FOLIAR AND SOIL-APPLIED MICRONUTRIENTS IMPROVE YIELD AND QUALITY OF KINNOW (*Citrus reticulata* Blanco)

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The current study was carried out in order to assess the effects of micronutrients (B, Fe, Zn) on the yield and quality of Kinnow (*Citrus reticulata* Blanco). Citrus is an important fruit crop of Pakistan and micronutrients are not only important for yield and quality of citrus but also for the human health. This experiment consisted of different soil treatments and foliar applications of B, Zn and Fe either alone or in combinations. The results revealed that the highest concentrations of these three micronutrients in soil were found where 20 g B, 40 g Zn and 100 g Fe were applied per plant, respectively through soil application. The highest concentrations of these micronutrients in the leaves of Kinnow were found in the individual treatments of foliar spray of 0.03 % B, 0.1 % Zn and 0.25 % Fe, respectively, followed by the combined application of B, Zn and Fe with concentration of 0.03%, 0.1% and 0.25%. Soil that had combined treatment, B: 10 g plant⁻¹, Zn: 20 g plant⁻¹, Fe: 50 g plant⁻¹ gave the highest yield of fruit and juice content. Fruit drop was the highest in control and the lowest for 20 g B soil application. Total sugars, reducing sugars and TSS were the highest for 40 g Zn as soil application, followed by the combined treatment of B:10 g plant⁻¹, Zn: 20 g plant⁻¹, Fe: 50 g plant⁻¹. The non-reducing sugars were the highest in the case of combined soil application of B, Zn and Fe. It is concluded that the micronutrients application is a suitable approach for increasing the quality and yield of Kinnow.

Keywords: micronutrients, soil application, foliar application, kinnow yield, sugars, fruit quality, fruit juice.

INTRODUCTION

In the world, citrus is considered as one of the most important fruit trees. The total production of citrus is about 46.1 million tons (USDA, 2020). Pakistan ranks twelfth for citrus production among all the nations of the world. About 2.36 million tons of citrus is produced annually in Pakistan on about 206.5 thousand ha area (Anonymous, 2020). In Pakistan, more than 95% of total production of citrus is contributed by Punjab, among all the provinces of the country. Kinnow Mandarin (Citrus reticulata Blanco) variety of citrus has gained more importance compared with the other citrus varieties due to its higher export value. Better adaptability to local agro-climatic conditions, shiny look, aroma, better acidsugar combination and beautiful color are some other factors which are contributing in fame and importance to this citrus variety (Khalid et al., 2012). Citrus fruit contains a good amount of vitamins such as A, B, C, foliate and minerals such as Calcium (Ca), Iron (Fe) and phosphorous (P) (Khan et al., 2015). About 27,869 thousand tons of mandarin is being produced in the world. The major contributors in this production are China, Spain, Brazil, and Pakistan, which

produces 15185, 1974, 1206 and 640 thousand tons citrus, respectively (FAO, 2007).

In Pakistan, most of the soils have low fertility levels and deficiency of important nutrients. About 95% of Punjab soils are nutrient depleted facing the deficiency of Nitrogen (N), Phosphorous (P), Potassium (K), Zinc (Zn), Boron (B) and Iron (Fe) with area percentage of 90, 40, 57, 50, 21 and 1 percent, respectively (Rashid, 2009). Among various plant and soil management practices, micronutrients application is considered as the most important practice for improving and enhancing citrus yield and quality (Iglesias et al., 2007). Healthy yield and growth of citrus is due to the availability of the micronutrients in reasonable quantity which plant requires at the proper time (Rashid, 2009; Khan et al., 2012). Citrus fruits are highly depleted with micronutrients which are grown on sandy soil, calcareous soil, shallow soil where the level of the water table is higher and soil which are not cultivated previously (Khan et al., 2015).

