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MICRONUTRIENTS SUPPLEMENTATION TO AFFECT LEAF NUTRIENTS COMPOSITION AND FRUIT YIELD OF CITRUS

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ABSTRACT

Background Traditional use of macronutrients only, like NPK, without micronutrients in Citrus production may cause severe decline in yield and quality. Keeping this in view, a field experiment was conducted to evaluate and compare the farmer practices and recommended fertilizer doses with/without micronutrients on yield attributes of Kinnow grown at different sites of Sargodha District, Pakistan.

Methodology Zn, Cu, Fe, Mn and B were applied at rate of 100, 10, 75, 30 and 25 g plant⁻¹ as soil application, respectively, and their 0.2, 0.2, 0.1, 0.2 and 0.1 % of solution were used as foliar application. Two sprays were applied, first before flowering and second sooner after the fruit set was of pea size. Farmers were allowed to carry out their own traditional farm practices along recommended NPK dose (1000, 500 and 500g plant⁻¹) with and without combination of micronutrients. Data including fruit yield and quantity was collected at maturity, soil and plant samples of citrus plants were also analyzed for physiochemical properties.

Results The results indicated that the number of fruits per tree increased with the application of balanced dose of NPK along with micronutrient by 1.23 folds as compared to farmer practices (control). Similarly, weight of fruit per tree was ranged from 87.48 kg/tree in control to 114.92 kg/tree in the treatment receiving micronutrients.

Conclusion The quality of the fruits in terms of fruit size, fruit girth, fruit shine increased with the application of micronutrient and NPK either applied through soil or foliar.

INTRODUCTION

Citrus is the most important fruit crop of Pakistan with annual production of 2.2 million tons and with export worth of \$200 million (Zuberi, 2015). Kinnow is largely grown in different tehsils of district Sargodha (Bhalwal, Kot Momin and Silanwali) and extending to Mandi Baha-ud-din, and Toba Tek Singh. Surprisingly, Kinnow production is on rise in other areas which were previously not famous for Kinnow production like Multan, Khanewal and Layya etc. Citrus occupies third position among the sub-tropical fruits and has a great nutritional role in our daily life as a rich source of vitamin C.

Crop nutrition and disease management are of vital importance for plant health and orchard viability in term of yield and quality. It is likely that nutrient deficiencies in crop plants increase susceptibility to variety of diseases. A number of abiotic and biotic factors including soil type, soil

nutrition status, type of rootstock and disease status effect nutrient uptake in fruit trees. This disease severity can be reduced by enhancing chemical biological and genetic control of many plant pathogens through proper nutrition (Huber and Haneklaus 2007).

Citrus cosmetic quality has been deteriorating over time due to diseases like scab and citrus canker, the average yield of citrus is 10 tons ha⁻¹; and the commercial yield of Kinnow is 25 tons ha⁻¹, showing potential gap to increase yield and grower's income. The importance of cosmetic quality of fruit can be visualized from the farm gate prices of different grades, A-grade: Rs 650-700/40 kg B-grade Rs 250-300/40 kg; C-grade Rs 80-120/40 kg. Due to cosmetic quality issues, the farm gate rejection varied from 20 to 50% with some orchards are completely rejected by exporters. Among essential nutrients, micronutrient deficiencies in soil and plants are the worldwide nutritional problems and very severe in many countries (Alloway 2008;

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Mousavi et al. (2007). Plant varies in their demand for micronutrient as these are involved in almost all physiological functions. Some of these elements are redox-active and are cofactors in many enzymes. They have enzyme activating function and play role in stabilizing protein (Hansch and Mendel 2009). In Pakistan, micronutrients deficiencies are common due to alkaline pH, low organic matter and calcareous nature of soils (Rashid et al. 1997). By selecting appropriate fertilizer doses, the farmers can drive towards heavier fruit and earlier fruit setting (Alva et al. 2006) and micro nutrients like iron (Fe), copper (Cu), zinc (Zn), manganese (Mn) and boron (B) are very important for optimal growth of plant, biochemical and physiological pathway in citrus cultivation under agro climatic conditions of Punjab Pakistan.

