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The Impact of Integrated Plant Nutrition Management System on Some Morphological Characteristics and Nutrient Uptake in Citrus Seedlings

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Abstract— **Citrus is considered as one of the most important agricultural products in the world; that increasing its yield and product has attracted global attraction. The present researchhas been designed in the Randomize Completely Design inthe factorial plan in four replications. The main factor was includedsubstrate enriched (PGPR bio fertilizers) and without enriched substrate. Subplot was conventional 5 treatments: 1) control (without chemical fertilizers and phosphate bio fertilizer), 2) seedlings' root inoculated with Pseudomonas putida 41; 3) phosphate fertilizer based on soil test; 4) phosphate chemical fertilizer, 25% less than soil test + Pseudomonas putida 41 and 5) phosphate chemical fertilizer, 50% less than soil test + Pseudomonas putida 41 in 4 replicates .Overall 40 pots have been tested. The results analysis with MSTATC software using Duncan test showed that wet and dry weight of root and shoot and the P, Fe, Zn concentrations and the lateral branches number increased in substrate enriched in compared with control. The growth parameters include; rootstock length, scion length, rootstock diameter and scion diameter increased in different treatments of pseudomonas and phosphorus fertilizer.**

***Index Terms*—Bio Fertilizer, Citrus, Organic matter, Substrate.**

I. INTRODUCTION

Citrus is a large group which included orange, tangerine, lemon and grapefruit. Citrus production in various regions of the world and produce high levels of this fruit means that the product has made in the world of great economic importance. Today in global business, citrus fruits the second largest industry. The fruit is producing in tropical and subtropical climates [4].

Soil of these areas is calcareous and salinity is less. pH is over 7 and T.N.V is more than 10. Thus selective and use of appropriate substrate is important factor for high production. In these regions were used unbalanced fertilizers so we see the accumulation of phosphorus and the vegetative and reproductive balance disturbance [3]. Fe concentration is high in leaves but because of abundant bicarbonate in irrigation water, mainly this element inactive and deposits in the vessels and results of these abnormalities are decrease of the quantity and quality citrus fruit. Attention to planting substrate aimed to healthy and productive of orchid plants .

Integrated management is a novel approach for optimal management of soil fertility and plant nutrition and in this regard, FAO has done extensive research in many countries as Thailand, Pakistan, India, Zimbabwe and ... and proportionate with soil properties , and climatic and social conditions of the mentioned regions, it has presented comprehensive models for sustainable soils management in order to raise yield at unit level and produce healthy and nutritious product. In this management method, the organic and inorganic sources of nutrient elements have been applied to promote soil fertility level and supply the plant required elements in a balanced form to produce healthy product with optimal quality .Then it is essential to upgrade production and yield and plant trees in a suitable substrate. In different research showed that application of Organic and Bio fertilizers are very effective in fruit production. Maksoud and et al reported that Using of different compost levels contain of urban, wastewater, and manure in a calcareous soil increases yield, enhances vegetative and raises olive oil weight, and when these treatments have been done with PGPR, the plant growth has built up [11].He reported that due to the enriched substrate synergistic useful effects and PGPR has caused of increasing of plant growth [11].

Phosphorus is the essential element that absorbed as phosphate. The combination of P with the elements available in soil, limited P absorption in to the root, and then even when P concentration is high, maybe it isn't accessible for the plant. For adjusting with the conditions of such plants, various methods are applied for soil P desorption and adsorption.

Phosphate plays role in the groups' photosynthesis and activities regulation and through this way, it boosts the plant growth [2].Through a research, Mani and Prakash performed in 1964 on sour orange seedling in nutrient solution, they observed that via removing P from the solution, first a major part of the root rots and then the seedling growth declines [22].

Chapman and Broman (1941) studied P deficiency in Tamson Navel plantedin 2-8 liter pots with sandy loam sand calcareous soil. They found that the first characteristic of malnutrition isdeficiency symptom sand abnormal leaf falling of three years. In a number of the leaves, blight has been created and some other ones have turned into tan dark green and the leaves have mainly been smaller than the normal size. The trees' fruits suffering from P deficiency have been smaller and dehydrated [22].

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In Palestine, Bar-Akiva et al. (1986), studying on grapefruit trees and reported that if these trees suffer from P deficiency ,they have unhealthy and dull appearance and their leaves lose their transparency ,the leaves size get smaller, too [22].

