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Foliar application of zinc, manganese, copper and boron influenced the fruit growth, development and quality of Khasi Mandarin (*Citrus reticulata* Blanco)

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Abstract
An experiment was conducted during 2016 and 2017 at Mizoram University, Aizawl, Mizoram to study role of micronutrients on fruit growth and development of Khasi Mandarin (*Citrus reticulata* Blanco). Randomized block design was adopted with sixteen treatments such as, Foliar application of T_1: Zinc (Zn),T_2:Manganese (Mn),T_3: Copper (Cu), T_4: Boron (B), T_5: Zn + Mn,T_6: Zn + Cu,T_7: Zn + B,T_8: Mn + Cu,T_9: Mn + B,T_{10}: Cu +B,T_{11}: Zn + Mn + Cu,T_{12}: Zn + Mn + B,T_{13}: Zn + Cu + B,T_{14}: Mn + Cu + B,T_{15}: Zn + Mn + Cu + B,T_{16}: Control (no micro nutrients). From the pooled analysis of two consecutive years during the experiment, foliar application of Zn+Cu+ B (T_{13}) showed maximum fruit set percentage, maximum fruit yield, maximum number of fruits per tree, highest fruit retention percentage, highest ascorbic acid content, total sugar and minimum total fruit drop percentage, seed number per fruit and acidity percentage. Whereas, highest TSS and minimum seed weight and minimum peel weight were observed with treatment which received micronutrient combination of Zn + Mn + Cu+ B (T_{15}) against control. However, maximum fruit weight was observed with application of Zn + Mn + B (T_{12}) and maximum fruit juice content with foliar application of Zn + Mn + Cu (T_{11}).

Keywords: Khasi Mandarin, foliar micro nutrients, fruit quality, ascorbic acid

Introduction
Citrus belongs to the family Rutaceae, are rich in refreshing fragrance, thirst-quenching ability, and vitamin C (Ladaniya, 2008) [12]. Citrus production in India is 12.55 million tons from 1.003 million hectares under this crop (Anon., 2018b) [4]. The production of citrus fruit is 12.89 per cent of total fruit production in the country. Oranges constitute about 60 per cent of the total citrus output, Mandarins are the second most important fruit crop in Citrus group after Sweet Orange and has 40.66 % share of total citrus production (Anon., 2018b) [4]. 'Khasi' mandarin is commercially grown in North-East India, historically it is believed that it is the centre for the spreading citrus to other part of the World (Srivastava and Singh, 2006) [21].
Mizoram has the second highest area share under Khasi mandarin in NEH. Khasi mandarin production in Mizoram is 44.02 thousand MT from an area of 16.37thousand Ha (Anon., 2018a) [3]. As per data of National Horticulture Board (2001-2002), Khasi mandarin production was 32.1 thousand MT from an area of 6.90 thousand Ha which clearly showed that Khasi Mandarin production is declining at Mizoram even the area under cultivation has increased to 137%. Improper management of soil fertility and plant nutrition is the major shortcoming in NEH citrus industry apart from the disease or pests problems (Srivastava, 2012) [22]. Citrus is a highly nutrient responsive crop, the productivity of plants depends largely on fruit nutrition. However, due to depletion of soil carbon stock and emerged multiple nutrient deficiencies, the high yield expectancy on a long term basis has failed to sustain by soil application of inorganic fertilizer (Khehra, 2014) [11]. The depletion of the major and minor nutrients limits the productivity of major crops. Most of the applied fertilizer is lost from soil-plant system by leaching, run-off, denitrification and volatilization and pollutes the soil, water bodies and the atmosphere. Imbalance nutrient, soil fertility fast depletion and continuous deterioration in soil physical properties are some added disadvantages of chemical agriculture. Therefore proper management of plant nutrition involving foliar application of micronutrients has strong basis for remunerative Khasi mandarin production. Citrus requires 17 essential elements for the normal growth and production. Though micronutrients are required in small, yet they are very effective and important in fruit growth and development. Foliar application of micronutrients often gives a quicker response than soil application (Obreza *et al*., 2010; Anees *et al.*, 2011) [16, 2] since the plants readily absorbed the nutrients through the leaf surface.

