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Micronutrients Availability in Citrus with Trichoderma Application

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Abstract- An experiment on the citrus orchards was carried out to evaluate the effect of Trichoderma on the availability of micronutrients (Cu, Fe, Zn, Mn) in District Buner. For this purpose, three orchards were surveyed. Samples were collected from soil and plants before the application of Trichoderma in June 2015. The soil sample was collected from the depth of 0-30cm. The samples were analysed at ARI (N) Mingora Swat. The result showed that the entire orchards were adequate in macro and micronutrients expect nitrogen and manganese. The texture of the soil was sandy loam and almost all the orchards were deficient in organic matter. And the pH ranges from slightly acidic to slightly alkaline. In each orchard 9 plants were selected. Tree T_1 are control plant,.4 gram of Trichoderma in 2 litter were applied to plant T_2 T_5 T_8.and 8gram of Trichoderma in 4 litter were applied to plant T_3 T_6 T_9. After the interval of 7th to 14th days the result showed that the application of Trichoderma enhanced the availability of nitrogen (N) 27% and manganese 79%, respectively. Foliar application of iron chelate was also applied for the correction of iron deficiency in plants. 8gram of iron chelate was mixed in 10 litter water and applied to plants T_4, T_5, T_6 and 12gram of iron chelate in 10 litters was applied to plant T_7, T_8, T_9 in each orchard.

Keywords- *Trichoderma, micro-nutrients, orchard, foliar application, iron chelate*

I. INTRODUCTION

The District of Buner lies between 34-9 and 34-43 N latitude and 72-10 and 72-47 E longitude, bounded North by Swat district, West by Malakand Agency, South by Mardan District, East by River Indus and Hazara Division and North-East by Swabi District.172431 hectares of land is available for cultivation, but only 55457 hectares is actually cultivated (32%), while almost 9% is fallow land and remaining 40983 hectares of Buner's land comprises forest. This area is famous for production of fruits such as apricot, mulberry, fig, plum, wild persimmon, and orchard like pear, peach and citrus.

Citrus a major fruit of Pakistan is cultivated on 192.3 thousand hectares having 2.46million tons, annual production, with 12.78 tons per hectare average [1]. Sweet orange (*Citrus sinenesis* L.) is popular for its special taste [2] but unfortunately, citrus orchards of Buner are gradually deteriorating, because of diseases and other factors especially nutritional deficits that are necessary to be sufficed through balanced nutrients supply (manure and fertilizer application) to maintain health and production of orchards. Along with macronutrients (N, P and K), micronutrient deficits also affect the production and quality of citrus [3]. Nutrient deficits and toxicities impact health, vigour and productivity with symptomatic expressions on foliage, stems, roots and fruits.

Micronutrient availability is pH dependent as all micronutrients are available in acidic pH except molybdenum which is available in basic pH range. Iron is an important micronutrient and is present in appreciable amounts in soils but its deficiency is associated with calcareousness of soils causing non-lability, high P content, accumulated heavy metals, soil Cu, Zn and Mn deficits.

Zinc deficiency affects citrus growing in either acid or alkaline soils, but is usually more severe in alkaline soils. Excessive use of phosphate fertilizers can increase zinc deficiency. Copper deficiency is usually associated with large, dark green leaves on long soft angular shoots. Young shoots may develop into branches which appear curved or “S-shaped," referred to as “ammoniation” usually resulting from excessive nitrogen fertilization.

Bio fertilizers are substances that contain living microorganisms. Trichoderma is a fungi and usually present in most soil types and generally known as being the most established of the cultivable types of fungi; these are very fast growing fungi and are very active in the temperature range of 25-30°C.

Trichoderma is works as biofertilizer, biopesticide [4] and bioactivator depending upon nutrient availability, pH, temperature, light and iron content in soil.

Nutrients transfer from soil to roots by Trichoderma is through colonization of the interior of the roots, solubilisation of soil nutrients [5] and increased uptake of less soluble mineralization [6]. Present study was planned to investigate the effect of Trichoderma and foliar application of chelated iron on availability and uptake of micronutrients affecting yield of citrus.

II. MATERIALES AND METHODS

During the year 2015 the experimental trials were started on citrus orchard at Pir Baba Tehsil Gadazi district Buner. The trials were conducted in three different citrus orchards to assess the effect of Trichoderma on citrus and its physiochemical properties. 9 plants from each orchard (BusheniKalay, KalabutKalay and BataiKalay) were elected randomly and treated as given below.