At the time being, bio fertilizers are discussed as alternative option or supplement for chemical fertilizers in order to raise soil fertility in sustainable agricultural products [24], Caob et al., 2005; Poudel et al., 2002; Estiken et al., 2010, Sherma, 2002).

Plant Growth Promting Bacteria (PGPB) usually exist in many environments .The widest studied group of PGPB that they are making colony at the root surface and strongly adheres to the Rhizosphere (Kloepper et al., 1999). And they help better root system development and seed germination [15].

Glick et al. (1995) announced that some evidence indicate the plant nutrients availability increase in rhizosphere due to growth stimulating rhizosphere bacteria activity. The action mechanism in this case includes the nutrients solubility or chelating substances production like siderophores and organic acids. The production of organic acids such as gluconic results in the environment surrounding the root acidic and P solubilizing, other mechanisms suggested for P solubility are chelating substance production, inorganic acids production such as solphoric acid, nitric acid and carbonic acid by soil microorganisms. In a research by Farzana and Radizah (2005) on the effect of rhizospheric bacteria on potato yield, they've reported a meaningful increase in the shoot and root dry weight in the inoculated plants with bacteria. The nutrient elements uptake as N, K and P in the inoculated plants with Rhizospheric bacteria showed significant rise compared to the non-inoculated plants.

Karlidiag et al (2007) reported that apple root inoculation with PGPR strains increased K, P, N, Fe, Mn and Zn, the main reason of which is the build-up organic acids by the bacteria and the plant in rhizosphere as a result of which rhizospheric soil pH has decreased and these elements accessibility has improved (Karlidiag et al., 2007, [23].

The Integrated Nutrient Management (INM) is a proper strategy for simultaneous use of biologic and chemical fertilizers and organic material having high efficiency in the product increase. The Integrated Plant Nutrition Management (IPNM) simultaneously applies all mentioned resources for maintaining soil fertility and promoting its producing potential, which is ecologically compatible, socially acceptable and economically justifiable. The most important objectives of this kind of management is improve of efficiency of fertilizer use, reasonable capital return increase and enjoying a balanced system for optimal consumption management.

Chemical fertilizers and biofertilizres combined with the organic fertilizers enrichment plant rhizospheric surrounding, favorite physical and biological properties and increase elements use potential for the plants, that one of its remarkable results in healthy and high quality production (Tandon, 1992).Using novel management methods, leading to healthy product production in citrus gardens, is developing.

In other countries, lots of studies have been carried out about producing healthy product based on IPNM. In California, the apple orchards production potential compared under three condition: orchardman tradition management practices, INM and organic productioults showed that increase of yield, nutritional balance, soil quality and fruit quality after harvest have been studied , under IPNM treatment implying the significance sustainability and apple product quality and quantity promotion Karlidiag et al. (2007).

Regarding the importance of Tamson as a strategic and economic fruit, producing a seedling with better and stronger growth can be productive tree with better yield and stronger against live and environmental stresses. On the other hand, unbalanced chemical fertilizers use and not concurrently studying bed and nutrition management in seedling production, it has been discovered essential to do a research to investigate the enriched beds' efficiency and phosphate fertilizersout of bio and chemical sources on the morphologic features and nutrients' adsorption and citrus seedling growth.

II. MATERIAL & METHODS

An experiment carried out on study the effects of enriched substrate, biofertilizres and phosphate fertilizers on seedling growth of Tamson and reducing the phosphorus fertilizer consumption. This pot experiment was done in 2014-2015 in one of the Sari orchards with location coordinates: East Longitude 36 ° 35$'$ 30.55$''$ and North Latitude 53° 1$'$ 38.13$''$ and Laboratory of Mazandaran Agricultural and Natural Resources Research Center. Plastic pots have been employed with ten of soil and treatments.

This experiment was performed as factorial in Randomaize Competly Design with 10 treatments in 4 replications. The main factor includes 2 levels: - enriched substrate and without enriched substrate and the sub factor involves 5 levels as: 1) control treatment (without chemical and phosphate biofertilizers); 2) seedlings'root were inoculated with *Pseudomonas putida* 41; 3) phosphate fertilizer based on soil test (8 gr Triple super phosphate fertilizer in each pot); 4) 25% less than soil test (6 gr Triple super phosphate in each pot) and seedling root inoculated with *Pseudomonas putida* 41; 5) 50% less than phosphorus soil test (4gr Triple super phosphate in each pot) and seedling root inoculated with *Pseudomonas putida* 41.