Among various micronutrients, B, Fe and Zn are regarded more important for citrus yield and quality (Khan *et al.*, 2012, 2015). Zinc is a precursor of tryptophan (indole acetic acid precursor) which performs crucial role in plants for starch metabolism (Alloway, 2008). Zinc can enhance vegetative growth of plant through the improvement of enzymes activities, metabolism of carbon, photosynthetic activities, chlorophyll production and by improving differentiation (Alloway, 2008)). Both the enzymes and non-heme iron (Fe) proteins contain Fe as an important component. Iron in enzymes as a FeS and cytochromes is involved in the redox reactions and control the electron transfer in these reactions. Other processes of plant such as photosynthetic mechanism, respiration and the nitrogen fixation are also affected by the non-heme Fe proteins (Taiz and Zeiger, 1991; Chen and Lu, 2006). Faded tree branches, dry leaves (looks like dry paper), vellowing of leave veins, fruit falling in growing stage, and lower rate of fruiting all are the symptoms of Fe deficiency in plants (Imran and Gurmani, 2011). Similarly, Boron (B) is also having very important physiological roles such as enzyme activities, protein synthesis, pollen tube elongation, pollen grain germination and ultimately fruit setting and yield (Marschner, 1995; Swietlik, 2002). Growth reduction of young fruits, roots and shoots occurs due to B deficiency which affects the cell division of plants and leads towards growth retardation (Shalp, 1993). Boron deficiency is the most prevalent micronutrient deficiency in citrus in arid regions of the world (Papadakis et al., 2003).

Soil and foliar application are the major routes of micronutrients application. Efficacy of the soil applied fertilizers is low in the calcareous and alkaline soil due to high fixation and less mobility of the nutrients (Rashid *et al.*, 2002; Zekri and Obreza, 2003). To enhance or improve the concentration of the micronutrients in plant organs micronutrients feeding through foliar sprays is the fastest way to cope up with deficiency of micronutrients (Boaretto *et al.*, 2002).

In the past, there had been few studies regarding Zn and B applications to citrus (Sajid *et al.*, 2010; Khan *et al.*, 2012, 2015). However, effects of combination of B, Fe and Zn has not been explored yet in order to increase the production and quality of Kinnow. Therefore, the present field experiment was conducted to assess the effect of soil and foliar applications of B, Fe and Zn on yield and quality of Kinnow.

MATERIALS AND METHODS

Experimental plan: The experiment was carried out in the fields of Horticulture Department of Ayub Agricultural Research Institute, Faisalabad. Thirty-six Kinnow plants of 6-8 years old, with uniform size and vigor, sown with plant x plant and row x row distance of 3.35 m in square system were selected for experimental material. The treatments were applied using Randomized Complete Block Design (RCBD). Each tree was counted as one experimental unit. Soil analysis was done before and after the experiment. The leaves (1-2 month old) were collected from each tree (from all sides). These leaves were analyzed before the start of the experiment

and at harvesting time for macro and micro nutrient values. The experiment consisted of twelve treatments including control. Every treatment has three replications and one plant was considered as one replication. Recommended doses of NPK (1 + 0.5 + 0.5 kg plant⁻¹) were applied in all the treatments including control. Three micronutrients (Zn, Fe, B) in the form of ZnSO₄, Fe₂SO₄ and H₃BO₃ were applied in soil or foliar spray using following treatments.

Treatments: Following twelve treatments were applied in this field experiment: Control (T₁), soil treatment of B @ 10 g per plant (T₂), soil treatment of B @ 20 g per plant (T₃), soil treatment of Zn @ 20 g per plant (T₄), soil treatment of Zn @ 40 g per plant (T₅), soil treatment of Fe @ 50 g per plant (T₆), soil treatment of Fe @ 100 g per plant (T₇), soil treatment of B, Zn and Fe @ 10, 20 and 50 (g per plant) (T₈), foliar treatment of B @ 0.03 % (T₉), foliar treatment of Zn @ 0.1 % (T₁₀), foliar treatment of Fe @ 0.03 % + 0.1% + 0.25% (T₁₂).

Soil was cleaned and hoed under the canopy of each tree before applying fertilizers. For mixing the fertilizers in the soil properly, hoeing was done again after applying the fertilizers. After all the treatment applications, irrigation of the plants was done immediately. For foliar treatments, micronutrients were sprayed on plants by mixing with water. *Soil analyses*: From 0-15 cm and 15-30 cm depths, the soil samples were taken and analyzed for various physicochemical properties of soil following Ryan *et al.* (2001) (Table 1). Soil pH (8.10) revealed the alkaline nature of the soil. Soil texture analysis showed that soil was clay loam.