TABLE I. TREATMENT PLAN

Tree	Trichoderma in	Water in	Iron-chelate in	Water in	Trichoderma and Iron-chelate per plant ratio
T_1	Control	-	-	-	(0-0)
T_2	4	2	-	-	(4-0)
T_3	8	4	-	-	(8-0)
T_4	-	-	8	10	(0-8)
T_5	4	2	8	10	(4-8)
T_6	8	4	8	10	(8-8)
T_7	-	-	12	10	(0-12)
T_8	4	2	12	10	(4-12)
T_9	8	4	12	10	(8-12)

Drench the soil around the tree with Trichoderma powder mixed in water with 2 feet distance away from tree trunk. Iron-chelate is mixed with various water levels in litter and applied to the plant leaves as a foliar application.

A. Sampling preparation and analysis

Soil samples were collected from 30 cm depth, ground sieved through 2 mm mesh sized sieve and analyzed for various soil properties and extractable micronutrients (Zn, Cu, Fe, Mn) and other properties are Soil pH [7], EC, Lime content [8], Organic matter [9]. AB-DTPA extract of soil for phosphorus and AB-DTPA extract of soil for potassium [10] and total nitrogen.

B. Analytical Procedures

1) Soil pH

Soil pH was determined in 1:5 soil water suspension follow 15 minutes stirring and read on pH meter (glass and calomel electrodes) [7].

2) Electrical conductivity

Total soluble salt was determined by measuring soil EC. Soil water suspension 1:5 was used to determine the EC of soil using the electrical conductivity meter [8].

3) Lime content

5 gram of soil was treated with 50ml of 0.5N HCL and back treated, in titrated with 0.025N NaOH, using phenolphthalein as indicator by acid- neutralization method [8].

4) Organic matter

One gram of soil was treated with 10mL of 1N K Cr_2O_7 rand added 20ml of concentrated H_2 SO_4. After cooling, first added 200ml of distilled water then filtrate in 500ml flask. Then add 2-3 drops of Ortho-phenolphthalein and titrate against 5 N Ferrous sulphate and note the reading each sample respectively. A blank titration was also run along with samples [9].

5) Soil texture

Soil texture was determined by the [11]. In brief 50 g air-dry soil was dispersed with 5ml 10% sodium hexametaphosphate solution in a mechanical dispersion machine for 5 min. After quantitative transfer of the suspension to a 1 litter bouyoucos cylinder. Filling the cylinder with distilled water to 1 litter mark. After through mixing carefully inserted a hydrometer in the suspension and took the hydrometer reading after 40secs for silt +clay and after 2 hr for clay. Also note temperature of the suspension with each hydrometer reading and necessary corrections in hydrometer readings. Percent silt and clay were calculated from hydrometer readings while % sand was calculated by difference, percent sand, silt and clay were used to determine soil textural class on the USDA soil textural Triangle.

6) Total Nitrogen

In this method block digester was used containing the digestion mixture composed of K_2SO_4 $CuSo_4$ and Se. 0.2 g of soil sample was treated with 3 ml concentrated H_2SO_4 in this block digester in the presence of digestion mixture [12]. Digestion mixture was done at gradual increase in temperature from 5O C^o to 350 C^o at the rate of 50 C^o increases in each step. It was then maintained for one hour. The sample became colorless or greenish at this stage. After cooling, this digested material was put in the 100 mL volumetric flask, distilled water was used to make the volume. Twenty mL of digest was distilled into 50 ml of boric acid mixed indicator solution in the presence of 5 mL of 40 % NaOH solution, the solution was titrated with slandered 0.005 M HCL.

$$\text{N}\ (\%) = \frac{(\text{mL sample} - \text{mL blank})\ \text{x N of HCL x meq N) x 100}}{\text{Wt of sample x volume distilled}}$$

III. AB-DTPA EXTRACTABLE PHOSPHOROUS

AB-DTPA extractable phosphorous concentration in soil samples will be determined by extracting it in soil solution as described by [10]. In this method, 10g of soil was shaken in 20 ml of AB-DTPA solution for 15 minutes in open conical flask. Suspension will be then filtered through Wattman 42 filter paper.

Then 2mL of filtrate will be added in 25 ml volumetric flask. Then 3ml of distilled water will be added. After it 5 mL of ascorbic acid mixed indicator will be added and volume will be adjusted up to 25 mL by adding distilled water. These 25 mL volumetric flask will be placed in dark in dark for development of blue color for 15 minutes. Absorption curve will be developed on spectrophotometer for 0,2,4,6,8, and 10ugP/ml standards which will be then used for calculation of AB-DTPA extractable P in samples.

AB-DTPA extractable P (mg/kg)

= concentration *ml of AB-DTPA/2*(Wt of soil)

A. AB-DTPA Extractable potassium

AB-DTPA extractable K will be extracted in solution by AB-DTPA determined by [10] and will be determined by flame photometer. Standard solution of K which will be 20.40.60.80.and 100 mg/l will be tested absorbance graph will be developed before analysis of sample. One blank will be also run in start on the machine in which sample will be absent and AB-DTPA extract will be only present.