The enriched substrate (ANIMAC brand) was formulated with Organic Matter and the Plant Growth Promoting Microorganisms applies in this study. Amount of ANIMAC enreached substate was 20% of the pots weight. *Pseudomonas putida* 41 has been inoculated on seedlings root with 6×10^{8} CFU on 2 hours and then were transferred into the main pots.

Some morphologic properties as rootstock length and diameter, scion length and diameter and the number of the lateral branches were measured in 4 times almost almost every two month. The data analysis has been done by ANOVA by MSTATC 6.2 software. Mean comparison has been carried out based on Duncan test and correlation.

III. RESULTS AND DISCUSSION:

An experiment carried out on study the effects of enriched substrate, bio fertilizers and phosphate fertilizers on seedling growth nutrients uptake of Tamson. Morphologic properties were measured at four times (every two mount). Presented results were achieved from the difference between the measured parameters of the final stage and the first stage. Table 1 shows the morphologic parameter results.

A: Morphologic parameters as Rootstock length, Scion Length, Rootstock Diameter, Scion Diameter and Lateral Branches Number.

A. Rootstock length:

Based on the results obtained we can conclude that among the treatments studied there were significant differences in 5% level. The maximum rootstock growth has been seen in treatment (9) with 6.25 cm. For This treatment showed that according to Duncan test ($p \leq 0.05$) with others treatments (Table1).

B. Scion length:

At scion length index except for maximum rate of scion length (5) treatment with 59.5 cm placed at A level. For This treatment showed that according to Duncan test ($p \leq 0.05$) with others treatments, the rest of the treatments have been placed at level B. Second treatment (2) showed the minimum growth among them. At this index, better effect is clearly distinguishable related to simultaneously using *Pseudomonas* and using less Phosphorus (Table1).

C. Rootstock diameter:

Based on the results obtained we can conclude that among the treatments studied there were significant differences in 5% level among some treatments. Seven treatments placed at level A where (5) has had the maximum growth and treatment (3) have been placed at the lowest level (Table1).

D. Scion diameter:

Ninth treatment showed that according to Duncan test ($p \leq 0.05$) with some treatments and placed at the level A. At this index, better effect is clearly distinguishable related to simultaneously using *Pseudomonas* and using less Phosphorus (Table1).

E. Lateral Branches Number:

At lateral branches, tenth treatment has had the highest growth and fifth treatment (5) has revealed the minimum difference in the final stage measurement compared with the initial measurement (Table1).

Table1: Results of morphologic parameters (Date of final stage - first stage)

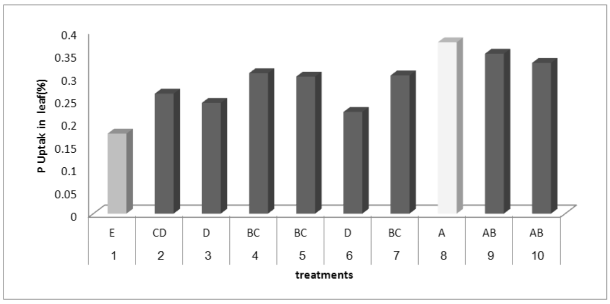
Number of the lateral branches	Scion (cm) diameter	Rootstock diameter (cm)	Scion length (cm)	Rootstock length (cm)	Sources	Substrate
14.75 BC	4.45 BC	4.8 A	33.75 B	3.5 C	Control(1)	Common substrate
19.25 ABC	4.7 BC	4.5 A	26.75 B	3.75 C	*Pseudomonads Putida* 41(2)	
19.25 ABC	4.125 C	3.125 B	36.5 B	3.5 C	Triple super phosphate(3)	
17.75 ABC	4.7 BC	4.225 AB	34.25 B	4 C	*Pseudomonas Putida* 41+ 25% less than of TSP(4)	
13 C	4 C	4.975 A	59.5 A	4.25 BC	*Pseudomonas Putida* 41+ 50% less than of TSP(5)	
23.5 A	3.625 C	4.45 A	40.25 B	3.25 C	Control(6)	Enriched substrate
18.75 ABC	4 C	4.65 A	30 B	3.5 C	*Pseudomonas Putida* 41(7)	
17.25 ABC	4.5 BC	3.85 AB	29.5 B	4 C	Triple super phosphate (8)	
20.5 AB	6.175 A	4.6 A	37 B	6.25 A	*Pseudomonas Putida* 41+ 25% less than of TSP (9)	
24 A	5.45AB	4.75 A	40.75 B	5.5 AB	*Pseudomonas Putida* 41+ 50% less than of TSP (10)	

