Ahmed Talib Joody /J. Pharm. Sci. & Res. Vol. 11(7), 2019, 2770-2773

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Effect of NPK and Organic Fertilizers on Increasing Medicinally Active Components and Limiting Heavy Metal Uptake in Pomegranate Trees

Ahmed Talib Joody
College of agriculture, University of Baghdad/Iraq

Abstract

The experiment was conducted in the pomegranate orchard of college of agriculture/ university of Baghdad on the first of April 2014 to study the effects of sewage sludge, sheep and poultry remnants and NPK on pomegranate fruit vitamin C levels, anthocyanin and nitrate as well as leaf heavy metal content, also the effect of animal remnants in decreasing heavy metal uptake from sewage sludge. A simple experiment was designed (RCBD) Randomized complete block design which included sapling fertilization with three organic fertilizers, Sewage sludge, Poultry and Sheep remnants using 8 kg. sapling $^{-1}$ for every fertilizer type, a chemical fertilizer NPK (50-50-100) g.sapling^{-1} was also used and the interaction between 50% chemical fertilizer and previously mentioned Organic fertilizers, as well as the interaction effect between Sewage fertilizer and both sheep and poultry remnants were included in this experiment. Main results revealed that experimental factors increased vitamin C levels, anthocyanin and decreased nitrate levels in fruits except NPK fertilizer which increased nitrate levels. Fertilization using sewage sludge ended up increasing heavy metal levels in leaves, as for sheep and poultry remnants both alone or in interaction with sewage sludge decreased heavy metal leaf content.

Keywords: Anthocyanin, V.C., Nitrate, Heavy Metal, Sewage Sludge, Animal Remnants, Pomogranate

INTRODUCTION:

Pomegranate a member of the Punicacea family originated in Iran and later on was brought to the Arabian Peninsula and later on taken to Spain and America by the Arabs. The importance of pomegranate returns to its nutritional value, chemical constituents and anti-oxidant compounds which gave this fruit a large role in the Pharmaceutical and food industry (Naser, 1996). Every 100 grams of sweet pomegranate fruit contains 18.7 g. of carbohydrates 13.8 g sugar, 4.0 g fiber, 2.1 g fat, 1.7 g protein, phenolic compounds, vitamins A, B, C, small amounts of Iron, phosphorous, Zinc, Potassium, Calcium, and Manganese, and is also a rich source of phenolic compounds such as anthocyanin, Tannins and Pectin (Langhout et. al., 1999; Lansky and Newman, 2007; USDA, 2010). Anthocyanin has a similar anti-oxidant effect as does vitamins E and C and may have an increased efficiency over them, as it ties down free radicals in the body, protects DNA and Lipids, improves eye sight, calms infections and protects blood vessels (Zuluaga, 2008).

Vitamin C is considered an important element in increasing immunity in the human body, provides protection against disease such as cancer, helps heal wounds, and protects the body from high cholesterol levels, heart diseases, and Arteriosclerosis etc.

The purpose behind fertilizing with chemical or organic fertilizers is to increase tree vegetative and root growth and as a result increase yield, and because of the negative effect of chemical fertilizers on the environment, human and plant health, a decrease in use of chemical fertilizers as well as the replacement of these fertilizers with organic fertilizer substitutes in many countries around the world, much research has also been done in this field. Neri et. al. (1998) referred to an increase in plant growth when organic fertilization was applied with decreased damage in plant tissue. Abd Al-Hadi (2005) obtained similar results of increased vegetative growth when pomegranate trees were fertilized with sewage sludge, poultry and camel remnants.

Nitrate as it is isn't considered carcinogenic but can be reduced to nitrite (a toxic form) which can react with amines in food or inside the human body to form nitrosamines which are strong carcinogenic compounds. The main source of Nitrate in vegetables is fertilization, Nitrate contamination in foods increase with the increase of agricultural crop fertilization.

Heavy metals are naturally occurring elements that have a high atomic weight and a density at least 5 times greater than that of water. Their multiple industrial, domestic, agricultural, medical and technological applications have led to their wide distribution in the environment; raising concerns over their potential effects on human health and the environment. Their toxicity depends on several factors including the dose, route of exposure, and chemical species, as well as the age, gender, genetics, and nutritional status of exposed individuals. Because of their high degree of toxicity, arsenic, cadmium, chromium, lead, and mercury rank among the priority metals that are of public health significance. These metallic elements are considered systemic toxicants that are known to induce multiple organ damage, even at lower levels of exposure. They are also classified as human carcinogens (known or probable) according to the U.S. Environmental Protection Agency, and the International Agency for Research on Cancer.