Formula:

$$\text{AB} - \text{DTPA extractable K } \left(\text{mg} / \text{kg}^{-1}\right) = \frac{\left(\text{I.R} - \text{blank}\right) * \text{vol} * \text{D.F}}{\text{Factor} * \text{ wt of sample}}$$

B. Extractable micronutrients in soil

The concentration of extractable micronutrients (Zn, Cu, Fe, Mn) in soil was determined by the AB-DTPA extraction procedure [10]. In this method 10g soil sample was shaken with 20 ml AB-DTPA extract in an open Erlenmeyer flask for 15 min. After filtration the extract was read for Zn, Cu, Fe and Mn on an Atomic Absorption Spectrophotometer (Perkin Elmer Analyst-200 USA).

C. Plant sample analysis

Leaf will be washed with distilled water blotted with tissue paper and air dried in open air under shade. The sample was then oven dried at 70^0C for 48 hr to a constant weight. The leaves were then chopped with a stainless steel razor.

The wet digestion of leaves samples will be done using procedure [13] as described in [14], with minor modification. In brief 1-gram day leaf sample treated over night with 10 ml conc HNO_3 followed by treatment with 4.0 ml of pherchloric acid. After preliminary digestion with acids, placed the tubes in a cooled block digester and raised the temperature to 150^0C for 1 hour and then to 235^0C until dense white fumes of per chloric acid appeared in the tube. From this point, continued digestion for another 30 min, after cooling, the digest was being filtered.

IV. RESULTS AND DISCUSSION

A. Soil Texture

Soil texture refers to the size of the individual soil particles. Soil particles have different size, depending on the kind of parent rocks and degree of weathering. The particle size diameter ranges are categorized as sand (0.05-2.0 mm), silt (0.002-0.05mm), and clay (<0.002 mm). On the basis of the proportions of these fractions, the soils are divided into twelve textural classes.

TABLE II. TEXTURAL CLASSES OF THE SOIL

Orchard No	Location Name	Sand%	Silt%	Clay%	Textural Classes
O_1	Bushenikalay	76	14	10	Sandy loam
O_2	Kalabutkalay	66	20	14	Sandy loam
O_3	Bataikaly	80	8	12	Sandy loam

Soil analysis (Table II) show that all orchards O_1, O_2 and O_3 are sandy loam, so proper nutrients leaching occur during this condition and will need regular addition of organic matter and nutrients for maintaining soil fertility.

B. Soil pH

Soil pH is the unit of scale used to measure the level of acidity or alkalinity of chemicals is called Soil pH. The unit start to the number of H^+ ions in a solution and range from pH 0 (more acidic) to pH 14 (more alkaline) while pH 7 Neutral.

TABLE III. RESULT OF SOIL PH VALUES

Orchard No.	Location Name	Soil pH	Acidic/Alkalinity
O_1	Bushenikalay	6	Slightly acidic
O_2	Kalabutkalay	7.6	Slightly alkaline
O_3	Bataikalay	7	Neutral

C. Electrical conductivity

Electrical conductivity has come into use for measuring the amount of total salts in solution. It is determined by the conductivity meter. Electrical conductivity increases with increases salt concentration.

TABLE IV. ELECTRICAL CONDUCTIVITY OF SOIL

Orchard No.	Location Name	EC	Ranges	Category
O_1	Bushenikalay	2	<3,0	Slightly calcareous
O_2	Kalabutkalay	6	3-15	Moderately calcareous
O_3	Bataikalay	1.5	<3.0	Slightly calcareous

The result (Table IV) shows that orchard O_1 and O_3 are slightly calcareous and orchard O_2 is moderately calcareous.

D. Organic Matter

Soil organic matter includes all materials of either plant, animal or microbial origin produced in the soil or added to it regardless of their degree of decomposition. SOM is one of the major key factors to soil productivity. The amount of organic matter that may accumulate in a soil from plant tissue depends upon the temperature, moisture, aeration, soil reaction, and the amount and chemical nature of the plant tissues returning to the soil. Organic matter affects many soil properties directly or indirectly, supplies plant nutrients.

TABLE V. SOIL ORGANIC MATTER

Orchard No.	Location Name	OM%	Ranges	Category
O_1	Bushenikalay	0.276	< 0.86	Low
O_2	Kalabutkalay	0.414	< 0.86	Low
O_3	Bataikalay	1.104	0.86-1.29	Medium

Result (Table V) show that two orchards O_1 and O_2 are low in organic matter and orchard O_3 are medium amount of organic matter so its required organic matter.