Based on obtained results enriched substrate had positive effect on morphologic parameters. In this study we used ANIMAC Brand Organic matter as enriched substrate, bio fertilizers and chemical fertilizer as ssupplement. The treatments used in our experiment showed positive effect on growth and morphologic parameters of citrus seedling. However, they showed different in their potential to enriched substrate or soil and enhance plant growth. These results confirmed previous findings. Beneficial effects of inoculation with Plant Growth Promoting Rhizobaceria of many crop plants have been described by numerous authers [24]. Awad et al (1993), Reported also about incease of plant hight and morphologic parameters of various crop plants owing to microbial inoculation. Several authors have reported that plants have initiate developmental and biochemical adaptations to low and uneventlt distributed phosphate supply [18], [9], [8].

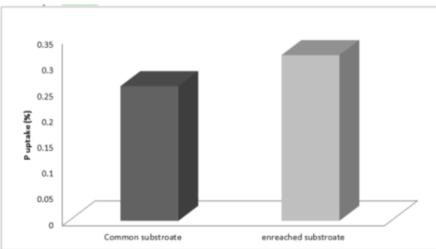
Developmental responses mostly involve changes in root architecture that enhance the root surface/soil volume ratio and, consequently, the ability of the plant to access soil phosphate. It includes increases in the root-to-shoot growth ratio, in the number of lateral roots and in the number and length of root hairs [25], [16], [19]. Also, when the root systems developed in a soil, where P ions were distributed in patches and lateral roots proliferated in areas with high concentration of P ions [20], [10].

Nutrients (P, Fe and Zn) uptake:

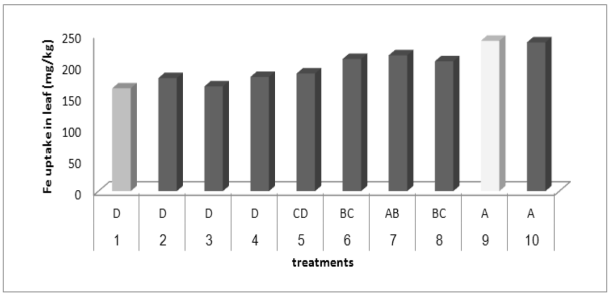
According to Duncan test ($P < 0.05$), P, Fe and Zn uptake was significantly affected in some treatments. Maximum quantities of P uptake in Eighth treatment as *Pseudomonas putida*41 + enriched substrate, Maximum Fe uptake was obtained from Ninth treatment and Maximum Zn uptake was showed in seventh treatment. However we had seen better effect in enriched substrate (charts 1, 2,3,4,5 and 6).



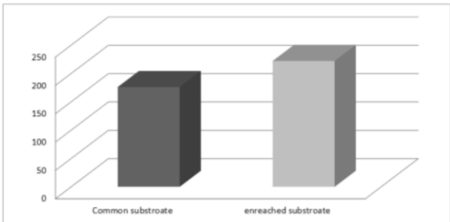
charts1: Results of P uptake in citrus plant (Interaction of enriched substrate and *Pseudomonas putida* 41 and chemical fertilizer)



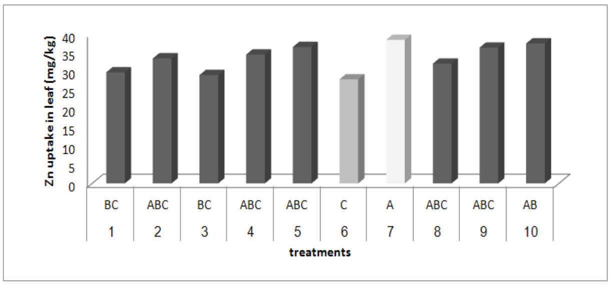
charts2: Results of P uptake in enriched substrate and common substrate