The contamination of the soil with toxic chemicals may occur as a result of unsuitable agricultural practices, irrigation using polluted water, addition of solid and liquid remnants and air pollutants (Zeid, 2001). Tissue analysis revealed the increase of heavy metal content in soil results in an increase of these elements in the plant as a result of absorption from the soil.

Given the above the aim of this research was to increase anthocyanin and Vitamin C, and decrease nitrate content, this research also aimed to minimize the use of chemical fertilizers and minimize chemical absorption by the plants found in the sewage sludge used by using Organic fertilizers which are considered low cost and considered safe to the environment and to our health, sewage sludge is obtainable at a low cost in Iraq only it contains high heavy metal contents.

MATERIALS AND METHODS

The research was conducted in the pomegranate orchard of college of agriculture/ university of Baghdad on the first of April 2014. A simple experiment was designed (RCBD) Randomized complete block design which included sapling fertilization with three organic fertilizers, Sewage sludge Table (1), Poultry Table (2) and Sheep Table (3) remnants using 8 kg. sapling $^{-1}$ for every fertilizer type, a chemical fertilizer NPK (50-50-100) g.sapling^{-1} was also used as recommended (Kessel, 2003) and the interaction between 50% chemical fertilizer and previously mentioned Organic fertilizers, as well as the interaction effect between Sewage fertilizer and both sheep and poultry remnants which makes 10 treatments in total with three replicates including two saplings for every experimental unit, studied samples were taken in mid-October and differences between means were compared using (LSD) least significant differences at a 5% test level (Al-Sahooky and Wahayib, 1990).

Treatments: 1- Control 2- NPK100% 3- Sewage 8kg.tree^{-1} 4- Poultry 8kg.tree^{-1} 5- Sheep 8kg.tree^{-1} 6- NPK50%+Sewage 7- NPK50%+Poultry 8- NPK 50% + Sheep 9- Poultry+Sewage 10- Sheep +Sewage .

Measured Parameters:

Vitamin C (mg. 100 ml^{-1} juice)

Pomegranate fruit Vitamin C levels were measured using 1 ml of concentrate, EDTA+ Oxalic acid was added and left over night. The solution was then filtered and +Acetic acid Metaphosphoric was added, 1 ml of concentrated sulfuric acid and 2 ml of Ammonium Molbydate were added and the volume was brought up to 25 ml using distilled water and was later measured using a spectrophotometer at a wavelength of 760 nanometer (Hussein et. al. 2010)

Anthocyanin:

10 fruits were taken from every experimental unit and squeezed to obtain 100 ml of the fruit concentrate which was filtered with filter paper and placed in brown colored glass jars and closed tightly. 5 ml of the filtered juice was then taken and placed in a 10 ml test tube and an extraction solution was added (ethanol 95% hydrochloric acid 1.5 m at a percentage of 15:85 for each respectively) the final solution was mixed well and placed in a centrifuge for 3 minutes at a speed of 3000 rotation per minute, the sedimentation was excluded and the remaining solution was brought up to 10 ml using the extraction solution, light absorbance was measured using a spectrophotometer at a wave length of 535 nanometers (Ranganna, 1986).

$$Anthocyanin\ \left(\frac{mg}{100\ ml\ juice}\right) = \frac{device\ reading\ \times solution\ volume}{sample\ volume\ \times 98.2} \times dilution\ \times 100$$

Nitrate in fruit:

Ten individual fruits were collected from every experimental unit, arils were extracted from fruits and nitrate levels were measured by what was mentioned by (Cataldo et.al., 1975).

Estimation of elements Lead (Pb) Cadmium (Cd) Cobalt (Co) and Nickle (Ni)

From each experimental unit twenty leaves were dried in an electrical oven at 65^{0}c until a stable weight was obtained, samples were grinded and 0.5 g were digested using sulfuric acid and hydrogen peroxide, Elements were then measured using an Atomic absorption spectrophotometer (Chapma and Partt, 1961).