Despite of some shortcomings, it is regarded as the best under certain conditions (Marschner and Marschner, 2012) [14]. Micronutrients help in uptake of macronutrients, helps in cell wall development, formation of chlorophyll, respiration, photosynthesis, activity of enzyme, synthesis of hormone, fixation of nitrogen and reduction (Das, 2003) [7]. Deficiencies of micronutrients are very prominent in citrus reducing crop productivity, stability and sustainability in many Indian soils. In view of this, there is a need for systematic work to evaluate the role of foliar application of micro nutrients in Khasi Mandarin in Mizoram condition to improve the fruit growth and development.

Materials and Method

The experiment was laid out during 2016 and 2017 at farmer's field situated at Thiak, Aizawl district, Mizoram, situated at 23.47^0N latitude & 92.71^0E longitude having an altitude of 1070 m above mean sea level (MSL). The experiment was laid out in randomized block design (RBD) with sixteen treatments namely, Foliar application of T_1: Zinc (Zn),T_2: Manganese (Mn),T_3: Copper (Cu), T_4: Boron (B), T_5: Zn + Mn,T_6: Zn + Cu,T_7: Zn + B,T_8: Mn + Cu,T_9: Mn + B,T_{10}: Cu +B,T_{11}: Zn + Mn + Cu,T_{12}: Zn + Mn + B,T_{13}: Zn + Cu + B,T_{14}: Mn + Cu + B,T_{15}: Zn + Mn + Cu + B,T_{16}: Control (no micro nutrients). Recommended dose of fertilizers N:P:K (Nitrogen: Phosphorus: Potassium) 600:300:600 g per plant per year were applied in all the plants. Full dose of phosphorus and potassium and half split dose of nitrogen in the form of urea, Single Super Phosphate (SSP) and Murate of Potash (MOP) were applied in the month of March during flowering and the remaining half split dose of nitrogen were applied in the month of September, while foliar micronutrients were applied during April and October. Zn was given @0.5%, Mn @0.4%, Cu @ 0.4%, B @ 0.1%. Total number of fruits per plant was counted and expressed in number. Yield per tree (kg tree^{-1}) was calculated by multiplying the average weight (50 fruits) of the fruits with total number of fruits per plant produced at harvest whereas, the percentage of fruit set was calculated as Fruit set (%) = (Number of fruitlets per branch/ Total number of flower per branch) X 100. Data on fruit drop was recorded from date of fruit set to till harvest at specific interval and was expressed in percentage. The percentage of fruit drop was calculated as Fruit drop (%) = {(Fruit Set-Fruit Retention) / Fruit Set} X100, data on the fruit retention was recorded under every treatment at different intervals and at the time of fruit harvesting. The fruit retention percentage was worked out by using the following formula:Fruit retention (%) = {(Total number of fruit set – total no. of fruit drop) / Total no. of fruit set} X 100.The fruit weight was measured by taking 50 representative fruits at random from each replication; these were weighed and expressed in g (average fruit weight). Total number of seeds per fruit were counted and expressed in number. The fruit was cut out and seeds were separated and washed with water, weighed and expressed in gm. Weight of the peel was measured using a digital balance and expressed in gm. After peeling and extracting the seeds, fruit was squeezed to obtain juice and was measured using a measuring cylinder and given in ml. Total Soluble Solids (TSS) content of freshly harvested matured fruit was measured by Handheld Refractrometer which was calibrated at 20^{o}C and expressed in terms of °Brix. Total titratable acidity was determined by titrating the extracted juice against N/10 NaOH using phenolphthalein as indicator and expressed in percentage (AOAC, 1990) [1]. The total sugar content of fruits were estimated by standard procedure of AOAC (1990) [1] using Fehling's A and Fehlings B reagents with methylene blue as an indicator through copper reduction method and to estimate the ascorbic acid content of the fruit, 2, 6 – dichlorophenol indophenol dye titration method was used (AOAC, 1990; Ranganna 1997) [1, 19] and expressed as mg / 100mg of aril.

