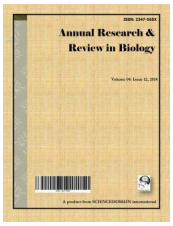

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Organic and Bio- fertilizers Improve Vegetative Characteristics and Nutrition Status of Young Pomegranate Trees (*Punica granatum* L.)

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Authers' contributions

This work was carried out in collaboration between all authors. Author OAA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors EAMA and EAAAEM managed the analysis of the study. Author EAAAEM managed the literature searches. All Authors read and approved the final manuscript.

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ABSTRACT

This investigation was conducted during 2014 & 2015 on pomegranates young trees cv. 116 grown in sandy soil, irrigated by drip irrigation, at the farm of National Research Center located at El-Nobaria District, El-Behaira Governorate. This study aimed to improve both vegetative characteristics and nutrition status of pomegranate by using different sources of fertilizers (organic and Bio-fertilizers). In this respect organic N and natural raw mineral rocky materials of PK mixtures were used at concentration of (500, 1000.1500 and 2000 g/plant) individual or in combination with bio- NPK mixture. Vegetative growth parameters (plant height, stem diameter, shoots length, no. of shoots/plant, no. of leaves/shoot and leaf area) as well as mineral composition (N, P, K, Fe, Mn and Zn content) and total chlorophyll were determined. Result showed that, mixing between organic and bio-fertilizers (organic N and PK raw mineral rocky materials at 2000 g plus NPK bio-fertilizations mixture at 300 ml/ plant) resulted in the greatest improvement of all the studied growth parameters. A promotive effect was noticed in term of mineral composition as well as total chlorophyll for pomegranates young trees treated with mixed organic (NPK at 2000 g/plant) and bio-fertilizers (NPK at 300 ml/ plant).

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Keywords: Bio and organic fertilizers; mineral composition; pomegranates and vegetative growth.

1. INTRODUCTION

Pomegranate (*Punica granatum* L.) is one of the important fruit crops of the world. Pomegranate orchards are now grown in many regions of the world, particularly in the Mediterranean Basin, where high quality fruits are obtained, It mainly belongs to semi-arid mild-temperate to subtropical climates, it considered one of the most valuable fruits for its nutritive, industrial and medicinal values [1]. Recent scientific findings corroborate traditional usage of the pomegranate as a medical remedy and indicate that pomegranate tissues of the fruit, flowers, bark, and leaves contain bioactive phytochemicals that are antimicrobial, reduce blood pressure, and act against serious diseases such as diabetes and cancer. These findings have led to a higher awareness of the public to the benefits of the pomegranate fruit, and consequently to a prominent increase in the consumption of its fruit and juice [2].

The increasing impacts on the environment due to agricultural practices in the world have gradually affected the quality of the soil in terms of structure and biological equilibrium, which has required the development of alternative practices to minimize and mitigate those impacts, parallel to the improvement on the yield per cultivated area and economical benefits for producers and farmers. Pomegranate plants require regular fertilization in order to assure their best performance in a commercial production system. Continuous use of chemical fertilization leads to the deterioration of soil characteristics and fertility and might lead to the accumulation of heavy metals in plant tissues, affecting the fruit nutritional value and edibility [3]. Use of various organic manures and fertilizers is a good practice to maintain physico-chemical and biological properties of the soil, improve aeration, and provide better scope for root growth and production. It is a sound practice for sustainable horticulture base on low chemical input. Organic fruit production methods are determined to a significant extent by the certification standards. Thus, the organic grower's toolbox is greatly restricted relative to what a conventional grower has. Organic production technology has gained momentum in recent years both in an increase consumer demand and a genuine desire of many fruit orchardists to sustain crop production and soil health. Moreover, organic produce commands and fetch a higher price than traditional commodity and thereby prompting producers to grow fruit crops organically [4].

Biological fertilization is based on the use of natural inputs including fertilizers, decaying remains of organic matter, excess crops, domestic sewage, animal manure, and microorganisms such as fungi and bacteria [5]. Recently, bio-fertilization is considered an important tool to maintain, improve soil quality and productivity levels at low input costs. They are known to improve fixation of nutrients in the rhizosphere, produce growth stimulants for plants, improve soil stability, provide biological control, biodegrade substances, recycle nutrients, promote mycorrhiza symbiosis [6]. Additionally, the use of bio-fertilizers can improve productivity per unit area in a relatively short time, consume smaller amounts of energy, mitigate contamination of soil and water, increase soil fertility, and promote antagonism and biological control of phytopathogenic organisms [7]. Many reporters studied the application of soil organic and biological fertilizers [8] on fruit crops, [9] on Pomegranate, [10] on Pomegranate, [11] on Pomegranate, [12] on banana, [13] on kiwi fruit, [14] on apple and [15] on Pomegranate.