Table 1. Heavy Metals in Sewage sludge

Element	Co	Ni	Cd	Pb
Concentration Mg.kg^{-1}	2.47	1.28	3.73	6.11

Table 2. Chemical characteristics of decomposed sheep remnants

Parameters	N g.kg-1	P g.kg^{-1}	K g.kg^{-1}	Organic carbon g.kg^{-1}	C/N	EC 1:5 ds .m^{-1}	Ph 1:5
Concentrations	22.3	1.77	2.22	252	18.77	7.9	7.22

Table 3. Chemical characteristics of decomposed Poulty remnants

Parameters	N g.kg-1	P g.kg^{-1}	K g.kg^{-1}	Organic carbon g.kg^{-1}	C/N	EC 1:5 ds .m^{-1}	Ph 1:5
Concentrations	26.7	14.8	16.4	273	10.34	4.05	7.33

Table 4. Effect of NPK and Organic Fertilizers on Vitamin C, Anthocyanin and Nitrate Levels in fruits

Characteristics / Treatments	V.C mg.100ml^{-1}	Anthocyanin mg.100ml^{-1}	NO3%
T1= Control	4.20	3.10	0.013
T2=100% NPK	5.10	4.56	0.044
T3= Sewage 8kg	4.56	3.73	0.030
T4= Poultry 8kg	4.86	4.26	0.033
T5= Sheep 8kg	4.70	4.00	0.015
T6=50% NPK + Sewage 8kg	5.00	4.30	0.035
T7= 50% NPK + Poultry 8kg	5.23	4.46	0.038
T8= 50% NPK + Sheep8kg	5.03	4.30	0.030
T9= Poultry8kg + Sewage8kg	4.83	4.16	0.032
T10= Sheep8kg + Sewage 8kg	4.80	3.96	0.024
LSD	0.48	0.38	0.004

Table 5. Effect of NPK and Organic fertilizers on Lead Cadmium Cobalt and Nickel in Pomegranate leaves (mg)

Characteristics / Treatments	Pb mg	Cd mg	Co mg	Ni mg
T1= Control	2.80	0.113	0.480	2.300
T2=100% NPK	2.87	0.200	0.503	2.533
T3= Sewage 8kg	14.33	1.900	3.400	6.467
T4= Poultry 8kg	2.50	0.113	0.387	1.733
T5= Sheep 8kg	2.37	0.100	0.347	1.733
T6=50% NPK + Sewage 8kg	13.33	0.193	3.033	6.033
T7= 50% NPK + Poultry 8kg	2.70	0.153	0.380	1.500
T8= 50% NPK + Sheep8kg	2.77	0.133	0.323	1.533
T9= Poultry8kg + Sewage8kg	7.13	1.067	1.767	3.433
T10= Sheep8kg + Sewage 8kg	6.73	0.867	1.600	3.233
LSD	2.73	0.225	0.461	0.785

RESULTS AND DISCUSSION:

Effect of NPK and Organic fertilizers on Vitamin C concentration, anthocyanin, and nitrate content in fruits

As noticed from table (4) all treatments differed significantly in vitamin C concentration from the comparison treatment which gave the least amount reaching 4.20 mg.100ml^{-1} except for the Sewage Sludge treatment which did not differ significantly from the comparison treatment, the highest level was obtained by the treatment 50% NPK + Poultry 8 kg reaching 5.23 mg.100 ml $^{-1}$ followed by the treatment NPK 100% recording 5.10 mg.100 ml $^{-1}$, as for the treatment 50% NPK + Sheep 8 kg recorded an average of 5.03 mg.100 ml $^{-1}$ as well as Sewage 8kg + NPK 50% which recorded an average of 5.00 mg.100 ml $^{-1}$.

Also noticed from table (4) all treatments differed significantly in anthocyanin levels from the comparison treatment which gave the least average reaching 3.10 mg.100 ml $^{-1}$, The highest average recorded was by the treatment 100% NPK reaching 4.56 mg.100 ml $^{-1}$ followed by the treatment 50% NPK +Poultry 8 Kg recording the average of 4.46 mg.100 ml $^{-1}$ As for the treatments both 50% NPK + Sheep 8 kg and 50% NPK + Sewage 8 Kg gave an average anthocyanin level of 4.30 mg.100 ml $^{-1}$.

As for treatment effects on Nitrate levels in fruits as can be noted from table (4) that some treatments greatly increased Nitrate levels and others not so much, As the treatment NPK 100% gave the highest nitrate percentage reaching 0.044% as for the treatments 50% NPK +Sewage 8kg , 50% NPK + Poultry 8 kg and the treatment 50% NPK + Sheep 8 kg recoring 0.035%, 0.038% and 0.030% respectively decreased nitrate levels in comparison to 100% NPK treatment, as for the least Nitrate percentage recorded was by the comparison treatment 0.0135% as well as Sheep 8 Kg treatment reaching 0.015 % which did not differ from the comparison treatment significantly.