Results and Discussions

Influence on fruit set, fruit drop percentage, total fruit retention and yield

Significant increase was observed with different treatments in perusal of the pooled data presented in Table 1. Foliar application of Zn + Cu + B (T_{13}) recorded highest fruit set percent (61.89%), highest yield per tree (19.99 kg per tree) along with highest percentage of fruits retention per plant (65.40%) and lowest total fruit drop percent (34.17%) compared with control. Quaggio (2011) [18] reported that Zinc (Zn), manganese (Mn) and boron (B) are important micronutrient for citrus production. Garcia *et al*., 1984 [8] reported that as leaf Zn and Mn content increased, fruit let drop decreased. Karim *et al*., (2017) [9] reported that consequences for tree health and crop production depends on boron (B) in citrus. Foliar application appeared to increase leaf boron concentration which influenced in vivo and in vitro pollen germination in many crops, increased fruit yield which might be due to transportation of applied B to the flowers where it exerted its influence of increased fruit set through an effect on pollen viability and/or pollen tube growth. The increase in fruit no and yield might be due to reduction in fruit drop where Nijjar (1985) [15] reported that Zn is required for prevention of abscission layer formation which thereby decrease the preharvest fruit drop. Perveen and Rehman (2000) [17] also concluded that spray of Zn, Mn and B helps in correcting the deficiency symptoms and improved the citrus fruit yield. B alone spray could not give satisfactory yield, but when it was applied in combination with Zn and Mn, yield was increased.

Table 1: Influence of micronutrients on fruit set, total fruit drop percent, total fruit retention percent and yield

Treatment	Fruit set (%)	Total fruit drop (%)	Total fruit retention (%)	Fruit yield (kg per tree)
T_1	58.37	42.66	57.34	12.21
T_2	57.08	40.65	59.35	11.96
T_3	58.10	42.90	57.10	12.23
T_4	56.66	44.74	55.26	10.26
T_5	58.74	38.75	61.25	13.51
T_6	57.55	43.12	56.88	11.25
T_7	60.97	36.71	63.29	16.87
T_8	58.74	39.96	60.04	13.20
T_9	59.25	40.58	59.42	13.65
T_{10}	61.30	40.72	59.28	14.73
T_{11}	59.89	36.38	63.62	15.90
T_{12}	59.96	35.64	64.36	17.38
T_{13}	62.13	34.17	65.83	19.99
T_{14}	60.96	37.69	62.31	15.19
T_{15}	59.86	35.72	64.28	19.74
T_{16}	54.07	49.10	50.90	8.98
SEm(±)	0.409	2.364	2.725	1.363
$CD_{(0.05)}$	1.183	6.829	7.870	3.937

Influence on number of fruits per plant and fruit physical properties

The data presented in Table 2 revealed that treatment which received micronutrient mixture of Zn + Cu + B (T_{13}) had maximum number of fruits per plant (140.55nos.) and

minimum seed number (12.14nos.) Whereas, plants which received micronutrient mixture of Zn + Mn+ Cu + B (T_{15}) resulted in lowest peel weight (29.89g) and seed weight (1.12g). However, application of Zn + Mn + B (T_{12}) recorded maximum weight of fruit (153.25g). Due to the involvement of Zn and B in hormonal metabolism, increase in cell division, and cell wall expansion, fruit weight increased with the spray of $ZnSO_4$ and Boric acid. B stimulates rapid metabolization of water and sugar in the fruit thereby increasing accumulation of dry matter within the fruit (Bhatt *et al*., 2012) [6]. Tariq *et al*., (2007) [23] recorded minimum peel percentage with spray of Zn + Mn in Sweet Orange. Kazi *et al*., (2012) [10] reported in Sweet Orange that NPK bulk recommended dose + multi micronutrient observed number of fruits per tree significantly higher over other treatments. Singh *et al.,* (2018) [20] supported our findings on his report on Sweet Orange that trees which received combined treatment of Zn+ B + Cu observed minimum number of seeds and minimum peel thickness.

Table 2: Influence of micronutrients on number of fruits per plant and fruit physical properties

Treatment	No of fruits per plant	Fruit weight(g)	Peel weight(g)	Seed No	Seed weight(g)
T_1	108.89	137.73	38.31	17.87	2.75
T_2	110.17	101.18	39.78	17.59	2.60
T_3	102.01	128.86	35.41	17.62	2.08
T_4	100.91	98.74	34.70	17.69	3.21
T_5	114.80	132.43	35.91	17.84	3.02
T_6	100.14	106.51	35.19	17.51	2.53
T_7	127.08	138.64	41.90	16.87	1.85
T_8	105.71	139.01	39.42	16.94	2.12
T_9	100.59	143.55	34.52	15.98	1.47
T_{10}	112.28	134.00	38.81	17.41	1.99
T_{11}	114.96	149.47	33.25	14.56	2.27
T_{12}	124.55	153.25	32.42	17.33	2.01
T_{13}	138.95	143.86	31.92	12.14	1.17
T_{14}	125.85	134.19	37.29	17.37	2.70
T_{15}	126.95	149.95	29.89	17.46	1.12
T_{16}	90.69	97.52	47.14	17.98	3.17
SEm(±)	1.247	1.164	2.329	0.667	0.278
$CD_{(0.05)}$	3.602	3.363	6.726	1.926	0.803