E. Macronutrient in soil (AB-DTPA extractable N, P and K)

TABLE VI. RESULT OF MACRONUTRIENT IN SOIL (AB-DTPA EXTRACTABLE N, P AND K)

Orchard No.	Location Name	Nitrogen %	Category	AB-DTPA Extractable P mg Kg^{-1}	Category	AB-DTPA Extractable K mg Kg^{-1}	Category
O_1	BusheniKalay	0.0138	Low	7.3289	High	154	High
O_2	KalabutKalay	0.0207	Low	1.2763	Low	70	Medium
O_3	BataiKalay	0.0552	Low	11.605	High	80	Medium

From the result of macronutrient N, P and K in soil compare with the critical value with (Table VI). It is concluding that Nitrogen is deficient in all orchards O_1, O_2, O_3. While Phosphorous is deficient in orchard O_2 and high in O_1 and O_3 and potassium is high in O_1 and adequate in $O_2$and O_3.

F. Micronutrient in Soil (AB-DTPA extract Cu Fe Zn Mn)

TABLE VII. RESULT OF MICRONUTRIENT CONCENTRATION IN SOIL (UG G^{-1} SOIL) IN AB-DTPA EXTRACT (CU, FE, ZN, MN)

Orchard No.	Location Name	Cu	Category	Fe	Category	Zn	Category	Mn	Category
O_1	BusheniKalay	0.56	Marginal	58.5	Adequate	5	Adequate	4	Adequate
O_2	KalabutKalay	0.42	Marginal	18.0	Adequate	5.5	Adequate	3.84	Adequate
O_3	BataiKalay	0.72	Adequate	31.0	Adequate	7.7	Adequate	5.62	Adequate

The result (Table VII) shows that all the micronutrient (AB-DTPA extract Cu Fe Zn Mn) in soil are Adequate. By comparing the data with critical value (Table No 3.3) it is found that all the micronutrient is adequate in soil of orchard O_1, O_2 and O_3 while Cu are marginal in orchard O_1 and O_2.

G. Micronutrient in citrus Leaves (AB-DTPA extract Cu Fe Zn Mn)

TABLE VIII. RESULT OF MICRONUTRIENT CONCENTRATION IN CITRUS LEAVES (UG G^{-1} DM) IN AB-DTPA EXTRACT (CU, FE, ZN, MN)

Orchard No.	Location Name	Cu	Category	Fe	Category	Zn	Category	Mn	Category
O_1	BusheniKalay	10	Adequate	175	High	27	Adequate	9	Low
O_2	KalabutKalay	11	Adequate	194	High	45	Adequate	8	Low
O_3	BataiKalay	11	Adequate	210	High	32	Adequate	9	Low

By comparing the result of micronutrient (AB-DTPA extract Cu Fe Zn Mn) concentration in citrus leaves with critical value (Table VII) [15] adapted from [16] it is found that Cu and Zn were adequate in leaves of orchard O_1 ,O_2 and O_3 while Fe were High and Mn is low in all orchard O_1 , O_2 and O_3..

H. Trichoderma Results

As Trichoderma species considered as nutrient enhancing and improving the physical and chemical properties of soil. According to [5]. While [17] examined that the application of *Trichoderma harzianum* strain increased the availability of nitrogen (N), phosphorus (P) and potassium (K) by 27, 65 and 44%, respectively. Availability of some of the micronutrients viz., Cu, Fe, Mn and Zn were also enhanced, respectively by 6, 100, 79 and 66%.

I. Iron Chelates result

Folier application of iron chelate is done for the correction of the iron deficiency in citrus. After the analysis of the citrus plant leaves before the application of Iron chelates the plant were adequate in Fe.

TABLE IX. RESULT OF IRON CONCENTRATION IN CITRUS LEAVES (UG G^{-1} DM) AND SOIL (UG G^{-1} SOIL) BEFORE THE APPLICATION OF IRON CHELATE

Orchard No.	Location Name	Fe in leaves (ugg^{-1} DM)	Category	Fe in soil (ugg^{-1} soil)	Category
O_1	BusheniKalay	175	High	58.5	Adequate
O_2	KalabutKalay	194	High	18.0	Adequate
O_3	BataiKalay	210	High	31.0	Adequate

V. CONCLUSION

The following conclusions were drawn from the research work conducted.

- The soil samples collected from Orchard O_1, O_2 and O_3 had Sandy loam texture, low organic carbon, marginal fertility and adequate Cu, Fe and Zn contents.
- Soil of orchard O_1 is slightly acidic, Orchard O_2 is slightly alkaline and orchard O_3 are neutral in pH.
- Orchard O_1 and O_3 are slightly calcareous and orchard O_2 are moderately calcareous.
- Nitrogen and manganese availability is increased with the application of trichoderma by 27and 79% respectively.

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