Effect of NPK and Organic acids on Lead Cadmium Cobalt and Nickel in leaves

Table (5) reveals that the highest Pb levels were recorded by Sewage 8 Kg treatments reaching 14.33 mg as for the least level which was recorded by the treatment Sheep 8 Kg reaching 2.37 mg as for the comparison treatment which gave a level of 2.80 mg. What was also noticed the interaction between Poultry or Sheep remnants with Sewage sludge greatly decreased Lead levels in leaves reaching 7.13 mg and 6.73 mg respectively. As for Cadmium Table (5) reveals the highest level was recorded by the treatment Sewage 8 Kg reaching 1.9 mg, the lowest concentration was recorded by Sheep 8 kg reaching 0.100 mg, Poultry 8 Kg gave a level of 0.113 mg as well as the comparison treatment which gave a level of 0.113 mg also. What can also be noticed the interaction between Poultry or Sheep remnants with Sewage sludge greatly decreased Cadmium Cd levels in leaves reaching 1.067mg and 0.867 mg respectively.

Treatments also had an effect on Cobalt levels recorded in table (5), the highest level was recorded by Sewage 8 Kg reaching 3.400 mg as for the lowest level which was

recorded by 50% NPK + Sheep 8Kg reaching 0.323 mg followed by Sheep 8 Kg reaching 0.347 mg, the comparison treatment recorded 0.480 mg, What can also be noticed the interaction between Poultry or Sheep remnants with Sewage sludge greatly decreased Cobalt levels in leaves reaching 1.767 mg and 1.600 mg respectively. Results from table (5) also point out that treatments also effected Nickel content in leaves where the highest content was recorded by Sewage 8 Kg reaching 6.467 mg as for the lowest content recorded by 50% NPK + Poultry 8kg reaching 1.500 mg followed by 50% NPK + Sheep 8kg reaching 1.533 mg, the comparison treatment recorded 2.300 mg, as for treatments Poultry 8 Kg and Sheep 8 Kg which gave the same level reaching 1.733 mg, the interaction between Poultry or Sheep remnants and Sewage sludge largely reduced Nickel content reaching 3.433 mg and 3.233 mg respectively.

CONCLUSION

It can be concluded from this research that NPK and organic fertilizers increased anthocyanin and Vitamin C and decreased Nitrate in fruits with an exception of NPK which increased Nitrate as for treatments involving both 50% NPK and Organic fertilizers which gave results similar to that given by 100% NPK except for a lower cost and is safer on the environment, while Sheep and Poultry decomposed remnants which minimized the uptake of heavy metals found in Sewage sludge, which gives the possibility to use low cost beneficial Sewage remnants when mixed with Sheep and Poultry remnants.