Influence on fruit biochemical properties

The data depicted in Table 3 revealed that application of micronutrients significantly differ in fruit quality. The spray of micronutrient mixture of Zn + Mn+ Cu + B (T_{15}) resulted in highest TSS (10.92 ° Brix), while combination of Zn + Cu + B (T_{13}) recorded highest total sugar (9.18 %) highest ascorbic acid content (51.40mg/100mg of aril)and minimum acidity (0.49%). However, fruit juice content was observed highest (75.25ml) with spray of Zn + Mn + Cu (T_{11}). The findings are in line with Singh *et al*., (2018) [20] in Sweet Orange cv. Mosambi who reported that improved TSS, ascorbic acid, total sugar and minimum acidity were observed with treatment which contained Zinc+ Boron+ Cupper. Previous studies revealed that translocation of photosynthates to the fruit was efficient by regulation of copper, boron and zinc (Ullah *et al*., 2012) [24]. They also reported that acidity of Mandarin was reduced due to higher synthesis of nucleic acid. Cu (copper) increase photosynthetic activity and when combined with Zn and B it increased the sugar compounds and brought more accumulation of total soluble solids in fruit juice. The findings are in accordance with Babu and Yadav (2005) [5] in Khasi Mandarin. Mann *et al*., (1985) [13] found that micronutrients (Zn, Cu, Fe and Mn) application on the leaves of sweet orange resulted improved juice percentage, ascorbic acid content and reducing sugar .

Table 3: Influence of micronutrients on fruit biochemical properties

Treatment	Fruit juice content (ml)	TSS (ºBrix)	Acidity (%)	Total sugar (%)	Ascorbic acid (mg/100g of aril)
T_1	60.59	9.75	0.62	9.23	44.86
T_2	55.25	9.49	0.64	8.73	43.67
T_3	62.75	9.72	0.64	8.86	44.86
T_4	56.08	9.46	0.64	9.20	43.67
T_5	64.42	9.80	0.62	8.97	46.05
T_6	53.17	9.65	0.64	8.79	44.86
T_7	68.58	10.24	0.60	9.18	46.64
T_8	69.83	10.19	0.60	9.07	46.64
T_9	73.58	10.40	0.60	9.14	46.64
T_{10}	56.50	10.44	0.60	9.15	7.83
T_{11}	75.25	10.47	0.58	9.24	49.02
T_{12}	59.78	10.55	0.56	9.27	51.40
T_{13}	64.81	10.66	0.44	9.31	51.40
T_{14}	62.75	9.79	0.62	9.03	46.05
T_{15}	63.16	10.88	0.53	9.29	49.62
T_{16}	42.23	9.34	0.68	8.39	42.77
SEm(±)	2.106	0.198	0.024	0.177	0.871
$CD_{(0.05)}$	6.081	0.572	0.068	0.511	2.516

Conclusion

The investigation revealed that foliar application of combined micronutrients with treatment T_{13} [Zn (0.5%) + Cu (0.4%) + B(0.1%)] recorded highest fruit set %, number of fruits per plant, yield (kg per tree),total fruit retention percentage along with total sugar percentage and ascorbic acid content and minimum acidity, total fruit drop percentage and minimum seed number. While T_{15} [Zn (0.5%) + Mn (0.4%) + Cu (0.4%) + B (0.1%)] recorded maximum TSS and minimum peel weight and seed weight. Hence, it was concluded that the treatment T_{13} [Zn (0.5%) + Cu (0.4%) + B (0.1%)] was the best treatment in terms of fruit growth and development which was next with T_{15} [Zn (0.5%) + Mn (0.4%) + Cu (0.4%) + B (0.1%)] as compared with control plant (T_{16}).

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