickness (cm) was measured.

2.3.2 Chemical properties of fruits

Total soluble solids (T.S.S. %), total acidity percentage (expressed as mg citric acid/100 cm3 juice), total soluble solids/acidity ratio and vitamin C (as mg ascorbic acid was determined and estimated per 100 ml fruit juice) were determined according to A.O.A.C [14].

2.4 Leaves analyses

Forty leaves (fourth or third leaf from the top) of six months old from non-fruiting and non flushing shoots, were collected as described by Jones and Embleton [15] to determinant the following analyses:

2.4.1 Photosynthetic pigments

Fresh leaves were extracted with dimethyl formamide (D.M.F) solution [HCON $(CH_3)_2$] and placed overnight at cool temperature (50C). Chlorophyll a, b, total chlorophyll and total carotenoids were determined according to Moran [16].

2.4.2 Proline concentration

Proline concentration of leaves was determined calorimetrically as described by Bates et al. [17] modified and adapted accordingly Naqvi et al. [18].

2.4.3 Minerals determination

Mandarin leaves were collected, cleaned, dried in an oven at 650 C and digested with sulphuric and perchloric acids for nutrients determination. Total nitrogen (%) was determined using microkjeldahl method. Phosphorus (%) was determined colorimetrically using ammonium metavanadate method. Potassium, sodium, and calcium (%) were determined using the flame photometric method. All mentioned elements were measured as described by Cottenie et al. [19]. Then, the ratios of Na/ K, Na/Ca, K/Ca and Ca/ (K+Na) were calculated.

2.4.4 Leaf osmotic pressure

Adequate fresh leaf samples were immediately frozen, and then the cell sap was extracted with a piston pressure in the laboratory when the frozen tissue has been thawed. The sap total soluble solids were measured by refractometer and the equivalent values of the osmotic pressure (in bars) were determined as described by Gusov [20].

2.5 Root distribution

Horizontal and vertical of roots were measured, and then the horizontal and vertical root ratios were calculated at the end of the second season.

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2.6 Soil EC and pH

At the end of the two seasons, soil samples were collected from two depths (0-30 and 30-60 cm) to determine the soil-EC (expressed in dSm^{-1}) using an electrical conductivity bridge (in 1:5; soil : water extract) and soil-pH was recorded (in 1:2.5; soil : water suspension) using pH-meter as described by Page et al. [13].

2.7 Experimental design and statistical analysis

The complete randomize block design (CRBD) of eight treatments and three replicates (three trees/each) was used, with total number 72 trees. The obtained data were statistically analyzed using the COSTAT computer program. The multiple comparisons of means were performed according to Duncan test [21, 22].

3. RESULTS AND DISCUSSION

3.1. Flowering and fruit set

Data in Table (2) revealed that, in the second season the highest values of leafy inflorescences were obtained when magnetite either alone or in combination with biotic and EM was used compared with control, while, the highest values of woody inflorescences were obtained in control when compared with other treatment. The increment of leafy inflorescences percentage in response to magnetite treatment may be due to its effect on salinity moderate and stimulation of plant growth and finally enhanced flowering percentage.

In another study, magnetic field had a positive effect on the number of flowers of both strawberry [23] and pea [24].

In this respect, the increase in the percentage of plant growth was due to the effect of magnetic field on cell division and protein synthesis in paulownia node cultures [25]. The formation of new protein bands in plants treated with magnetite may be responsible for the stimulation of all growth parameters, and promoters in treated plants [26]. In addition, effective microorganisms (EM) have hormonal effects similar to the gibberelic acid [27].

Concerning to fruit set percentage, the highest values of fruit set percentage were obtained by the combination of biotic and EM, the combination between magnetite, biotic and EM as well as magnetite treatment respectively when compared with control at the two experimental seasons.

Similar results were obtained by Sheren [28] on Sukkary mango trees as well as EM foliar application under saline stress conditions enhance fruit set percentage of "Hayany", "Sewy" and "Zaghloul" cultivars date palm [29, 30]. Also, Atawia and El-Desouky [31] noticed that, in Washington navel orange, spraying yeast extract (one of biotic components) at 100 - 200 ml/L and some growth regulators were improving fruit set percentage and reducing June drop. Abd El-Motty *et al*. [32] reported that, spraying Keitte mango trees with algae (another component of biotic) at 2% combined with yeast at 0.2% improving fruit set, yield as well as number and weight of fruits.