This paper scoped on the improvement of vegetative growth and mineral composition of young pomegranate trees cv.116 using two different sources of organic and bio-fertilizers, specially, the huge application of chemical fertilizers for intensive crop cultivation which are not only in short supply but also expensive and resulted in pollution of the environment, soil and water too.

2. MATERIALS AND METHODS

2.1 Experimental Material

The present experiment was conducted during two successive seasons (2014 & 2015) on young pomegranate trees cv.116 (*Punica granatum* L.), one-year-old, planted at 3x5 meters apart in new reclaimed sandy soil under drip irrigation system using Nile river water at the Experimental Station Farm of National Research Center located at El-Nobaria district, El-Behara Governorate, Egypt. Alexandria Road, Egypt. Before starting 1st season (2014) physical and chemical analysis of orchard soil surface (0.40 cm. depth) were determined after the methods described by [16]

and [17] as shown in Table 1. The selected plants were uniform in vigor as possible.

2.2 Preparation of Fertilizers

Three NPK sources i.e., granulated organic N fertilizer and granulated natural raw mineral rocky materials for either P or K fertilizers were mixed together at an equal proportion (1:1:1by weight) for being used as a composite fertilizer mixture soil applied at four doses (500, 1000, 1500 and 2000 g/plant) either solely or in combined with three bio-fertilizers mixture, moreover three bio-fertilizers (Nitrobein, Phosphorene and Potassein) were also mixed together at equal proportions (1:1:1by volume) for being soil drench applied at the rate of 300 ml /plant either individual or in combination with the four investigated doses of the three alternative NPK sources.

All plants in this the experiment were subjected to the same horticultural practices. Soil fertilization treatments were applied three times at mid of Feb., March and April every treatment was divided to three equal doses to be applied during each season.

2.3 Fertilization Treatments

The selected young trees were subjected to ten treatments as in Table 2.

Table 1. Physical and chemical properties of the experimental soil

Properties	Value	Properties	Value
Clay %	5.00	P%	0.44
Silt %	5.00	K%	0.57
Sand%	90	Ca mg/L	2.65
Texture%	Sandy	Mg mg/L	2.40
PH	8.2	HCO_3 mg/L	3.85
EC	1.5	Cl mg/L	53
N%	Trace	SO_4 mg/L	55.65

Table 2. Fertilization treatments

Treatments	Treatments details
Cont.	Recommended NPK fertilization program adopted at 400,400 200 g/plant from $(NH4)_2SO_4$, superphosphate and K_2SO_4, respectively according to the Ministry of Agric.
T1	Natural NPK fertilizations mixture (organic N and PK raw mineral rocky materials) at 500 g / tree.
T2	Natural NPK fertilizations mixture (organic N and PK raw mineral rocky materials) at 1000 g / tree.
T3	Natural NPK fertilizations mixture (organic N and PK raw mineral rocky materials) at 1500 g /tree.
T4	Natural NPK fertilizations mixture (organic N and PK raw mineral rocky materials) at 2000 g /tree.
T5	Natural NPK fertilizations mixture (organic N and PK raw mineral rocky materials) at 500 g combined with NPK bio-fertilizations mixture at 300 ml / tree.
T6	Natural NPK fertilizations mixture (organic N and PK raw mineral rocky materials) at 1000 g combined with NPK bio-fertilizations mixture at 300 ml/tree.
T7	Natural NPK fertilizations mixture (organic N and PK raw mineral rocky materials) at 1500 g combined with NPK bio-fertilizations mixture at 300 ml/tree.
T8	Natural NPK fertilizations mixture (organic N and PK raw mineral rocky materials) at 2000 g combined with NPK bio-fertilizations mixture at 300 ml/ tree.
T9	NPK bio-fertilizations mixture alone at 300 ml/plant.