Soil and foliar application of Cu stabilize chlorophyll by protecting chlorophyll protein lipid complex (Tumolo and Marquez 2012) and restrict the chloroplast bound to plastocyanin in plants and improves electron transport chain because Cu is part of it. Maksymiec (1997) and Stenico et al. (2009) also concluded that Cu is indispensable for carbohydrates and nitrogen metabolism in citrus. Zinc improves the citrus fruit yield and juice quality, activation of enzyme protein synthesis (Ashraf et al. 2013, 2014). Zinc is also involved in photosynthesis, carbohydrates translocation (Tsonev and Lidon 2012). Application of Zn enhances the photochemical reactions occurring in thylakoid membrane, electron transport through PS-II and increases photosynthetic rate (Roach and Liskay 2014) and chlorophyll content (Alloway 2009). Soil or foliar supply of Zn increases the biosynthesis of chlorophyll and carotenoid synthesis that are important for proper performance of photosynthesis process (Mousavi 2011). Foliar application of Zn had positive impact on fruit yield and quality of Kinnow, mandarin sweet orange and grapes (Razzaq et al. 2013).

The foliar application of B and Zn combination significantly enhanced fruit yield and juice content total soluble solids ascorbic acid and non-reducing sugar (Asad et al. 2003). Boron application impact the yield by its role in pollen germinating and elongation (Abd-Allah 2006). Naz et al. (2012) also reported that B increases growth and flowering in tomatoes. Iron is involved in various physiological process of plant system namely chlorophyll formation and degradation synthesis of protein which contains chloroplast and electron carrier in enzyme system (Somasundaram et al. 2011).

Manganese play important role in plant physiological processes like photosynthesis, and respiration and Nitrogen metabolism/assimilation, is one of the most important micronutrient for plant growth. Manganese primarily functions as part of plant enzyme system, activating several metabolic functions (Somasundaram et al. 2011). It involve in

oxygen evolving step of photosynthesis and membrane function, as well as serving as an important activator of numerous enzyme in the cell. Average yield of citrus fruit is 9.2 tons per hectare that reflects poor exploitation of production potentials. In Pakistan, citrus yield and quality is far behind from well managed farms and other citrus growing countries of the world. Our exporting potential is merely 8% due to a big chunk going waste on account of poor soil crop and fertilizer management, poor quality (fruit with nutrient deficiency and disease symptoms) and poor management during harvesting, packaging, transportation, and storage (Zaman and Schumann 2006; Tariq et al. 2007).

It is need of the time to increase the production of exportable fruit quality by using non-conventional methods alone or in combination with conventional approaches. The integrated use of micronutrients (Zn, Cu, Fe, Mn and B) in association with different methods of application can render good production of crop year after year. Therefore, a study was conducted to manage the nutritional requirement of Kinnow to get potential yield and quality of fruit on different site of Sargodha.

MATERIALS AND METHODS

The field experiments were conducted to assess the efficacy of different techniques of micronutrients application to improve yield and fruit quality of citrus orchards in district Sargodha, Punjab, during year 2015-16. For this, uniform Kinnow plants of about 15 years of age were selected on the basis of visual observation of micronutrient deficiencies on two different farmer's fields. Soil and plant samples from selected orchard were collected and analyzed for physicochemical properties of soil and micro nutrient status in plants leaves.

Soil analysis

To determine the physicochemical properties of soil, composite soil samples were taken from the top (0-60 cm) soil layer of the experimental site. Samples were air dried, and plant twigs were removed. The soil samples were grinded and sieved through 2.00 mm sieve, labelled and stored in plastic containers until ready for analysis. Soil samples were analyzed using the method described by Homer and Pratt (1961).

Determination of micronutrients in soil

The concentrations of available micronutrients (Zn, Cu, Fe and Mn) in soil were determined by the DTPA extraction procedure (Lindsay and Novell 1978). Soil sample (10 g) was shaken with 20 mL DTPA in an open Erlenmeyer flask for two hour, the extract filtered and was read for Zn, Cu, Fe and Mn on an Atomic Absorption Spectrophotometer (Varian-Spectraa 220).

