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Combined Iodine, Iron and Zinc Biofortification of Tomato Fruit

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ABSTRACT: Deficiencies of zinc (Zn), iron (Fe) and iodine (I) are major malnutritional health problem in the devoloping countries. Biofortification of vegetables with I, Fe and Zn can become an alternative strategy of introducing these elements for human dietary intake. The purpose of this study was to determine the effect of combined I (KIO_3), Fe ($FeSO_4.7H_2O$) and Zn ($ZnSO_4.7H_2O$) supply on I, Fe and Zn concentrations of tomato plants, which is stem and leaf, and their fruits (*Lycopersicon esculentum* L. cv. Swanson). Tomato cultivar was grown in glasshouse conditions with four replications in 10 kg soil and 5% peat mixture. The treatments as contain: contol, each element applied at 10, 20 and 40 mg I-Fe-Zn kg^{-1}, respectively. Concentrations of I, Fe and Zn and essential elements (P, K, Ca, Mg, S, Cu, Mn, Mo, Cl, Si and Ni) as well as non-essential elements (Al, Co, Ti, Br, Rb, Sr, Ba, Cr, Sn, Sb, Te, Ge, Cs, Ce, Ga, Ta, Hf) were determined by Polarized Energy Dispersive X-ray Fluorensence (PEDXRF). Effect of combined I-Fe-Zn treatments on fresh and dry weights of plant and fruit were found statistically important. Iron and Zn concentrations of fruits and plants were increased by combined I-Fe-Zn treatment except for Fe concentration in plant. Application of I-Fe-Zn were not significant effect on essential element concentrations in both plants and fruits, out of Ca, Na and Si concentrations in fruit. No influence of I-Fe-Zn treatment on the measured non-essential elements concentrations with the exception of plant Br concentration and fruit Sr concentration. This study revealed that combined I-Fe-Zn treatment can be used effectively for I, Fe and Zn biofortication of tomato fruits for the dietary intake for human.

Key words: Biofortification, iodine, iron, zinc, tomato (*Lycopersicon esculentum*)

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INTRODUCTION

Micronutrient malnutrition is an inadequate daily diet of iron (Fe), zinc (Zn) and iodine (I) (Stein, 2010; Clemens, 2014) and deficienciey of these nutrition is a reason of serious health problem on world population especially in devolopping countries (Welch et al., 2013; Cakmak and Kutman, 2018; Zou et al., 2019). There are some methods to combat nutrient deficiency such as biofortification, specific plants, transgenic plants or conventional breeding and etc. (Dimkpa and Bindraban, 2016; Kumar et al., 2019) but biofortification is the more impactful, sustainable, low-cost and easier method to enrich the micronutrient content of crops than the other methods for developing countries (Bouis et al., 2011; Diaz-Gomez et al., 2017; Sazawal et al., 2018).

Iodine, Fe and Zn are essential micronutrient for human health and unfortunately, deficiencies are common in both developing and developed countries. Iodine necessity of people is about 150 µg day^{-1} which is especially need for activity of thyroid hormones, besides infant mortalities, mental retardation (Lin et al., 2004; Smolen and Sady, 2012). Anemia is the one of the common health problem by the reason of Fe deficiency, especially about 40-45% of prescholl-age children are anemic, which more than half of the Fe in the human body is bound to hemoglabine (Grillet et al., 2014). The recommended human dietary of Fe varies between 8-18 mg day^{-1} depending on the age, body weight, gender and pregnancy (Anonymous, 2009). Zinc is structural role on thousands of proteins for microorganisms, plants, animals and humans. People daily Zn requirement is 1.5-2.5 mg day^{-1} and due to the deficiency of Zn may occur retarded growth, skeletal abnormalities, hypogonadism, diarrhea, immune dysfunction, delayed wound healing etc. (Salgueiro et al., 2000; Anonymous, 2009; Anonymous, 2017).