Common mineral NPK fertilization regime that was recommended by Ministry of Agriculture was used as control. Moreover, both organic and bio-alternate NPK fertilizers sources were used as follows:

A- Organic fertilization:

Granulated organic N fertilizer of 18-20% actual N*, Natural raw rocky materials as P fertilizer of 18-20 % actual P2O5*and Natural raw rocky materials as K fertilizer of 10-12% actual K2O*

*The organic fertilization was prepared, purified and salad by AlAhram Minning Company.

B- Bio- fertilization**:

Nitrobein: - is a commercial nitrogenous bio-fertilizer contain specialized bacterial strains for N fixation, phosphorene: is a commercial phosphorus bio-fertilizer containing some active bacterial strains which facilate P uptake through changing the insoluble tri-calcium phosphate (unavailable form) into available soluble one (mono- Calcium phosphate), Potassein: is a commercial potassium bio-fertilizer that facilitates potassium releasing from clay complex components or between their mineral platelets layers.

**Bio-fertilization was Prepared and marketing by Ministry of Agriculture.

Three NPK sources i.e., granulated organic N fertilizer and granulated natural raw mineral rocky materials for either P or K fertilizers were mixed together at an equal proportion (1:1:1by weight) for being used as a composite fertilizer mixture soil applied at four doses (500, 1000, 1500 and 2000 g/plant) either solely or in combined with three bio-fertilizers mixture, moreover three bio-fertilizers (Nitrobein, Phosphorene and Potassein) were also mixed together at equal proportions (1:1:1by volume) for being soil drench applied at the rate of 300 ml /plant either individual or in combination with the four investigated doses of the three alternative NPK sources.

All plants in this the experiment were subjected to the same horticultural practices. Soil fertilization treatments were applied three times at mid of Feb., March and April every treatment was divided to three equal doses to be applied during each season. The complete randomized block design with three replications was used. Evaluation of all treatments and their effect on both vegetative growth characteristics and nutritional status was done at late October.

1- Vegetative growth

At the end of growing season, during Sept. the selected shoots were measured to determine the following:

(Plant height, stem diameter at 10.0 cm above soil surface, average shoot length in cm.), (no. of leaves and no. of shoots). Whereas average leaf area was estimated according to the following formula by [18]: Leaf area =0.41(leaf length x its width) + 1.83

2- Nutritional status

At the end of July a sample leaves was randomly selected from the middle part of non-fruiting shoots of each replicate tree in both seasons. The selected leaves were washed with tap water, rinsed with distilled water and oven dried at 70°C to constant weight and then ground.Macro and Micronutrients (N, P, K, Fe, Mn and Zn) as well as total chlorophyll contents were determined as follows: -

a. Leaves total (N) was determined by the modified micro Keldahl method mentioned by [19]
b. Leaves total P, K, Fe, Mn and Zn were determined after [20] .
c. Leaf total chlorophyll contents were determined in fresh leaves by using Minolta meter SPAD-502.

2.4 Statistical Analysis

The complete randomized block design with three replications was used for different fertilization treatments and each replicate was represented by two plants. All data obtained during both seasons were subjected to analysis of variance according to [21] and significant differences among means were distinguishing according to the Duncan's, multiple test range [22].

3. RESULTS AND DISCUSSION

3.1. Vegetative Characteristics

3.1.1 Plant height Stem diameter and shoot length in (cm)

Results illustrated in Fig. 1 represent the effect of different sources of fertilization i.e. organic and bio fertilizers on vegetative growth characteristics

(plant height, stem diameter and shoot length) of young pomegranate trees cv.116 during two successive seasons. It was clear that, all fertilization treatment had a promotive effect on plant height, stem diameter and shoot length of young pomegranate trees cv.116.However, mixing both organic and bio-fertilization treatments as (organic N and PK raw mineral rocky materials at 2000 g plus NPK bio-fertilizations mixture at 300 ml. per tree) was a superior treatments as it scored (168.2 and 191.2) (59.83 and 60.40) and (2.07 and 2.65) for plant height, stem diameter and shoot length respectively, followed by T7 (organic N and PK raw mineral rocky materials at 1500 g plus NPK bio-fertilizations mixture at 300 ml. per tree) and T6 (organic N and PK raw mineral rocky materials at 1000 g plus NPK bio-fertilizations mixture at 300 ml. per tree) respectively during both seasons of study compared with control. Meanwhile, the other treatments were between range in this respect.