In this respect, the presence of minerals, some growth regulators, protein, carbohydrates, vitamins, lactic acid bacteria, actinomycetes, photosynthetic bacteria and fungi in yeast, algae and EM may be the reason of improving the nutritional status of the trees, which reflected on increasing fruit set [28]. Microorganism application is an important strategy that has been used in order to decrease the harmful effect of salinity on plant growth [33].

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Table (2): Effect of some soil amendments on flowering and fruit set of Murcott mandarin in the two experimental seasons

Treatments	Leafy inflorescences (%)		Woody inflorescences (%)		Fruit set (%)	
	1 st	2 nd	1 st	2 nd	1 st	2 nd
Control	77.98 a	75.86 b	12.90 a	24.14 a	12.90 d	15.62 e
Mag	91.56 a	92.00 a	8.45 a	9.44 b	17.91 bc	24.98 abc
EM	88.91 a	88.22 ab	11.09 a	11.78 ab	16.91 bc	22.28 bcd
B	92.15 a	90.40 a	7.85 a	9.60 b	17.56 bc	20.89 cde
Mag +B	92.19 a	91.44 a	7.81 a	8.56 b	15.56 cd	19.38 de
Mag +EM	90.48 a	82.31 ab	9.54 a	17.69 ab	17.12 bc	22.45 bcd
B +EM	93.29 a	93.12 a	7.73 a	6.88 b	21.89 a	28.29 a
Mag+B+EM	91.89 a	92.37 a	8.11 a	7.63 b	19.15 ab	26.35 ab

Means in each column fallowed by the same letters did not differ at $p<0.05$ according to Duncan's multiple range tests.

3.2. Average yield

It is clear from the obtained data in Table (3) that, using magnetite either alone or in combination with EM or biotic as well as the combination of magnetite, biotic and EM had a significant promotion effect on the number of fruit per tree, the average of fruit weight (g) and the final yield (ton/ha) compared with control in both experimental seasons. The highest yield values were obtained by biotic plus EM, the combination of magnetite, biotic and EM or magnetite, respectively in the two experimental seasons.

Similar results were obtained by Nadia, *et al*. [34] who found that, the ground application of Mag (138 kg/ha/year) increased the number of fruit/tree, fruit weight thus the final yield compared with untreated Mandarin trees. Also Eman, *et al*. [35] found that, applied of 1000 g magnetite at December induced the highest values of yield, and leaf mineral content of Le-Conte pear trees.

In this respect, there are many benefits resulted from the addition of magnetic iron such as improving soil structure, increasing soil content of organic matter, improving water holding capacity and become more energy and vigor and this known as "Magneto biology', improving cation exchange capacity (CEC), enhancing crop nutrition. Furthermore, the magnetic iron application separates chlorine, toxic gases from soil, increases salt solubility and nutrients movement, moderates the temperature, and thus enhances plant growth [36].

Concerning to the effect of EM and B, the results were consistent with those attained by Fornes, *et al*. [37] who found that, the yield of orange was increased by using algae and yeast extract, [38] found that, adding EM mixed with the irrigation water to peach trees increase yield, as well as Sheren [28] showed that, adding EM to the soil increased yield as weight and fruit number of “Sukkary” mango tree fruits compared to untreated trees.

Table (3): Effect of some soil amendments on Murcott mandarin fruits yield in the two experimental seasons

Treatments	Fruit number/tree		Fruit weight (g)		Fruit yield (ton/ha)	
	1 st	2 nd	1 st	2 nd	1 st	2 nd
Control	120.00 d	120.00 d	120.89 f	134.67 d	7.98 d	8.87e
Mag	201.37ab	208.81ab	165.61bc	167.98abc	18.52ab	19.29b
EM	158.48 c	168.33 c	160.00bcd	166.90abc	14.06c	15.43c
B	169.46bc	160.00 c	147.39 de	166.59abc	13.59c	14.67cd

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Mag +B	213.00 a	193.00 b	131.72ef	149.30cd	15.43bc	15.85c
Mag +EM	154.33cd	149.67 c	155 .00 cd	164.28bc	13.16c	13.52d
B +EM	208.33 a	228.00 a	182.87 a	185.54 a	20.96a	21.57a
Mag+B+EM	204.67 ab	201.33 b	174.17ab	172.01 ab	19.61a	20.54a

Means in each column fallowed by the same letters did not differ at $p<0.05$ according to Duncan's multiple range tests.