The concentration of available-B in soil was determined by the dilute hydrochloric acid method (Ryan et al. 2001). For this, 10 g air dry soil was shaken with 20 ml of 0.05 N HCl for 5 min. After filtering, B concentration in the extract was measured by the Azomethine-H method at 420nm on Spectrophotometer (PG/T70, UV/VIS).

Plant analysis

Approximation old leaves of six month age (15-20 in numbers) from different plants were collected. Samples of plant leaves were dried in a drying oven at 60°C for 48 h (Liu et al. 2006). Dried samples were ground in a mill (2mm of mesh size) fitted with a stainless steel chamber and blades. Subsequently, finely ground 1.0 g samples was put in a digestion tubes and kept overnight after adding a di-acid (HNO₃: HClO₄ ratio of 2:1) digestion mixture (Jones and Case, 1990). After 24 h, samples were digested on a digestion block at 150 °C until all the material was digested. After digestion, the material was cooled and diluted upto 50 mL by adding de-ionized water. Digest was then filtered with Whatman filter paper No. 42 and stored in air tight plastic bottles. Micronutrients concentration in the digested samples was determined by atomic absorption spectrophotometer (Varian, Spectraa 220). All samples for micronutrients determinations were prepared and analyzed in duplicate.

At maturity, the crop was harvested and weighed for determination of yield. Fruit weight for each treatment was recorded by digital balance and expressed in Kilogram per plant (kg plant⁻¹).

Treatments description and experimental design

The experiment was designed in a randomized complete block design (RCBD) with four replications (Table 1). The data obtained was analyzed statistically. Simple means and averages were calculated using MS Excel Sheet. LSD test was performed using free version of Statistix 8.0 application.

Leaves analysis of citrus of site 48 NB Sargodha

The analysis of the leaf samples (Table 4) showed that there was lowest concentrations 1.87%, 0.071%, 1.71% of N, P, K and 10.7, 22, 2.1, 11.7 ppm of Zn, Fe, Cu, Mn in control (FP) in which N, P and Zn, Fe, Cu, Mn were deficient while K 1.71% and B (29.5 ppm) were in sufficient range. Concentration of Zn, Fe, Cu, Mn and B in leaves increased with application of these nutrients. The analysis indicated that use of Zn, Fe, Cu, Mn and B also increased the uptake of N, P and K. Similar findings were also reported by Nanaya et al. (1985). Mamm et al. (1985).

Leaves analysis of citrus of site 83 SB, Sargodha

The analysis of the leaf samples (Table 5) showed that the concentration of 1.66%, 0.064% of N, P and

12.7, 23.3, 2.3, 13.7, 17.4 ppm of Zn, Fe, Cu, Mn and B, respectively, were deficient while K (1.90%) was in sufficient range in control (*FP). Concentration of Zn, Fe, Cu, Mn and B in leaves increased with application of these nutrients. Similar to site-1, use of Zn, Fe, Cu, Mn and B also increased the uptake of macro nutrients.

RESULTS AND DISCUSSION

It is well known that order of nutrient requirement for citrus is nitrogen > phosphorus > potassium > among macronutrients while Zn > Fe > B > Cu > Mn. Method and amount of fertilizers application also improved the yield and quality of fruit. Because citrus is a tree and, it has growth in cycles, therefore until and unless, fertilizer application correctly designed according to growth cycle, otherwise it's impossible to improve the plant health and fruit production (Yaseen et al. 2004; Alva et al. 2006; Zaman and Schumann 2006). Micronutrients application significantly increased the fruit yield.

Two demonstration trials were conducted at farmer's field at Sargodha Tehsil, Data in tables showed the effect of application of micronutrients in comparison with farmer' practice, application of micronutrient markedly increased the number of fruits and yield of kinnow and extent of increase was 20 to 40 % compared to farmer practice during (2015–16). Data showed that application of micronutrients in right amount and at right time increased the yield of kinnow.