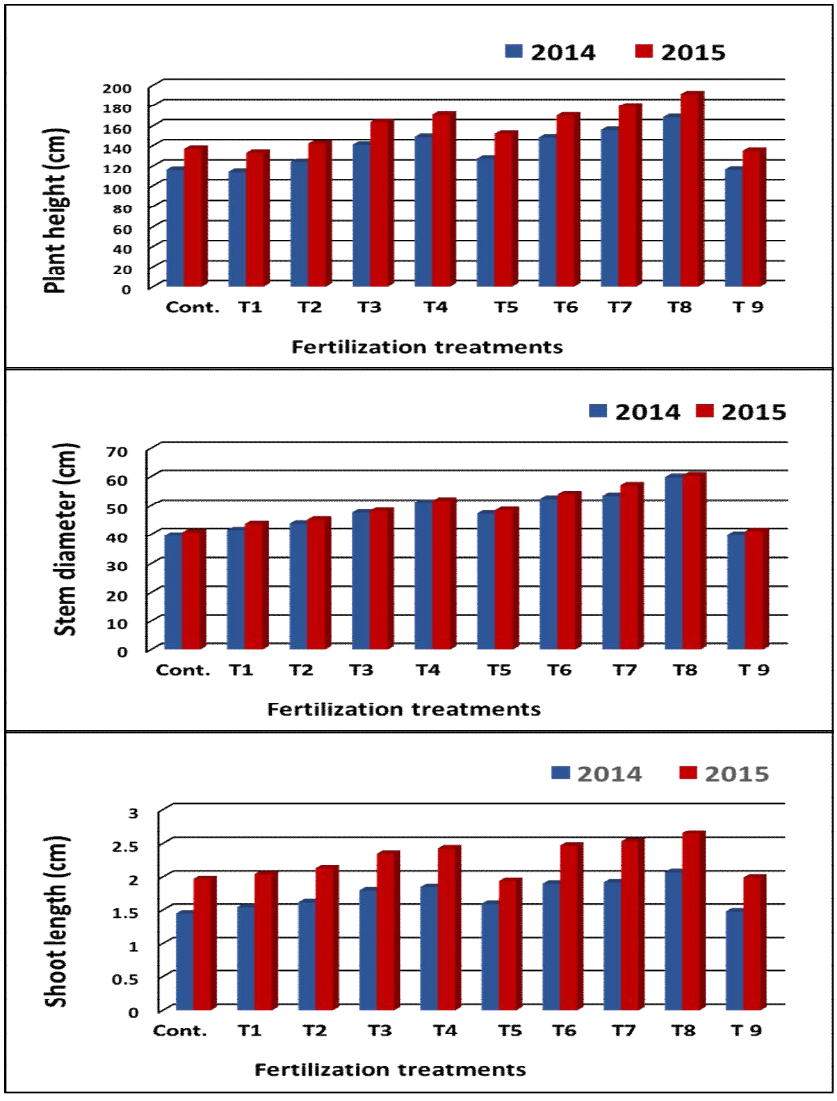

Fig. 1. The effect of different fertilization treatments on plant height (cm), stem diameter (cm) and shoot length (cm) of young pomegranate trees cv.116

Cont.=control(recommended NPK),T1=organic NPK at 500g/tree,T2=organic NPK at 1000g/tree,T3=organic NPK at1500g/tree,T4=organic NPK at 2000g/tree,T5=T1+NPK bio-fertilization at300ml/tree ,T6=T2+ NPK bio-fertilization at300ml/tree,T7=T3+ NPK bio-fertilization at300ml/tree,T8=T4+ NPK bio-fertilization at300ml/tree T9= NPK bio-fertilizations mixture alone at 300 ml/tree

3.1.1.1 No .of shoots/trees, no. of leaves/shoots and Leave area (cm^2)

Results presented in Fig. 2. showed that, how can mixing between two different sources of fertilizers (organic and bio) resulted in the highest significant no. of shoots/tree and no .of leaves /shoot. In this respect ,it was noticed that, T8 scored about four fold number of shoots/tree (20.00 and 24.67) compared with control (5.67 and 9.67) during both season of study, followed by T7 (14.67 and 19.00) and T6 (13.00 and 17.67) respectively during both seasons of study. Moreover, same trend was obtained for no of leaves/shoots as T8 surpassed other treatments scoring the greatest no. of leaves/shoots (55.00 and 57.33) followed by T7 (50.00 and 55.67) and T6 (48.33 and 51.67) respectively compared with control (33.33 and 36.33). As for the other treatments, they were in-between range. Statistical analysis showed that, there were no significant differences among all fertilization treatments during the first season in term of Leaf area (cm_2). On the other hand, during the second one, it can be noticed that, T8 recorded the greatest significant value of leaf area (5.32) followed by T7 (5.24) and T6 (5.14) compared with control since it was (4.29) respectively.