In this respect, the higher citrus yield, which resulted from EM treatments were somewhat correlated with the improvement of soil physical and chemical characteristics. The use of biofertilizers may help in improving tree productivity and fruit quality by increasing the availability of nutrients and stimulating the natural hormones [39]. The mixture of yeasts, photosynthetic bacteria, lactic acid bacteria, actinomycetes and fermenting fungi can mitigate salinity stress by adjustment both biosynthesis of endogenous phytohormone and photosynthetic mechanisms [11].

3.3 Fruit quality

3.3.1 Physical properties

All applied treatments significantly increased fruit size when compared with control, with some exceptions. The highest values either were recorded by EM in combination with biotic or with magnetite treatment, respectively at the two experimental seasons (Table 4).

It was noticed that, EM treatment increased significantly fruit shape index when compared with control at the two experimental seasons.

Concerning the peel thickness, at the both seasons, all applied treatment significantly increased peel thickness when compared with control. The highest value of fruit peel thickness was obtained by the combination of magnetite, biotic and EM treatment when compared with control.

In this respect, EM application can act to 1) suppressing soil disease; 2) accelerating the mineralization rate of soil organic matter, 3) releasing nutrients, amino acids and other organic compounds for plant absorption, 4) increasing the number of nitrogen-fixing bacteria and photosynthetic bacteria and 5) enhancing the plant's photosynthetic rate and efficiency, Thus improving plant growth, crop yield, and its quality [9]. Fruit quality of date palm improvement due to yeast and EM application may be due to improve the synthesis of protein and nucleic acids which enhanced cell division and enlargement leading to fruit weight and size increases. In addition, photosynthesis enhanced and hormone promotion, which advanced the fruit maturity [40].

Table (4): Effect of some soil amendments on physical properties of Murcott mandarin fruits in the two experimental seasons

Treatments	**Fruit size (cm^3)**		**Fruit shape index**		**Peel thickness (cm)**	
	1 st	**2 nd**	**1 st**	**2 nd**	**1 st**	**2 nd**
Control	131.00 f	139.33 c	0.76 c	0.73b	0.28 c	0.30d
Mag	153.17 de	160.67bc	0.78abc	0.81 ab	0.32bc	0.35 abcd
EM	163.33 cd	174.00 b	0.80 a	0.88 a	0.31bc	0.32 cd
B	142.25 ef	145.00 c	0.79ab	0.80 ab	0.34ab	0.37 abc

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Mag +B	174.67 bc	178.67ab	0.80 a	0.80 ab	0.31bc	0.35 abcd
Mag +EM	183.67ab	186.00ab	0.79 ab	0.86 ab	0.31bc	0.34 bcd
B +EM	199.33 a	200.00 a	0.76 c	0.81 ab	0.35 ab	0.41 ab
Mag+B+EM	177.33 bc	181.33 ab	0.77bc	0.79 ab	0.38a	0.42 a

Means in each column fallowed by the same letters did not differ at p<0.05 according to Duncan's multiple range tests.

3.3.2. Chemical properties

Data represented in Table (5) demonstrated that, biotic treatment significantly increased T.S.S (%) and T.S.S/ acid ratio, while decreasing acidity at the two experimental seasons when compared to control, with some exceptions.

All treatments increased vitamin C compared with control treatment in the two experimental seasons. Whereas, the highest value of vitamin C was obtained by magnetite in combination with biotic treatment followed by biotic plus EM treatment. Similarly, Ismail, *et al.* [41] found that, the lower rates of magnetite were significantly increased fruit quality of Superior cv. grapevines as compared with the other treatments. Also, Higa and Wididana [9] found that, EM significantly increase vitamin C and sugar in fruit over that of the control. Furthermore, Abd El-Messeih, *et al*. [42] showed that, adding EM to the soil improved yield, total sugars, T.S.S and decreased acidity and fruit drop in Le-Conte pear tree as compared with control. Sheren [28] noticed that, added EM to the soil increased vitamin C of mango trees.