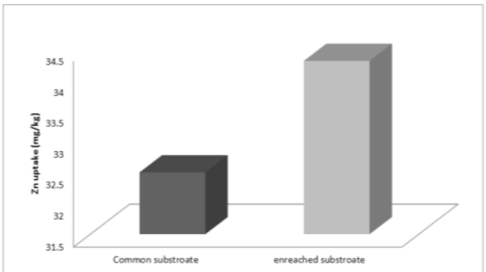
charts3: Results of Fe uptake in citrus plant (Interaction of enriched substrate and *Pseudomonas putida* 41 and chemical fertilizer)



charts4:Results of Fe uptake in enriched substrate and common substrate



charts5:Results of Zn uptake in citrus plant (Interaction of enriched substrate and *Pseudomonas putida* 41 and chemical fertilizer)



charts6:Results of Zn uptake in enriched substrate and common substrate

Biochemical responses serve two main functions: increasing the endogenous and soil P availability by the increase of P uptake capacity through the induction of high affinity P transporters, and increasing P mobilization and recycling activity through the Induction of soil-secreted and endogenous phosphatases and the increased release of organic acids and protons [18].

Datta et al (1982), reported that phosphate solubilizing and hormone-producing organisms increase P uptake and crop yields. They found that a phosphate solubilizing and indole3-acetic acid producing strain of *Bacillus firmus* increased the grain yield and P-uptake of rice in a P-deficient soil amended with rock phosphate.

Based on the observed results of our work and soil analysis, we conclude that INM system was able to improve P – deficient soil and increase P uptake by plant mainly due to the solubilization of phosphorus in soil. Although the exact mechanism(s) by which phosphate solubilizing rhizobacteria stimulate plant growth remains unclear, we consider that it does not diminish the potential use of these organisms as biofertilizers.

Influence of interaction between the pot soils inoculated with *Pseudomonas putida* 41 and enriched substrate on Fe and Zn uptake by plant of citrus. The results obtained have shown that according to Duncan test ($P < 0.05$) interaction of pot soils inoculated with *Pseudomonas putida* 41 and enriched substrate had statistically different significance for Fe and Zn uptakes (Chart 3,5) . Maximum rates of Fe and Zn uptake by plant observed during ninth treatment. Minimum rates of Fe and Zn uptake by plant observed for uninoculated soil (control).

The results presented showed that ANIMAC enriched substrate improve Fe and Zn uptake by plant and grain.

Environmental conditions of rhizosphere determine which pseudomonads species would predominate. Since the efficacy of fluorescent pseudomonads strains could be eliminated by concomitant addition of Fe^{3+} , plant growth-promoting fluorescent pseudomonads appear to exert their beneficial effects by producing under iron-limiting conditions extracellular siderophores what efficiently form complex with environmental iron.

The influence of microbial siderophores on plant iron nutrition depends on the ferric-cheating properties of the siderophores, as well as on the iron acquisition mechanism of the plant. Berker et al., (1985a) demonstrated negative effect of 10µM of the pyoverdin siderophore of *pseudomonads sp.* Strain B10 on iron nutrition in pea (*pisum sativum*). In contrast, the catechol siderophore of *agrbacterium tumefaciens* stimulated chlorophyll synthesis in pea [17], pseudobactin 385, the pyoverdin siderophore of *pseudomonas putida* strain WCS358, increased iron uptake and stimulated chorophyll synthesis in barley [6], but had differential effects on the carnation (*Dianthus caryophyllus*) Cultivars Lena and pallas [5].The difference was attributed to iron-deficient plants of cultivar lena producing more and longer root hairs than iron-deficient plants of cultivar pallas, and the ferric-reducing activity of cultivar Lena being higher than that of cultivar pallas.

In conclusion, INM system that was used had the better effect on improving shoot growth and other growth parameters of seedling citrus and nutrients uptake in plant. It results in increased nutrients uptake and plant growth, what will attract the attention for practical application.

IV. CONCLUSION:

Results were obtained showed that INM system (Application of less use of phosphorus fertilizer with pseudomonas putida 41 in ANIMAC brand Organic fertilizers as enriched substrate) to non INM systems had better effect on morphological parameters and nutrients uptake in plant. Than application of Integrate Nutrient Management System is a method for have a seedling health and finally increase of yield.

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