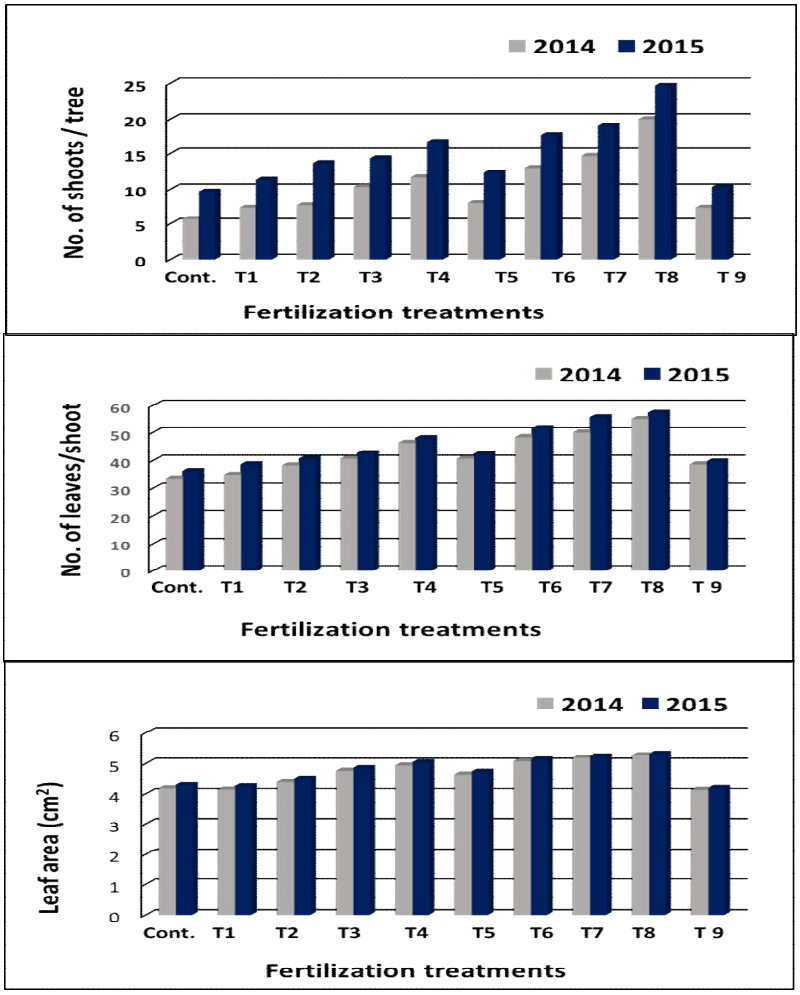

Fig. 2. The effect of different fertilization treatments on no. shoots, no. leaves and leaf area (cm^2) of young pomegranate trees cv.116

Cont.= control (recommended NPK), T1=organic NPK at 500 g/tree, T2=organic NPK at 1000 g/tree, T3=organic NPK at 1500 g/tree, T4=organic NPK at 2000 g/tree, T5=T1+NPK bio-fertilization at 300 ml/tree, T6=T2+ NPK bio-fertilization at 300 ml/tree, T7=T3+ NPK bio-fertilization at 300 ml/tree, T8=T4+ NPK bio-fertilization at 300 ml/tree, T9= NPK bio-fertilizations mixture alone at 300 ml/tree

3.2 Total Chlorophyll

Results presented in Fig. 3 showed the effect of different sources of (organic and bio) fertilizers on the total leaf chlorophyll content of pomegranates trees cv.116. It was cleared that, T8 treatment had a promotive effect on total leaf chlorophyll content since it was (52.14 and 53.59) compared with control treatment (43.40 and 44.14) during both seasons of study respectively.