Table (5): Effect of some soil amendments on chemical properties of Murcott mandarin fruits in the two experimental seasons

Treatments	TSS (%)		Acidity(%)		TSS/Acid ratio		Vit. C (mg/100ml juice)	
	1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd
Control	10.33 b	11.00 b	0.970 a	0.976 a	10.66 d	11.27 e	31.97 c	32.23 c
Mag	11.67 a	11.33ab	0.921 a	0.819bc	12.81 cd	13.82 cd	33.83bc	36.90bc
EM	11.50 a	12.33ab	0.739 b	0.830bc	15.58ab	16.25 b	36.03bc	38.20 b
B	10.75ab	12.83 a	0.614 c	0.696 d	17.59 a	18.44 a	37.33ab	38.50 b
Mag +B	10.83ab	12.83 a	0.768 b	0.819bc	14.13bc	15.68bc	40.60 a	44.10 a
Mag +EM	10.83 ab	12.17ab	0.787 b	0.730 cd	13.89 bc	16.69 ab	36.40 ab	38.65 b
B +EM	10.50 b	12.33ab	0.749 b	0.920 ab	14.05 bc	14.48 d	38.03 ab	40.20ab
Mag+B+EM	11.17 ab	12.33ab	0.730 b	0.786 cd	15.32abc	15.73 b	34.53bc	36.73 bc

Means in each column fallowed by the same letters did not differ at p<0.05 according to Duncan's multiple range tests.

In this respect, yeast composition might be playing a considerable role in the orientation and translocation of metabolites from the leaves to the productive organs. However, its improved cell division, metabolism and other biological reactions, as well as the activation effect of these components on photosynthesis and promoting protoplasm formation including DNA and RNA that essential for cell division and it play a vital role in the synthesis of nucleic acid, and protein [32, 43]. The increase in fruit quality could be to the effective components of algae and yeast such as major and minor elements, growth regulators, cytokinins content, and high content of vitamin B5 and minerals. Also, EM has benefits in increasing yields, improving fruit quality. The concept of EM is based on the

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inoculation of beneficial microorganisms into the soil where they create the microbiological equilibrium and produce a suitable environment for the growth and health of plants [9].

3.4 Leaves analyses

3.4.1 Plant pigments

Data presented in Table (6) obviously reveal that, EM in combination with magnetite or biotic showed significant increment effect on chlorophyll a, b as well as total chlorophyll, for both seasons as compared to control, with some exceptions.

In this respect, EM foliar application under saline stress enhances leaf chlorophyll content and fruit set [29, 30]. The positive effect of magnetic treatment (magnetite) may be attributed to paramagnetic properties of some atoms in plant cells and some pigments such as chloroplasts [44].

Table (6): Effect of some soil amendments on plant pigments (mg/g f.wt.) of Murcott mandarin leaves in the two experimental seasons

Treatments	Chl. (a)		Chl. (b)		Total chl.		Total carotene	
	1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd
Control	0.635 b	0.665 c	0.305 b	0.332 c	0.938 b	0.995 c	0.077 b	0.134 b
Mag	0.757ab	0.826 a	0.427 ab	0.665 ab	1.174 ab	1.486 ab	0.095 ab	0.134 b
EM	0.650 b	0.668 bc	0.315 b	0.561 abc	0.963 b	1.004 c	0.097a	0.117 b
B	0.758ab	0.777 a	0.432 ab	0.466 bc	1.174 ab	1.240 bc	0.093 ab	0.111 b
Mag +B	0.717ab	0.768 ab	0.392 ab	0.457bc	1.095 ab	1.222 bc	0.090 ab	0.109 b
Mag +EM	0.797a	0.794 a	0.504 a	0.577abc	1.298 a	1.368 ab	0.102 a	0.904 a
B +EM	0.805 a	0.842 a	0.529 a	0.761 a	1.359 a	1.600 a	0.094 ab	0.138 b
Mag+B+EM	0.738ab	0.801a	0.412 ab	0.564 abc	1.150 ab	1.362ab	0.091 b	0.136 b

Means in each column fallowed by the same letters did not differ at $p<0.05$ according to Duncan's multiple range tests

3.4.2 Proline concentration

All treatments led to decreasing proline concentration in leaves compared with control in both seasons. Generally, the values of the second season are lesser than that first one (Fig. 1). This depression may be attributed to the effect of irrigation water on decreasing soil salinity irrespective of treatments' effect. Nadia, et al. [34] described similar results.

Regardless control treatment, the highest value was obtained from B and Mag + EM treatments, but the other treatments decreased proline concentration in leaves as follows: EM, Mag + B, Mag, B + EM, Mag + B + EM.

The maximum treatments in their effect on reducing proline concentration are B+EM and Mag + B + EM whereas the decrease percentage were 36 and 40% for B + EM and 43 and 34% for Mag + B + EM compared to control in 1st and 2nd seasons, respectively.