Number of fruit per plant

Table 6 shows, site located at Chak 48N.B. In treatment T2, where micronutrients were applied (Zn, Cu, Fe, Mn and B) in the soil. Increase of about 9.8% in number of fruits as compared to T1 (where no micronutrients were applied). While in T4, number of fruits increased by 20.92% as compared to T1 (In T4, micro nutrients were applied with recommended dose of NP and K). In T3, number of fruits increased by 14.14 % as compared to T1 while in T5, 11.39% number of fruits increased as compared to T3. Number of fruits also increased by 12.35% in T4 as compared to T2. Over all foliar application of micronutrients slightly increased the number of fruits over soil application (Table 6).

Our results are similar with the findings of Chiu and Chang (1986) who reported 3-15% increase in fruit yield following B application. Although B alone could not gave satisfactory results over Zn and Mn but yield was increased from 89 to 95 kg tree⁻¹ when it was applied in combination with Zn, Fe, Cu and Mn. This may be attributed to the cumulative effect of Zn, Fe, Cu, Mn and B in reducing heavy drop of young fruits and increased fruit set. These results are in line with Sato (1962).

Fruit yield plant⁻¹ also increased with application of micronutrients as shown in Table 6 at

Table 1 Description of the treatments plan

Treatments	N	P ₂ O ₅	K ₂ O	Micronutrients (Zn, Cu, Fe, Mn and B)
T1	FP*	FP*	FP*	Without micro nutrient application
T2	FP*	FP*	FP*	Soil (100, 10, 75, 30, 25 plant ⁻¹)
T3	FP*	FP*	FP*	Foliar sprays (0.2, 0.2, 0.1, 0.2 and 0.1%)
T4	1000	500	500	Soil (100, 10, 75, 30, 25 plant ⁻¹)
T5	1000	500	500	Foliar sprays (0.2, 0.2, 0.1, 0.2 and 0.1%)

*FP: farmer's practice

Table 2 Leaf analysis for micronutrients before application of micronutrients

Micronutrient in plant leaves before application	Experimental sites	
	Chak 48 NB	Chak 83SB
Zn (mg kg ⁻¹)	11.45	12.34
Cu (mg kg ⁻¹)	3.70	4.30
Fe (mg kg ⁻¹)	65.5	78.40
Mn (mg kg ⁻¹)	13.40	12.80
B (mg kg ⁻¹)	75.65	70.37

Table 3 Soil characterization of experimental sites

Soil characteristics	Chak 48NB	Chak 83 SB
Soil texture	Loam	Loam
EC(dS m ⁻¹)	1.72-2.28	1.26-1.48
pH	7.72-7.77	7.8-7.9
Organic matter (%)	0.90-0.66	0.8-0.6
P (mg kg ⁻¹)	7.5-5.8	7.6-6.2
K (mg kg ⁻¹)	180-110	160-140
Zn (ppm)	0.68	0.74
Fe (ppm)	4.8	4.50
Cu (ppm)	0.44	0.37
Mn (ppm)	1.52	1.20
B (ppm)	0.44	0.40

Table 4 Nutrients concentrations in citrus leaves at site-1 (48 N/B Sargodha)

Treatments	N (%)	P	K	Zn (ppm)	Fe	Cu	Mn	B
T1	1.87	0.071	1.71	10.7	66	2.1	11.7	29.5
T2	1.92	0.082	1.99	19.4	102	3.5	13.5	30.6
T3	1.95	0.090	2.20	24.5	110	5.8	33.2	37.5
T4	1.90	0.084	2.42	14.4	90	4.5	23.4	36.7
T5	1.90	0.088	2.35	26.0	140	9.7	36.7	46.2

Table 5 Nutrients concentrations in citrus leaves at site-2 (Chak 83 NB Sargodha)

Treatments	N (%)	P	K	Zn (ppm)	Fe	Cu	Mn	B
T1	1.66	0.064	1.90	12.7	70	2.3	13.7	17.4
T2	1.95	0.066	2.43	22.7	89	3.9	15.5	28.1
T3	1.97	0.079	2.54	25.3	90	6.8	36.2	36.2
T4	1.99	0.078	2.61	20.7	112	5.5	27.4	34.5
T5	1.97	0.079	2.63	23.2	155	12.7	38.5	43.4