 Table 1. Soil
 and
 leaf
 analyses
 before

application	-		
Soil analysis	0-15 cm	15-30 cm	Leaf analysis
pH	8.10	8.08	-
EC (dS m^{-1})	1.20	1.10	-
Organic matter (%)	0.34	0.17	-
N (%)	0.17	0.10	2.90
P (mg kg ⁻¹)	45.0	76.0	0.45
K (mg kg ⁻¹)	32.0	34.0	1.14
Zn (mg kg ⁻¹)	0.35	0.30	11.1
Fe (mg kg ⁻¹)	1.97	1.70	30.9
B (mg kg ⁻¹)	0.30	0.28	18.6

treatment

Leaf analyses: The leaves of similar age were collected randomly from experimental plants and analyses were done for macro and micronutrient contents two times, firstly at the start of experiment and secondly at the time of harvesting of fruits. For this purpose, 100 leaves were collected carefully from each plant (Smith, 1966). Care was taken that leaves had no obvious deficiency symptoms nor injured or destroyed through insect bites and other diseases. After sampling, these collected samples of leaves were carefully packed in paper bags with proper labeling for analyses.

Sample preparation: For surface disinfection, leaves after collection were washed properly with detergent after that with tap water. Thereafter, leaves were washed by dipping them in distilled water. Leaves were kept under shade for about 24 h for drying purpose. After 24 h, dried leaves were packed in properly labeled bags. These bags were punched and kept in an oven at temperature of 65° C for 48 h for drying. These samples were grinded in electric grinder and fine material was kept in labelled bottles made up of plastic which were air tight and were stored at room temperature.

Determination of Fe, Zn and B: The method of Chapman and Parker (1961) was used for the determination of total nitrogen content. For potassium, phosphorous and micronutrient determination, digestion was done in tri-acid mixture of HNO₃, HClO₄ and H₂SO₄ following the process described by Yoshida *et al.* (1976). Potassium concentration was determined on flame photometer and phosphorus was determined on spectrophotometer. The concentrations of micronutrients (Zn and Fe) were determined by atomic absorption spectrophotometer (Shimadzu, Japan). Dry ashing method was used for the determination of B level in plants on UV/visible spectrophotometer (U2020; IRMECO, GmbH, Germany) using Azomethine-H (Bingham, 1982).

Fruit analyses: Ten fruits were randomly picked from each tree for the physico-chemical analyses. All the fruits from each plant were picked and calculated. After every month, droppage percentage of fruits was calculated by fruit counting from the branches previously tagged. Following formula was used for the calculation of droppage percentage of fruits:

Fruit drop % =
$$\frac{Total fruit drop \times 100}{Tatal fruit act}$$

Total fruit set

Fruit juice was extracted from each sample then this juice was sieved in order to separate the juice from the fruit pulp. Each sample weight was also obtained. Following formula was used to calculate the juice percentage:

Juice weight % = $\frac{Average \ juice \ weight \times 100}{Average \ fruit \ weight}$

Fruit juice was extracted from 10 fruits for chemical analyses using manual extractor. Fruit juice total soluble solids (TSS) were determined with the help of a digital refractometer ATAGO PAL-1 (Atago, Japan). The method described by Khan *et al.* (2009) was used to estimate the total sugars, reducing and non-reducing sugars.

Statistical analysis: The experiment was done using Randomized Complete Block Design (RCBD). The computer software package "Statistix" was used for the statistical analysis of the collected data. Analysis of variance was applied on data, while Least Significant Difference (LSD) test with ($P \le 0.05$) was used (Steel *et al.*, 1997) for the comparison of differences among treatments.