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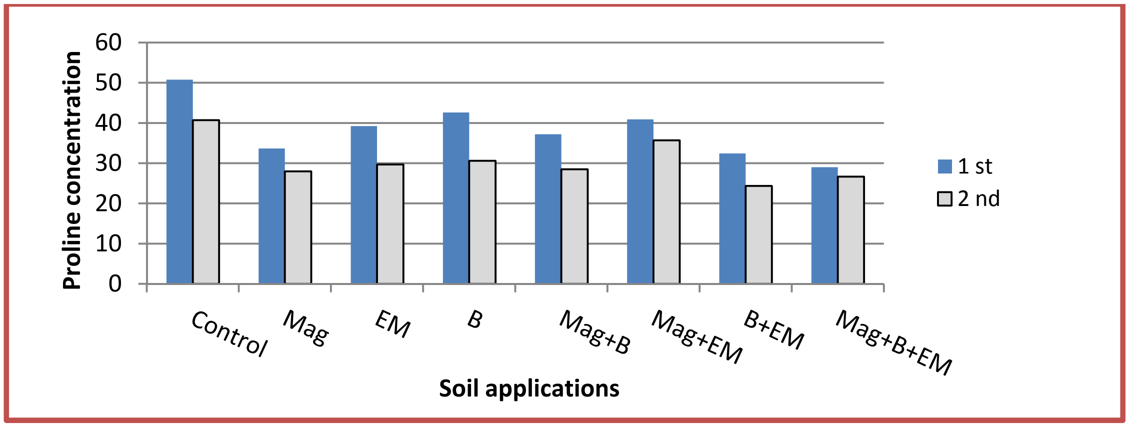

Fig (1): Effect of soil amendments on proline concentration of Murcotte leaves at the two experimental seasons.

3.4.3 Minerals concentration

Effective microorganisms combinations with magnetite or biotic increased significantly the nitrogen concentration in the two experimental seasons when compared with control and other treatments (Table 7). No significant difference between the highest three treatments (B+ EM, Mag + EM and Mag +B +EM) in their effect on nitrogen concentration.

In this respect, soil microorganisms are important in decomposing organic materials and recycling their nutrients for uptake by plants [9]. The beneficial effect of EM on improving leaf mineral contents may be attributed to its microbes rule in enhancing natural fertilizing processes within the soil and act as abio- stimulant that directly increases the resident nitrogen fixation capacity through activation of N fixing bacteria, and indirectly by increasing nutrients uptake [45]. Nitrogen, phosphorus and potassium forms available to plants were increased in the soil treated with EM [46]. Effective microorganisms mixed with other biofertilizers or organic materials can be added to the soil to stimulate the supply and release of nutrients in the soil [47]. In addition, magnetic field plays an important role in cation uptake capacity and has a positive effect on the immobile plant nutrient uptake, such as Ca and Mg [48]. Magnetite might have a stimulating effect on nutrient absorption [8].

Concerning to phosphorus concentration, Mag+B treatment produced highest leaves P concentration. Statistically, no significant difference was found between treatments compared with control in the two experimental seasons. In contrast to nitrogen, the lowest values were obtained in Mag+EM and B+EM treatments, this could be due to 1) the translocation of P from leaves to fruits and seeds 2) the dilution effect, whereas these treatments are the superior in most growth parameters so their leaves have large volume that resulted in the dilution of their element contents. Abou-Baker, *et al*. [49] confirmed that, high plant growth might cause a dilution of some nutrient concentrations. As for potassium, all treatments led to increase K concentration in leaves compared to control. As shown in phosphorus trend, Mag+B treatment increased K in both seasons compared with other treatments. It can be observed that, the highest three treatments in their effect on P and K concentration are Mag + B followed by EM and the next is B. This trend was true in both seasons. Like P and K, Ca concentration increased by the addition of all treatments compared with control and Mag+B treatment was the superior. Concerning Na, control treatment has the highest concentration of Na compared with other treatments. This could confirm that all treatments led to decrease the harmful effect of salinity by decreasing Na

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concentration in the leaves. The treatment of B + EM that formed highest fruit yield produced the lowest concentration of Na.