REFERENCE

1. Abd AL-Hadi,S and Abd AL-Adeem .2005. Production of Pomegranate under organic and bio fertilized systems .
2. Abdu, N. 2010. Availability, transfer and balances of heavy metals in urban agriculture of West Africakassel university press GmbH.
3. Ajaikumar, K.B, I .Asheef ,.A. Babu and J. Padikkafa .2005.The inhibition of gastric mucosal in fury by Pomegranate .methanolic extract .J .Ethnopharmacol 96:171-176.
4. Al jumaily,A.A and M.O.Salom.2012.Effect of spray by humic ,potassium on growth and yield of potato under drip irrigation system.Journal of dyalla for agriculture science 4(1) 205-219 .
5. Al Sahooki, M and K .M.Wahaib.1990.Application in design and analysis experemintal .Ministry of higher education and scientific research.Iraq.
6. Al-Obaidi,A.J.H.2008. Response of apricot trees *prunus armeniaca* L .C.v. Zaini to organic and mineral fertilization. A Thesis. College of Agriculture,Uneversity of Baghdad .
7. and water". Univ. of Calif. Div. Agric. Sci.
8. Ashraf, M., M. Maah and I. Yusoff. 2012. Chemical speciation and potential mobility of heavy metals in the soil of former tin mining catchment. The Scientific World Journal 2012.
9. Cataldo, D.A; M.Haroon ; L.E. Schrader and V.L. Youngs.1975.Determinatin nitrate in plant tissues by nitrogen of salisylic acid Communication in Soil Science and Plant Analysis .6:71-80 .
10. Chapman, H. D. and Partt, P. F.1961. "Methods of analysis for soil, plant.
11. Dolgen, D., M.N. Alpaslan and N. Delen. 2007. Agricultural recycling of treatment-plant sludge: A case study for a vegetable-processing factory. Journal of Environmental Management 84: 274-281.
12. Eman, A.A.A ; M.M.S.Saleh and E.A.M.Mostafa (2008). Minimizing the quantity of mineral nitrogen fertilizers on grapevine by using humic acid , organic and biofertilizers.Research Journal of Agriculture and Biological Sciencs.4(1): 46-50.
13. Farage, S.G.(2006). Minimizing mineral fertilizers in grapevine farms to reduce the chemical residuals in grapes.M.Sc.Thesis, Institue of Environmental Studies&Research, Ain Shams University,Egypt.pp :67 .
14. Fawzi, M.I.F ; F.M.Shahin ; A.D.Elham and E.A.Kandil (2010). Effect of organic and biofertilizers and magnesium sulphate on growth yield,chemical composition and fruit quality of Lecont Pear trees .8 (12):273-280 .
15. Haggag, M. N. and H. A. EL – shany. 1987. Response of fig and Pomegranate fruit tress to NPK Fertilization . Alex. J. Agric. Res. 32 (3) : 199 – 208 .
16. Higazi, E.S ; M. R. Al-sonbaty; M. A. Eissa; D. M. Ahmed and T. F. El-Sharony (2007). Efeect of organic and bio-fertilization on vegetative growth and flowering of picual olive trees. World Journal of Agricultural Sciences 3(2) : 210-217 .
17. Hora, J.J.; E.R. Maydem, E.P. Lansky and C. Dwivedi . 2003 . Chemo- preventive effect of pomegranate seed oil on skin tumor development in CDI mice . J. Med. Food, 6 : 157 – 161 .
18. Hussein, I . L . Khan , M. Khan , S . Ayaz and F .U . Khan .2010. UV Spectrophotometric analysis profile of Ascorbic acid in medicinal plant of Pakistan . World Appi .Sci .J. 9(7) :800-803.
19. Kassem, H. A. and H. A. Marzouk (2002). Effect of organic and mineral nitrogen fertilization on the nutritional staus, yield and fruit quality of flameseedless grapevines grown in calcareous soils. J. Adv. Res, 7(3):117-126 .
20. Kessel , Christoph .2003. Fertilizing Stone fruit (Peach , Plum , Nectarines , Apricot , Cherries) and pears . Horticulture crop nutrition. Ministry of Agriculture , Food and Rural Affairs. Ontario -Canada.
21. Langhout , D .,T .B Schutt ., P. Vanleeliwen ., T .Wiebenga ., and S . Tamminga .1999.Effect of dietary high – and low methylated citrus pectin of the activity of for ilealmicroflora and morphology of the intestinal wall of broiler chicks .T .N . O .Nutrition and food research institute .Wageningen, the Netherlands .Pages 340 – 347 .
22. Lansky, E.P and R.A. Newman.2007.pomegranate (*Punica granatum*)and its potential for prevention and treatment of inflaming animation and cancer .J Ethnopharmacol 2007; 109:177-206.
23. Naser,E .1996. Determining Multiple Phenol amounts in Pomogranate peels. Journal of Agriculture and Food Chemistry. International academy of Press and Publishing. Beirut. Lebanon.
24. Neri, D.; G.Bonanomi; E. Cozzolino and F. Zucconi (1998). Study sugli apporti di sostanza organic net fragoleto. Frutiicalturea. 5: 47-54.
25. Ranganna, S. 1986. Hand Book of Analysis and Quality Control for Fruit and Vegetable Products. Tata McGraw-Hill Publishing Co. New Delhi.
26. Rettke , M . A.; T. R. Pitt ; N. A. Maier and J. A. Jones .(2006). Growth and Yield responses of apricot (CV . Moorpark) to soil-applied nitrogen . Australian journal of Experimental Agriculture.
27. Roccuzzo, G.; S. Fabroni; M.Allegra.; B. Torrisi; F. Camin.; P. Rapisarda; S. Canali and F. Intrigliolo (2010). Effect of organic fertilization on Valencia late orange bearing trees. Lisbona-Book of Abstract. 2 :639-640.
28. Sherene, T. "Mobility and transport of heavy metals in polluted soil environment." *Biological Forum—An International Journal*. Vol. 2. No. 2. 2010.
29. Tiruneh, A.T., A.O. Fadiran and J.S. Mtshali. 2014. Evaluation of the risk of heavy metals in sewage sludge intended for agricultural application in Swaziland. International Journal of Environmental Sciences 5: 197-216.
30. USDA,. 2010. Pomegranates, Raw. United States Department of Agriculture. http://www.nal.usda.gov. [Accessed September 2010].
31. Zeid, I. M. Biologia. Plantarum., 44(1) : 111-115., (2001).
32. Zuluaga, D.L. etal.2008.Arabidopsisthaliana MYB75/PAP1transcription factor induces anthocyanin production in transgenic tomato plants. Funct. Plant Biol. 35, 606–618.