Concerning the effect of using different sources of organic and bio fertilizers on vegetative characteristics and Chlorophyll content, it can be noticed that, application of organic and Bio-fertilizers resulted in significant increase in all vegetative characteristics. Our results go in line with those found by [23] on guava, [15] on Pomegranate, [12] on Grande Naine, [13] on kiwi fruit, [11] and [24] on pomegranate, [25] on Grapevine, [26] on Naval Orange, [9] on pomegranate and [27] on fig tree they reported that, combination between both organic and bio-fertilizers improved growth characteristics (Plant height, Stem diameter and shoot length in (cm), no. of shoots/trees, no. of leaves/shoot, Leaves area and total chlorophyll. Moreover, [23] reported that, bio-fertilizers encouraged better growth and accumulate optimum dry matter with induction of growth hormones, which stimulated cell division, cell elongation, activate the photosynthesis process, moreover, vigorous vegetative growth and increased chlorophyll content, which together activate the photosynthetic rate and thereby increased the supply of carbohydrates to plants which reflexed positively on vegetative characteristics; whereas the application of organic matter improves the soil health by improving physicochemical and biological activities of soil.

3.3 Macronutrient Contents

3.3.1 N content %

Results tabulated in Table 3 demonstrated that, during the first season of study, there were no significant differences among all fertilizers treatments concerning leaf N content %. On the contrary, it was cleared that, T8 surpassed other treatments in terms of N % (1.51) followed byT7 (1.46) respectively. Meanwhile, T9 treatment came at the last ranking in this respect (1.10).

3.3.2 P% and K %

Concerning leaf P% and K % results cleared that, during both seasons of study, the highest significant % of P and K content was obtained when pomegranates trees were treated with T8 followed by T7 (0.52, 0.57 and 0.48, 0.52) and (1.07, 1.16 and 1.03 and 1.14) for P and K % during both seasons of study respectively .On the other hand T9 treatment recorded the lowest significant value in this respect as it gave (0.21, 0.21) and (0.57, 0.63) for P and K % during both seasons of study respectively. Moreover, the other treatments were intermediate in this respect.

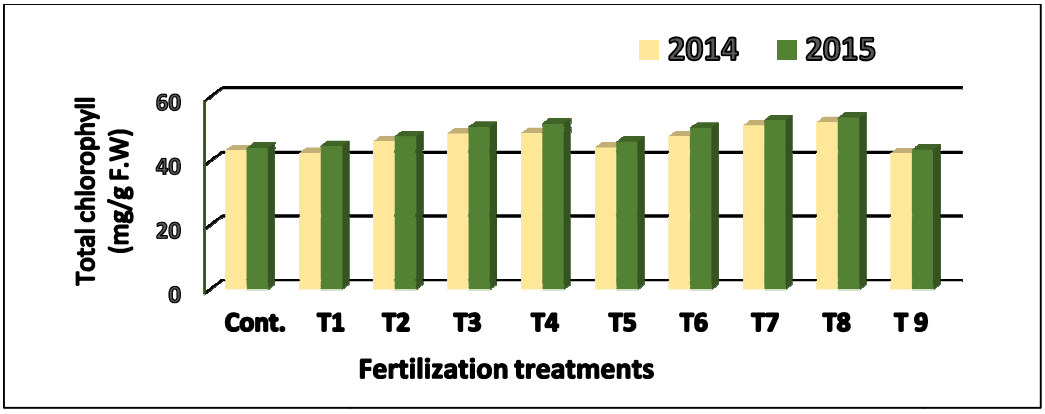

Fig. 3. The effect of different fertilization treatments on total chlorophyll (mg g^{-1} F.W) of young pomegranate trees cv.116

Cont.=control(recommended NPK), T1=organic NPK at 500 g/tree,T2=organic NPK at 1000 g/tree, T3=organic NPK at 1500 g/tree, T4=organic NPK at 2000 g/tree, T5=T1+NPK bio-fertilization at 300 ml/tree, T6=T2+ NPK bio-fertilization at 300 ml/tree, T7=T3+ NPK bio-fertilization at 300 ml/tree, T8=T4+ NPK bio-fertilization at 300 ml/tree, T9= NPK bio-fertilizations mixture alone at 300 ml/tree

Table 3. Effect of different sources of organic and bio- fertilizers on total chlorophyll (mg/g F.W) and macronutrient contents