Table (7): Effect of some soil amendments on minerals concentrations of Murcott mandarin leaves in the two experimental seasons

Treatments	N%		P%		K%		Ca%		Na%	
	1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd
Control	1.54d	1.59d	0.12b	0.18bc	0.44d	0.99c	1.27c	1.43b	0.26a	0.35a
Mag	1.85c	2.00abc	0.12b	0.25abc	0.89bc	1.11c	1.38bc	1.70ab	0.20c	0.31a
EM	1.64d	1.72cd	0.15ab	0.26ab	1.11ab	1.82ab	1.67a	2.16a	0.21bc	0.30a
B	1.59d	1.77bcd	0.15ab	0.26ab	1.06ab	1.80ab	1.57ab	2.05a	0.24ab	0.30a
Mag +B	1.62d	1.75bcd	0.21a	0.29a	1.16a	2.00a	1.70a	2.28a	0.25a	0.31a
Mag +EM	2.18ab	2.21a	0.09b	0.15c	0.91abc	1.46bc	1.59ab	1.81ab	0.21bc	0.21b
B +EM	2.31a	2.18a	0.11b	0.18abc	0.74c	1.80ab	1.32c	1.97ab	0.19c	0.20b
Mag+B+EM	2.10b	2.05ab	0.13b	0.25abc	1.01ab	1.70ab	1.59ab	2.21a	0.22abc	0.27ab

Means in each column fallowed by the same letters did not differ at $p<0.05$ according to Duncan's multiple range tests.

The average values of the determined elements ratios of both seasons showed a clear trend (Fig.2). Irrespective of control, all treatments decreased Na/K, Na/Ca and Ca/(K+ Na), but increased K/Ca ratio. As for Na/K ratio, the control produced high value (0.5) followed by the sole application of magnetite (0.3) but the others recorded the same value (0.2). This may be attributed to the high Na concentration in leaves under control treatment. The ratio of Na/Ca produced two values 0.2 for control, magnetite and biotic and 0.1 for other treatments. The values of Ca/(K+ Na) ratio ranged between 1.1 and 1.4. Regardless Na ratios (Na/K, Na/Ca and Ca/(K+ Na)), K/Ca ratio enhanced by application of any treatment compared with control (0.5). The highest record of K/Ca (0.8) was produced by four treatments; the soil application of EM and B, the combination between the B+EM in addition to Mag+B treatment. In another study, the addition of biofertilizer under salinity conditions led to a significant increased in K/Na, Ca/Na and Mg/Na ratios and enhanced the mineral status of the plant by decreasing Na absorption compared with Ca and Mg [49].

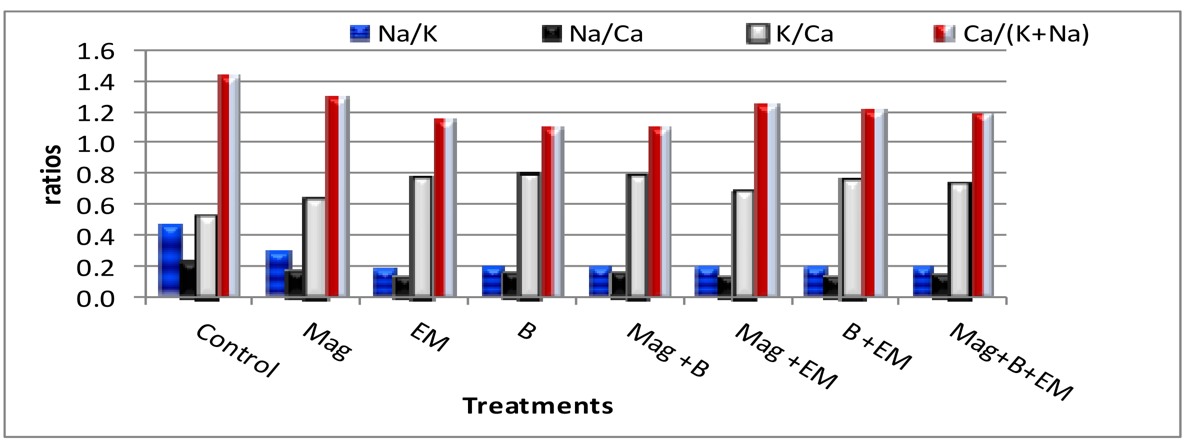

Fig. (2): Effect of some soil amendments on nutrients concentration of Murcott mandarin leaves in the two experimental seasons.

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In this respect, the presence of salts alters the nutritional balance of plants, resulting in high ratios of Na^+/K^+, Na^+/Ca^{2+}, Na^+/Mg^{2+}, Cl^-/NO_3^- and $Cl^-/H_2PO_4^-$ [50].

3.4.4 Leaves osmotic pressure

The data in Table (8) revealed that, at the two experimental seasons the lowest values of leaf osmotic pressure were obtained by B+EM, Mag+B+ EM followed by Mag treatments when compared with control.

