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EDTA CHELATED MICRONUTRIENTS FOR GROWTH OF RICE, ONION AND LEMON PLANTS AND ENHANCING THEIR FRUIT YIELDS

MUHAMMAD ABDUL QADIR, MAHMOOD AHMED[*], NAVEED-UL-IHSAN, ASRAR AHMAD[a] and RABIA WASEEM[b]

Institute of Chemistry, University of the Punjab, LAHORE, PAKISTAN
[a]Division of Science and Technology, University of Education, LAHORE, PAKISTAN
[b]University of Malaya, MALAYSIA

ABSTRACT

The estimation of 70% increase in food production all over the world to meet the requirement of population level in 2050 is being slow as global agriculture output forecast for next decade lessened than past decade. In spite of hike in feed prices, world produces enough feeds for population requirement but food insecurity and hunger still persist due to economic crises. Global agricultural production need to step up for nourishing one billion people, which are estimated to be undernourished. The present work emphasizes on the use of micronutrients to over come their deficiency caused by repeated crops on the same Pakistani land. By using micronutrients, an extraordinary increase of 14-25% yield in rice, onion and lemon has been noticed.

Key words: Micronutrients, Soil, Plants.

INTRODUCTION

Carbon, hydrogen and oxygen are non-mineral nutrients. They are synthesized by the plants by using carbon dioxide and water. Nitrogen, Phosphorous, Potassium, Sulfur, Magnesium and Calcium are designated as macronutrients while Iron, Manganese, Zinc, Copper, Boron, Molybdenum, and Chloride are designated as micronutrients because they are also required in minute amount for plant growth. Deficiency of micronutrients is caused by growing repeated crops. Since repeated crops are frequently practiced in Pakistani soil, the present work was conducted to overcome these micro-nutrient deficiencies. Micronutrients play a critical role in activating enzymes for photosynthesis, lignin biosynthesis, carbohydrate synthesis and transportation. Similarly, synthesis of fatty acids

[*]Author for correspondence; E-mail: mahmoodresearchscholar@gmail.com 0092 300 8819844*

and proteins are also regularized through micronutrients. Deficiency of iron (Fe) in crops harvested in calcareous soil has been linked to higher concentration of carbonates and bicarbonates in soil[1]. Some scientists observed that the main symptom of Fe deficiency is chlorosis or yellowing between the veins of new leaves. Fe deficiency limits legume production on black calcareous soils in Asia[2]. Zinc (Zn), boron (B) and copper (Cu) improve the roots of rice[3], which affect the over all growth of the rice plant. Manganese complex with ethylenediammine tetra acetic acid (Mn-EDTA) was found much more effective than manganese sulphate in supplying Mn to the soybean plant[4]. Application of molybdenum (Mo) on soybean yields more 239-299 lb/acre than controlled crop. Uptake of Zn with Zn-EDTA application was superior to other sources[5].

Micronutrients deficiencies also constitute malnutrition which is responsible for more deaths than any other cause, accounting for more than twenty million mortalities annually affecting more than two billion peoples, with Fe, Zn and vitamin A deficiencies as the most prevalent nutritional problem[6]. Nearly five million child deaths happen due to micronutrient malnutrition every year. Leading global economists have identified investing in strategies to reduce malnutrition, as the most cost-effective investments that governments can make[7]. Malnutrition arising from dietary deficiency of critically important mineral micronutrients such as Fe and Zn is a serious problem affecting nearly half of the world's population[8]. The bio-fortification is desired as an intervention strategy to address micronutrient malnutrition by producing food crops with enhanced levels of bio-available essential micronutrients and vitamins that will have measurable impact on improving the micronutrient status of target populations[9]. The present paper is to study the effect of EDTA chelated micronutrients on growth of plants of onions, rice and lemon, which in turn enhanced the crop yields.

EXPERIMENTAL

Reagents

All the reagents were of analytical grade purchased from Merck, Germany while distilled water was prepared in our own laboratory using Milli-Q® direct water purification system.

Preparation of metal complexes

The determination of the complexes of Fe (III), Zn, Mg, Mn, Co and Cu by EDTA complexometric titrations are well reputed. In the present work, these complexes were made by using concentrated reactants and modifying the procedure to get pure crystals of these

complexes. 0.05 mol/L Fe (III) and 0.05 mol/L EDTA were prepared by taking 12.0 g of ferric ammonium sulphate and 9.3 g of EDTA-Na in 500 mL of distilled water, respectively. The EDTA solution was added slowly to Fe (III) solution with continuous stirring. The bright red color of Fe (III) was changed into dark red color due to the formation of iron complex with EDTA at a pH of 2-3. The water from the product was then evaporated and cooled to 3^{o}C for three hours. Crystals of iron complex were formed, which were then filtered, washed with acetone and dried on filter paper and stored. Zn, Mg and Mn form stable complexes at pH 10 with EDTA. 500 mL of 0.1 mol/L of zinc sulphate solution pH was adjusted at 10 by using NH_3/NH_4Cl buffer. 0.05 mol/L solution of EDTA was then added slowly to the above solution in order to obtain Zn-EDTA complex.