Table 6 Effect of micronutrients application on number of fruit plant⁻¹ of kinnow mandarin Chak 48 NB Sargodha

Treatment	Number of fruits plant ⁻¹		Fruit yield plant ⁻¹	
	Chak 83 SB	Chak 48 NB	Chak 83 SB	Chak 48 NB
T1	508.88c	505d	76.97d	87.48e
T2	554.63c	559c	88.1c	94.19d
T3	559.38bc	582b	95.64b	98.24c
T4	611.13ab	638a	109.3a	108.91b
T5	625.88a	638a	111.1a	114.92a

location of Chak 48 NB 19.66% fruit yield increased where recommended dose of fertilizer (NPK) and micronutrients was applied in T4 as compared to T1. In T4, 13.51% yield increase was observed over T2, foliar application slightly increased fruit yield by 4.12% in T3 as compared to T2, and 10.94% in T3 in comparison with T1. Similar trends were also reported by Yaseen et al. (2015) and Aisha et al. (2015).

The data given in Table 6 also revealed that at Chak No. 83 SB in T2, the number of fruits increased by 8.24% as compared to T1. Number of fruits increase was by 9.24% in T4 in comparison with T2. In T5, where micronutrient applied (Zn, Cu, Fe, Mn and B) in the soil, increase of 18.23% of number of fruits was observed in comparison with T1 (where no micronutrients were applied). In T4, number of fruits increased by 8.46% as compared to T3. Whereas in T5, there was 10.62% increase in number of fruits as compared to T3. Over all, foliar application of micronutrients slightly increased the number of fruits over soil applied micronutrients. Foliar application of Zn, Cu, Fe, Mn and B has advantage over soil application Labanauskas et al. 1969; El-kassas et al. 1987.

In Table 6, results revealed that at 83 S.B, the treatment T4 had 19.38% yield increase over T2, foliar application also significantly increased the fruit yield by 7.88% in T3 as compared to T2, and 19.52% in T3 in comparison with T1 (FP). Fruit yield per plant also increased with the application of micronutrients, 29.56% fruit yield increase were achieved where recommended dose of fertilizer and micronutrients were applied as T4 as compared to T1. The result also reported by Yasin et al. (2010) and Aisha et al. (2015).

Overall application of micronutrients showed that number of fruits and yield increased per plant due to balanced fertilization. Micronutrient has definite role in production and health of citrus orchards. It need of time to apply micronutrient to citrus plants at critical growth stages to get quality and healthy fruit which could be exported to earn foreign exchange.

Foliar application of Zn, Fe, Cu, Mn and B significantly enhanced fruit yield in terms of fruit number and fruit size, weight and fruit shine. The results are in line with Ashraf et al. (2013) and Razaq et al. (2013) who reported that foliar application of Zn enhanced productivity with better fruit quality in Kinnow (Tariq et al. 2007; Alloway 2008; Ashraf et al. 2013). Ashraf et al. (2012) also reported that foliar application of micronutrients increased the number of fruits per tree and juice volume per fruit in sweet orange (*Citrus sinensis* L.) and in Kinnow. Boron application increases fruit set and yield in several fruit and nut trees, including almond, Italian prune, olive, and sour cherry (Slavko et al. 2001). The results are similar to Khurshid et al. (2008) when orange trees were foliar application

with Cu it significantly increased fruit yield tree⁻¹, fruit weight, total soluble solids, and fruit size as compared to the untreated trees. Fageria (2002) also observed that application of Cu increased yield of upland rice and common bean. Galrão (1999) also reported the significant effect of Cu on yield of annual crops.

CONCLUSIONS

Application of Zn, Fe, Cu, Mn, and B at rate of 100, 75, 10, 30 and 25g plant⁻¹, respectively as basal dose in soil while Foliar application of Zn, Fe, Cu, Mn and B at 0.2, 0.1, 0, 0.2 and 0.1% improved the photosynthetic activity and fruit yield with better fruit quality of citrus orchards.

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