RESULTS AND DISCUSSION

Soil B, Zn and Fe contents: The results of concentration of B in soil (Fig.1A, B) depicted that the highest concentration of B in 0-15 cm soil depth was found where soil was treated with B (20 g) followed by 10 g B and combination of B: 10, Zn: 20, Fe: 50 g/plant both being at par with each other. In the lower depth of soil (15-30 cm), the highest concentration of B was found where soil was treated with B (20 g) followed by B, Zn and Fe combination (10, 20, and 50 g/plant) and 10 g B soil application, respectively. We found that due to the slow movement of B within the soil profile, B concentration was lower in the lower depth of soil as compared with the upper depth of soil. During the last 60 years, the applications of B on 132 crops in 80 different countries have resulted in positive effects of B on soil (Shorrocks, 1997), and our experimental results showed similarities with these previous findings. In the fertilized soil, the concentration of B increases with respect to the dosage applied (Moura et al., 2013). Soil texture affects the B content in the soils for instance, the sandy soils are deficient in B (Fleming, 1980). Peryea (1994) and Kotze (2001) reported that the concentration of B was enhanced to 0.5-1 mg kg⁻¹ (optimal range) and 0.2-0.5 mg kg⁻¹ (adequate range) in pre and post experiments, respectively.

Zinc concentration in soil depicted that the highest Zn level was reported in both depth of soil from 40 g Zn application on soil (Fig.1C, D) followed by combined (B: 10, Zn: 20, Fe: 50 g/plant) application and 20 g Zn soil application, respectively.

The soil with no fertilizer application (control) contained the least level of Zn content. Dutta and Banik (2007) reported an increase in Zn content both in soil and plants by the application of Zn which increased fruit size due to improved physiology and increased uptake of water and other nutrients. Zinc availability is decreased at high pH and due to calcareousness of soils, presence of HCO_3^- ions in soil and adsorption of Zn on the particles of clay minerals. These factors contributed in lower Zn level in other treatments where zinc was not applied (Trehan and Sekhon, 1977; Dogar and Hai, 1980).

The highest concentration of Fe in both soil depths (0-15 and 15-30 cm) was reported where 100 g Fe was applied followed by the combined (10:20:50 g plant⁻¹) treatment and 50 g Fe application (Fig. 1E, F). In control, there was less concentration of Fe. In the lower depth, the level of Fe was lower than the upper depth because of less mobility of Fe in the deeper soil layers. Analysis of samples before applications showed that Fe content was low in soil therefore, we had to apply Fe in the soil. Due to the high pH (8.12 in our experiment), the solubility of Fe decreases which in return lowers soil Fe content. Iron solubility is suppressed with the



Figure 1. Effect of micronutrients application on the concentrations of B 0-15cm (A) and 15-30 cm (B), Zn in 0-15 cm (C) and 15-30 cm (D) and Fe in 0-15 cm (E) and 15-30 cm (F) soil depths. Vertical bars indicate mean values of three replicates \pm SE of means. Values not sharing the same letter differ significantly at $P \le 0.05$. The treatment details are given only in Figure 1-F.

increase of pH. The lowest solubility of Fe in soil was observed at pH of 7.5-8.5 (Lindsay, 1979). The findings of Rakkiyappan *et al.* (2002) supported our experimental results that the level of Fe enhanced in the soil through the application of the ferrous sulphate. Free calcium carbonate (CaCO₃) and the alkaline pH are the causes of Fe scarcity in soil (Anderson, 1982). These results are in line with our experimental values which showed the deficiency of Fe in soil which was not treated with Fe.

Leaf N, P and K contents: Nitrogen, P and K value in the leaves of Kinnow were fairly low before the treatment application (Table 1). Soil application of all the three micronutrients (B, Fe, Zn) mostly caused significant increase

in leaf N content (Table 2). On the other hand, the foliar application of micronutrients decreased the leaf N content. Leaf P content were either reduced or remained unchanged by both soil and foliar treatment of any nutrient (Table 2). Leaf K content was not enhanced significantly due to soil treatment of micronutrients separately. However, both the soil and foliar combined treatments and individual foliar treatments of Zn and B enhanced the level of K in leaves as compared with the control (Table 2). Improved N and K levels in leaves due to Zn and B application in citrus has been reported in the past (Ullah et al., 2012; Khan et al., 2015) and might be due to the synergetic relationship between these macro and micronutrients (Khan et al., 2012; Razzaq et al., 2013). In line with our results, application of these micronutrients has shown positive effects on macronutrients contents in the leaves of Washington Navel orange (Omaima and El-Metwally, 2007) and *Citrus reticulata* Blanco (Khan *et al.*, 2012).