The water from the above mixture was then evaporated and cooled. Crystals of the complex were then removed, washed with acetone and packed. Mn-EDTA and Mg-EDTA complexes were also prepared by using same methodology as for Zn-EDTA by the reaction of their sulphates with EDTA. Co and Cu form stable complexes with EDTA at a pH of 6. The pH of the Co solution was adjusted with acetic acid while Cu solution of 0.1 mol/L itself attains pH = 6. 0.1 mol/L concentrations of Co from cobalt sulphate and Cu from copper sulphate at pH = 6.0 were treated with 0.1 mol/L EDTA separately and the products were purified by crystallization after washing with acetone. All these complexes have a general formula of Na_2 [Metal-EDTA].

Analysis of soil and water samples

Soil test was performed to estimate the nutrient supplying power of soil before the crop was planted. 15 samples were taken randomly and composite sample was prepared for final analysis. Samples from 15-25 cm, 15-30 cm and 60-100 cm depth were obtained from rice, onion and lemon field, respectively in a grid pattern separately by stainless steel soil auger and stored in plastic (HDPE) bags until analysis. 500 mL water sample from area located for plant cultivation was taken and stored in plastic bottles, which were soaked with concentrated hydrochloric acid overnight, then washed with distilled water. The water samples were preserved by adding 2 drops of concentrated nitric acid. All the soil samples were crushed and ground in wooden pestle mortar and sieved through 2 mm plastic sieve. A saturation paste of soil was made by taking 300 g dry mass in enameled cup and stayed overnight to ensure the saturation. Then the saturation extract was obtained. The pH and electrical conductivity (salinity) bicarbonates (HCO_3^-), carbonates (CO_3^{2-}) and chlorides (Cl^-) of soil was determined using saturated extract. The metals were determined by digesting the 1.0 g dried soil in 4 mL in aqua regia[11,12].

Instrumentation

The soil and water pH and electrical conductivity (ECe) was determined by Orion 5 star multi-meter (Thermo scientific, UK); sodium (Na) and potassium (K) were analyzed by Flame photometer (Sherwood, UK); essential metals like calcium (Ca), magnesium (Mg) and trace metals (micronutrients) like zinc (Zn), iron (Fe), copper (Cu), cobalt (Co) and manganese (Mn) were estimated by atomic absorption spectrophotometer (PG-990, PG-Instruments, UK) and HCO_3^-, CO_3^{2-} and Cl^- were determined titrimetrically. The elemental analysis-carbon (C), nitrogen (N) and hydrogen (H)) of prepared complexes were performed by Flash HT Plus elemental analyzer, Thermo, UK.

Application of Micronutrients to Crops

In order to test the effects of micronutrients on plant growth, two plots of 90 x 90 feet were selected for each of the plant species. One plot was designated as control, while the other as sample. After 30 days of sowing the crop, the chelated micronutrients were applied to the crops. Foliar application was chosen for these experiments. These formulations were dissolved in 500 mL of water. 125 mL of each formulation was further diluted in eighteen liter capacity spray tank with water. In this way, all 500 mL of micronutrients were sprayed per 90 x 90 feet of the cultivated land. Second spray was conducted after 27-30 days. The growth of plant in sample and control was monitored. The third spray was conducted before the ripening of the crop. Plant height for all species and root weight ratio for onion crop was noted and at the end, the % increase in yield of fruit in rice, onion and lemon was calculated.

RESULTS AND DISCUSSION

Before application of micronutrients to selected fields, the respective field soil and water samples were tested. Physico-chemical characteristics of soil and water before the application of micronutrients are presented in Table 1.

Table 1: Physico-chemical characteristics of soil and water

	Tests													
	*ECe	pH	$^{**}Ca^{2+}$	$^{**}Mg^{2+}$	$^{**}Na^{+}$	$^{**}K^{+}$	$^{‡}Zn^{2+}$	$^{‡}Fe^{2+}$	$^{‡}Co^{2+}$	$^{‡}Cu^{2+}$	$^{‡}Mn^{2+}$	$^{**}CO_3^{2-}$	$^{**}HCO_3^{-}$	$^{**}Cl^{-}$
Soil	1.48	7.82	5.32	2.03	5.93	1.51	20.01	32.10	2.01	6.04	17.52	0.33	1.91	2.02
Water	0.88	7.41	3.81	1.74	2.95	0.90	1.78	0.81	0.10	1.10	1.03	0.03	0.90	1.81

Measuring units: * dSm^{-1}, ** mmol/L, ‡ ppm

Fe formed the complex with EDTA at pH: 2-3, Co and Cu complexes are stabilized at pH: 6 while Zn, Ca, Mg and Mn formed stable complexes at pH: 10. All the metals form the complexes with EDTA in 1:1 which were confirmed by AAS and elemental analysis. The metal and elemental confirmatory results are summarized in Table 2.