Citrus does not tolerate salinity well. Therefore, most citrus grows poorly in coastal environments. This because of high levels of salts in the water will raise the osmotic pressure and decrease water uptake [51]. Ferguson and Grattan, [52, 53] reported that, there are two ways can damage plants especially citrus: 1) direct injury due to specific ions, and 2) osmotic effects (the total concentration of salt in the soil solution produced by the combination of soil salinity, fertilization and irrigation water quality). In addition, the highest leaf osmotic pressure of valencia orange trees was presented in the absence of magnetite iron [5].

Table (8): Effect of some soil amendments on leaves osmotic pressure of Murcott mandarin trees at the two experimental season

Treatments	Leaves osmotic pressure (%)	
	First season	second season
Control	25.50 a	23.50 a
Mag	20.25 bc	18.50 cd
EM	23.00 ab	21.17 ab
B	23.50 ab	22.00 ab
Mag +B	22.50 ab	20.75 bc
Mag +EM	21.50 abc	20.00 bcd
B +EM	17.50 c	12.00 e
Mag+B+EM	19.50 bc	18.00 d

Means in each column fallowed by the same letters did not differ at $p<0.05$ according to Duncan's multiple range tests.

3.5 Root distribution

The effect of the tested treatments on root horizontal, vertical and the root horizontal/vertical (H/V) ratio of Murcott mandarin trees are presented in Table (9). The results showed that the biotic and EM together achieve the highest value of root horizontal distribution followed by Mag+B+EM where Mag+B came in the third order compared to the other tested treatments. Generally, all treatments included microorganism with or without magnetite showed positive effect on root horizontal distribution in comparison with the control. The same trend was observed with the root vertical extension and the ratio of H/V, where the highest values were abtained by Mag +B+EM followed by B+EM, B+Mag, Mag+EM.

Generally, EM can ferment soil organic matter which consequently releases sugar, alcohol, amino acid and other organic compounds that can be absorbed by plant roots [9]. The beneficial effect of EM was attributed to the ultization of plant root exudates and the solubilization and mineralization of certain soil nutrients into plant available forms [54].The addition of biofertilizers or live micro-organisms produce bioactive substances such as hormones and enzymes which promote active cell and roots division. In addition, it enhances the soil life that improves both the soil physical and chemical properties such as soil water

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retention and the availability of nutrients. These soil characteristics have direct positive effects on root extension both horizontally and vertically [6].

Moreover, the salt mitigation caused by adding magnetite to the soil improves the tree root growth and increase the extension area.

Table (9): Effect of some soil amendments on root parameters of Murcott mandarin trees at the end of second experimental season

Treatments	**Root parameters**		
	Horizontal roots extension (cm)	**Vertical root extension (cm)**	**H/V**
second season			
Control	75.0 e	53.3 b	1.430 c
Mag	91.6 d	58.3 b	1.604 bc
EM	98.3 cd	55.0 b	1.787 b
B	98.3 cd	60.3 ab	1.639 bc
Mag +B	108.3 bc	61.6 ab	1.769 bc
Mag +EM	101.6 bcd	55.0 b	1.853 ab
B +EM	123.3 a	56.6 ab	2.182 a
Mag+B+EM	110.0 b	65.3 a	1.703 bc

Means in each column fallowed by the same letters did not differ at $p<0.05$ according to Duncan's multiple range tests.

3.6 Soil EC and pH

Even control treatment there is a high depression in EC records compared with the initial soil sample (Fig.3). This could be attributed to the ordinary soil management, especially the irrigation with high-quality water that leads to decrease the soil salinity compared with the initial soil sample. The surface layer was more sensitive to the applications effect than the subsurface layer. The lowest EC values were observed in Mag+EM treatment. The decrease in EC percentages were 41 and 35% for control compared with initial soil in surface and subsurface layers, respectively. In another study, treated water by EM can be used to alleviate a saline soil [10].

Slightly reduction was observed in pH values compared with control and initial soil samples (Fig. 4). This may be attributed to the leaching of some basics by irrigation water or the weak acids that produced by root secretions and microorganisms. The decrease in pH values in subsurface layer was higher than the surface layer because the subsurface layer is more active chemically and biologically than the surface one. The lowest value of pH was recorded by EM+B+Mag treatment.