 Table 2. Effects of micronutrient application on the concentrations of N. P and K in kinnow leaves

Treatments	N (%)	P (%)	K (%)
Control	3.05c	0.47a	1.13c
10 g B	3.33b	0.12d	1.01c
20 g B	3.50a	0.26cd	1.11c
20 g Zn	2.60e	0.47a	1.13c
40 g Zn	3.4ab	0.47a	1.13c
50 g Fe	3.50a	0.40ab	1.12c
100 g Fe	3.30b	0.12d	1.04c
10:20:50(g) B:Zn:Fe	2.20f	0.44ab	1.65a
0.03% B	2.80e	0.30bc	1.39b
0.1% Zn	3.0cd	0.43ab	1.39b
0.25% Fe	2.60e	0.46a	1.13c
0.03:0.1:0.25(%) B:Zn:Fe	2.60e	0.42ab	1.39b

Leaf B, Zn and Fe contents: The concentrations of micronutrients in leaves were B: 18.6, Fe: 30.9 and Zn: 11.1 (mg kg⁻¹) before treatment application and leaves were apparently deficient in these micronutrients (Sauls, 2008). The highest concentration of B in leaves (Fig. 2A) was noticed where 0.03 % B was applied by foliar application (67 % more than control) followed by foliar application of the combined treatment of B: 0.03%, Zn: 0.1%, Fe: 0.25% (66% more than control). Our experimental findings were in line with those of Sourour (2000) and Khan et al. (2015) as they reported a positive relation between B foliar spray and B value in the leaves. Greater concentration of B induces cell division, enhances the carbohydrate accumulation and translocation of sugar (Khan et al., 2012), hence it improves yield and quality. The data depicted (Fig. 2B) that the highest concentration of Zn in Kinnow leaf was found in the foliar application of 0.1 % Zn (75% more than control) followed by the combined application of B: 0.03%, Zn: 0.1%, Fe: 0.25% plant (67% more than control). Khan et al. (2012; 2015) also found that the foliar Zn spray increased leaf Zn content and yield of different citrus varieties. Swietlik (2002) stated that Zn foliar spray positively affects the yield of grapes and citrus. It was founded that improvement of yield is due to the enhancement of the nutritional contents of leaves. Results of our experiment indicated that the combined treatments showed less increase in Zn content as compared with Zn alone as observed by Mann and Takkar (1983). They reported that multi-nutrient application does not increase the concentration of individual nutrients in the same manner because some nutrients have less absorption level as compared to the other nutrients.

The data regarding Fe concentration (Fig. 2C) showed that the highest leaf Fe concentration was found where 0.25 % Fe was

applied by foliar spray (82% more than control) followed by the combined application of B: 0.03%, Zn: 0.1%, Fe:0.25% (79% more than control). Our results showed similarity with



Figure 2. Effect of micronutrients application on the concentrations of B (A), Zn (B) and Fe (C) in kinnow leaves. Vertical bars indicate mean values of three replicates \pm SE of means. Values not sharing the same letter differ significantly at $P \leq 0.05$. The treatment details are given only in Figure 2-C.

the results of Rana and Sharma (1979) who reported that there was higher level of Fe in the leaves treated with Fe (0.5% iron sulphate) as compared with the untreated plants. Rakkiyappan *et al.* (2002) also concluded that iron sulphate spray enhanced Fe level significantly. In citrus leaves the intrinsic deficiency of B, Zn and Fe might be due to the lower levels of these micronutrients in our soil and their availability is further

decreased due to calcareous nature and high pH of soils (Rashid and Ryan, 2004; Alloway, 2008). Therefore, the foliar application of these micronutrients seems very promising approach for increasing their levels in citrus (Khan *et al.*, 2012, 2015).

Effects of micronutrient on fruit production: The highest fruit weight and number were found in the soil that was treated with combination of B: 10g, Zn: 20g, Fe: 50g followed by 40 g soil treatment of Zn (Fig. 3A, B). Results of our study are similar to many previous studies (Ibrahim et al., 2007; Yadav et al., 2013) in which fruit yield (fruits weight, number) was increased by the treatment of B, Fe and Zn. Omaima and El-Metwally (2007) figured out in an experimental study in Washington navel orange that the application of K with Zn resulted in an increase in synthesis of protein and photosynthetic activity, which positively affected the growth and weight of fruits. According to Khan et al. (2015), the nutritional status of Kinnow is balanced by the foliar treatment of B and Zn which improved the yield of fruit. We found that the separate application of micronutrients in particular was less effective in yield increase of citrus. Similar to our results, synergistic relationship among B and Zn was also noticed by Tarig et al. (2007) and Razzag et al. (2013) in sweet oranges and Kinnow mandarin which resulted in reduction in fruit drop, enhanced fruit setting and ultimately increased fruit yield.