Table 2: Analytical data of complexes

Complexes	Calc. (Found)%				Yield (%)
	H	C	N	Metal	
$C_{10}H_{12}MgN_2O_8.Na_2$ [358.51]	3.35 (3.39)	33.47 (33.43)	7.81 (7.88)	6.78 (6.72)	83.32
$C_{10}H_{12}CaN_2O_8.Na_2$ [374.29]	3.21 (3.28)	32.10 (32.08)	7.48 (7.34)	10.71 (10.66)	90.43
$C_{10}H_{12}MnN_2O_8.Na_2$ [389.15]	3.10 (3.03)	30.84 (30.89)	7.20 (7.31)	14.12 (14.19)	86.87
$C_{10}H_{12}FeN_2O_8.Na_2$ [390.05]	3.08 (3.12)	30.76 (30.87)	7.18 (7.11)	14.32 (14.22)	85.81
$C_{10}H_{12}CoN_2O_8.Na_2$ [393.14]	3.05 (3.09)	30.52 (30.44)	7.12 (7.07)	15.01 (15.11)	83.93
$C_{10}H_{12}CuN_2O_8.Na_2$ [397.76]	3.02 (3.12)	30.17 (30.15)	7.04 (7.11)	15.97 (15.88)	88.76
$C_{10}H_{12}ZnN_2O_8.Na_2$ [399.38]	3.00 (3.02)	30.03 (30.10)	7.00 (7.08)	16.36 (16.33)	94.54

Different amounts of micronutrients were added in the formulations for different crops as reported in Table 3, by using the rough estimates of micronutrient demand for specific crop through literature[10]. Different formulations were tested, but only, which produce best results are reported. Soil fertility increases with addition of micronutrients to soil. When these micronutrients are added in soil or given to plant directly, the plant shows a remarkable growth, which increases the crop yield. Similar effects have been observed in the present work. With the addition of micronutrients, the growth of the plant increases and the sample plants show remarkable difference as compared to control crop. The data (Table 3) indicate that the plant height was increased as compared to control plants.

Table 3: Amount of micronutrients required for Rice, Onion and Lemon

Complex	Rice	Onion	Lemon
	g/500 mL		
Zn-EDTA	5.6	3.9	2.6
Mn-EDTA	1.6	2.9	0.6
Fe-EDTA	2.3	2.3	1.3
Mg-EDTA	3.6	5.5	1.56
Cu-EDTA	0.1	0.05	0.04
Co-EDTA	0.04	0.03	0.007
Ca-EDTA	1.6	2.9	0.6

The results of rice crop prove that the micronutrients have significant effects on the plants growth and crop yield. The heights of the sample plants calculated at both stages were greater than the control plants. The crop was resistant to storms as most plants in control zone falls on the land while the plants in sample zone did not fall because of stronger stem. The crop yield data indicate that increase in crop yield was about 14-25% more than the control crop.

Table 4: Statistical data of crops

Crops ID	Plant height (inches)	Total amount of fruit (Kg)	Increase in fruit yield (%)
		Rice	
Sample	26	439	25.3
Control	24	351	
		Onion	
Sample	14	2459	18.28
Control	13	2079	
		Lemon	
Sample	74	674	14.2
Control	64	590	

The micronutrients applied to onion crop show that the weight of individual onion plants was also increased than that of control crop. The size of onion was increased by the addition of micronutrients, because micronutrients enhances the photosynthesis rate due to which more food was formed and stored in roots. As a result of this the crop yield was increased by 18.3% in test crops. Micronutrients increase the lemon product by 14.2%. The lemon plant gain around one foot more height than the control one and the growth of lemon plants was enhanced by the use of micronutrients and the plant leaves remain green while in control crop the leaves turn yellow. The height of plant, root weight gain and % increase yield of test and control crops are reported in Table 4.

CONCLUSION

These micronutrients can be used in fields to enhance the crop production. Since repeated crops are practiced in Pakistan, the use of micronutrients is even more essential to get desirable production. There is need to develop amino acid based essential micronutrient complexes and determine their doses for different crops in order to meet increasing demand of food.

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