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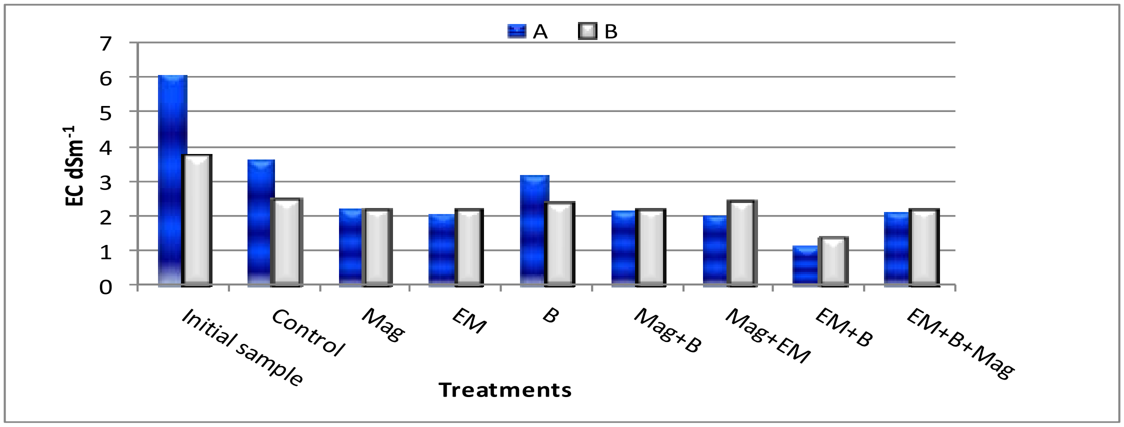

Fig. (3): Effect of soil amendments on soil EC in the two depth sample (A and B).

Microorganisms interact with plants and contribute to the living ecosystem, they are believed to be an integral part of the defense mechanism in plants against several stresses. Growth of plants that grow under salinity conditions are usually inhabited by microbes that are beneficial for the enhancement of their salinity tolerance mechanism. Under the salinity stress, microorganisms triggers rapid fluxes of cell water along the osmotic gradient out of the cell and accumulates large amounts of organic osmolytes as well as modulates the potassium transport. The organic osmolytes such as trehalose, proline, and glycine betaine, etc. offer an adaptive strategy to abiotic stresses, including high salinity [55]. Hydrogen peroxide and lipid peroxidation content were significantly increased in response to salinity, while they declined by the EM addition to both stressed and non-stressed plants [56].

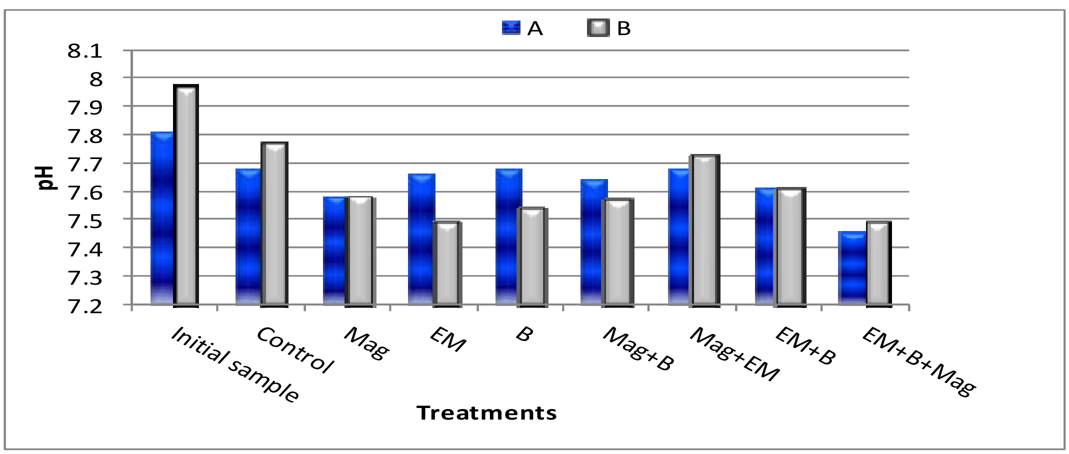

Fig. (4): Effect of soil amendments on soil pH in the two depths sample (A and B).

4. CONCLUSION

It could be concluded that B+EM, Mag+B+EM as well as magnetite are the best treatments where they led to increasing fruit yield, yield quality, root distribution, photosynthetic pigments and mineral status of Murcott mandarin leaves. Moreover, these treatments reduced proline concentration, the osmotic pressure of leaves, thus promoted mandarin plants to mitigate salinity stress.

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COMPETING INTERESTS

Authors have declared that no competing interests exist

AUTHORS' CONTRIBUTIONS

All authors collaborated to carry out this work and approved the final manuscript.

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