The results regarding juice percentage depicted that juice percentage in fruits was higher in the combined treatments of B + Zn + Fe as compared to individual treatments (Fig. 3C). The findings of this experiment have similarity with those of Babu and Yadav (2005) which showed that the percentage of the fruit juice increased in Khasi mandarin trees sprayed with Zn. These findings are in line with the study of Devi et al. (1997) who reported that the combination of ZnSO₄ and FeSO₄ application in soil and 0.5% micronutrients foliar application resulted in significant increase in fruit juice percentage. Increased juice concentration might be due to Zn which plays a crucial role in tryptophan production which positively affects the plant growth and fruit development (Razzaq et al., 2013; Khan et al., 2015). The findings regarding fruit drop (Fig. 3D) indicated that the highest droppage of fruits was founded in control. Droppage of fruits was minimum in the soil treated with 20 g B and by the combined treatment. Droppage of fruits was the highest in control due to the deficiency of micronutrients. Our results are supported by previous studies where the deficiency of B caused droppage of fruits. Soil sprays and foliar sprays decreased dropping percentage of fruits probably due to increased production of IAA that limits fruit drop by enhancing the auxin concentration at abscission zones (Nijjar, 1985; Khan et al., 2015). The findings of El-Baz (2003) showed similarity with this experimental finding, that fruiting efficiency is enhanced by micronutrients application. Micronutrient application (B, Zn and Fe) results in synthesis

and transportation of carbohydrate (cellulose) from leaves to fruits which strengthens cell wall and dropping of fruit is reduced (Shahin *et al.*, 2010; Ullah *et al.*, 2012; Razzaq *et al.*, 2013).





Fruits TSS were the highest in the treatment of soil application of 40 g Zn (Fig. 4A). Our findings are similar to those of Srivastava and Gupta (1996) who indicated that foliar treatment of Zn enhanced the TSS. Similarly, Monga and

Josan (2000) indicated that TSS concentration in mandarin fruits was increased due to the foliar Zn spray. However, only the soil zinc application was effective in this experiment and foliar application of Zn in this experiment did not increase the TSS. Results of this experiment are similar with the findings of Karim *et al.* (1996) for B that the TSS concentration was not affected by the application of B.



Figure 4. Effect of micronutrients application on total soluble solids (A), total sugars (B), reducing sugars (C) and non-reducing sugars (D) of kinnow. Vertical bars indicate mean values of three replicates \pm SE of means. Values not sharing the same letter differ significantly at $P \leq 0.05$.

Total sugars, reducing sugars and non-reducing sugars were the highest in treatment of soil application of 40 g Zn, followed by the combined treatment of B: 10%, Zn: 20%, Fe: 50% (Fig. 4B, C, D). Our results are similar to those of Babu and Yadav (2005) work, who indicated that the combined treatment of Zn, Mg and Mn on Khasi mandarin enhanced the level of total sugars. Findings of our experiment partially agree to the conclusions of Mann *et al.* (1985), who stated that the mandarin orange's reducing sugar concentration remained unchanged by Zn and B application. According to Khan *et al.* (2015), B and Zn foliar sprays significantly affected sugar contents of Kinnow fruits. Increase in sugar level may be due to the sprays of Zn and B that affected aldolase (enzyme) which is beneficial for the fruit sugar formation (Alloway, 2008; Ullah *et al.*, 2012).

Conclusions: It is concluded that the soil application of micronutrients (B:10, Zn:20, Fe:50 g per plant) is the most useful treatment for improving the quality and increasing the yield of Kinnow under the studied experimental conditions. The application of micronutrients increased the fruit yield and juice content and reduced the fruit drop (2%). Similarly, the use of micronutrients improved the quality attributes of Kinnow.

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