Fertilization treatments	Macronutrient contents%					
	N%		P%		K%	
	2014	2015	2014	2015	2014	2015
Cont.	1.093 a	1.190 f	0.253 h	0.287 h	0.600 h	0.677 h
T1	1.077 a	1.150 g	0.300 g	0.313 g	0.637 g	0.693 gh
T2	1.257 a	1.297 e	0.330 f	0.347 f	0.760 f	0.710 g
T3	1.320 a	1.380 d	0.400 d	0.400 e	0.950 d	1.013 e
T4	1.387 a	1.410 c	0.443 c	0.470 d	0.903 e	0.987 f
T5	1.280 a	1.303 e	0.353 e	0.397 e	0.957 d	1.067 d
T6	1.360 a	1.397 cd	0.457 c	0.500 c	1.007 c	1.100 c
T7	1.430 a	1.467 b	0.480 b	0.523 b	1.033 b	1.140 b
T8	1.457 a	1.510 a	0.520 a	0.573 a	1.077 a	1.167 a
T9	1.050 a	1.107 h	0.217 i	0.217 i	0.577 i	0.623 i

Means having the same letter/s within each column aren't significantly different at 5% level

3.4 Micronutrient Contents

Result in Table 4 showed the effect of different sources of fertilization on micronutrient contents of pomegranate trees cv116. It was obvious that, there was a clear trend obtained among all treatments, as T8 resulted in the greatest content of micronutrient (102.5, 108.1), (80.22, 85.28) and (25.26, 31.14) for Fe, Mn and Zn respectively followed by T7 (100.3, 106.5), (73.93, 79.40) and (24.32, 29.09) respectively compared with T9 (79.52, 80.63), (58.22, 59.26) and (17.57, 19.34) and control treatment (80.14, 83.72), (55.46, 59.92) and (16.64, 18.67) that came at the last ranking in this respect during both seasons of study.

As for macro and micronutrient contents, our results are in harmony with those found by [23] on guava, [15] on Pomegranate, [12] on Grande Naine, [13] on kiwi fruit, [11] and [24] on pomegranate, [25] on Grapevine, [26] on Naval Orange, [9] on pomegranate and [27] on fig tree, they reported that, combination between both organic and bio-fertilizers improved mineral composition of many fruit crops. It was clear that, application of organic and Bio-fertilizers resulted in significant increase in macro and micronutrients. The beneficial role of supplemented organic manures and bio-fertilizers in improving soil physical, chemical and biological role is well known, which in turn helps in better nutrient absorption by plants with lesser cost but longer duration. Moreover, the total chlorophyll content and photosynthetic rate of leaves were positively correlated with leaf N content thus it reflected positively with encourage vegetative growth characteristics.

Table 4. Effect of different sources of organic and bio- fertilizers on micronutrient contents

Fertilization treatments	Micronutrient contents					
	Fe ppm		Mn ppm		Zn ppm	
	2014	2015	2014	2015	2014	2015
Cont.	80.14 i	83.72 h	55. 46 i	59.92 h	16.64 f	18.67 g
T1	83.79 h	88.73 g	59.12 g	63.37 g	17.52 e	19.46 f
T2	87.21 g	92.89 f	61.50 f	66.41 f	20.94 d	23.41 e
T3	93.60 e	96.50 e	65.31 d	68.55 e	23.17 c	25.94 d
T4	95.97 d	100.0 d	70.34 c	73.98 d	23.52 c	27.34 c
T5	88.54 f	93.78 f	62.50 e	69.21 e	20.76 d	23.15 e
T6	98.01 c	104.2 c	70.42 c	76.48 c	23.65 bc	27.70 c
T7	100.3 b	106.5 b	73.93 b	79.40 b	24.32 b	29.09 b
T8	102.5 a	108.1 a	80.22 a	85.28 a	25.96 a	31.14 a
T9	79.52 i	80.63 i	58.22 h	59.26 h	17.57 e	19.34 fg

Means having the same letter/s within each column aren't significantly different at 5% level

4. CONCLUSION

It can be recommended from the present study that, young pomegranate trees received organic N and PK raw mineral rocky materials at 2000 g plus NPK bio-fertilizations mixture at 300 ml. per tree (eighth treatment) resulted in the greatest improvement of vegetative characteristics (Plant height, stem diameter, average shoot length, number of leaves and number of shoots, leaf area and leaf total chlorophyll content as well as a noticeable significant increase was observed in leaf macro and micronutrients (N, P, K, Fe, Mn and Zn).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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