Deficiency of reasons of I, Zn and Fe in soil and plant are soil texture, pH, tillage, water management, nutrient interactions, fertilization, type of nutriets and plant cultivars (Hetzel and Pandav, 1994; Lin et al., 2004, Smolen and Sady, 2012; Prasad et al., 2014; Patel et al., 2018; Gonzali et al., 2017; Lyons, 2018). In addition, main important reason of deficieny of I, Fe and Zn concentration is phytic acid. Phytic acid is a compound, which found especially in cereals and therefore has an important influence in daily human food consumption. Unfortunately, bioavailability of some element such as Zn, Fe are relationship with phytic acid. Because, phytic acid obstructed the availability of these element in cereals which there are many studies about it (Cakmak et al., 2010; White and Broadley, 2011; Sperotto et al., 2012; Shahzad et al., 2014; Guo et al., 2016; Maqbool and Beshir, 2018; Cakmak and Kutman, 2018). While vegetables have low phytic acid and high ascorbate content as well as phenolics and carotenoids that it is increased availability of these elements (Gillooly et al., 1983; Siegenberg et al., 1991; Garcia-Alonso et al., 2004; La Frano et al., 2014; Krzepilko et al., 2015; Woch and Hawrylak-Nowak, 2019; Giordano et al., 2019). In these way biofortification of vegetable is an alternative to suppress on the phytic acid metabolism (Majumber et al., 2019). Besides, vegetables such as spinach, lettuce, tomato etc. are short-term growing than the cereals which means that people can uptake nutrient is more quickly and easily. At the same time inceases of concentrations of I, Fe and Zn not only effect on the concentrations of deficit nutrients but also increase the antioxidant compound of plants and so increases of these nutrients will have a positive effect on human health (Blasco et al., 2008; Przybysz et al., 2016; Incrocci et al., 2019).

Among the vegetables, tomato is the most consumed and traded vegetables in the world and it has important nutrients and antioxidants which plays an important role in human diet, especially for vegetarian diet. Additionally, tomato is not only used as a fresh but also it uses as a souce, paste, dried, peeled etc. There is some study about Zn and Fe biofortification on most important cereal like maize, rice or wheat etc. (Cakmak et al., 2010; Sperotto et al., 2012; White and Broadley, 2011; Guo et al., 2016; Maqbool and Beshir, 2018; Cakmak and Kutman, 2018). Unfortunately, there is not any study on the combined I, Fe and Zn biofortification of edible plants (Kiferli et al., 2013; La Frano et al., 2014; Krzepilko et al., 2015; 2016; Giordano et al., 2019).

The aim of this study is to find out I, Fe and Zn biofortification with the supply of those elements and also determine the variations of essential (K, P, Ca, Mg, S, Cu, Mn, Mo, Cl, Si and Ni) and some non-essential (Co, Ti, Br, Rb, Sr, Ba, Cr, Sn, Sb, Te, Ge, Cs, La, Ce, Ga, Ta, Hf,) elements concentrations of tomato plants and fruits. This is the first study about combine I, Fe and Zn biofortification on vegetables and I expect this study to lead the new studies with other vegetables.

MATERIALS AND METHODS

Plant Growth Conditions and Treatments

Tomato plants (*Lycopersicon esculentum* Mill. cv. Swanson) were grown from May 23 to August 16, 2018 in a glasshouse condition at the Department of Soil Science and Plant Nutrition, Ankara University. The experiment was carried out in plastic pots (30cm×24cm×27cm) holding 10,000 g air-dried soil and 5% peat of total soil weight. The soil was taken from the 0-20 cm of experimental fields of the Agricultural Faculty, Ankara University and properties of the soil were determined by the Page (1982) (Table 1). For each element from I (KIO_3), Fe ($FeSO_4.7H_2O$) and Zn ($ZnSO_4.7H_2O$) were applied at the rates of 0, 10, 20 and 40 mg kg^{-1} of soil after the seedling transplantation, respectively. For the basal fertilization, 400 mg N kg^{-1} soil from KNO_3 and 100 mg N kg^{-1} from $CaNO_3$, 100 mg P kg^{-1} from $(NH_4)H_2PO_4$, which total amount of N, P, K and Ca was 545, 100, 1110, 170 mg kg^{-1} respectively, were applied during the plant growth period. The experiment was designed according to a randomized block design with four replications-one plant per one replicate in each treatment. Plants were irrigated with tap water until reached the 70% of field capacity. Plants were cultivated until the bud of the inflorescences in the four cluster was formed. Ripening fruits and leaves were collected for each cluster, weighed and dried during the experiment. Plants were harvested and separated into leaf and stem (plant) and unripened fruits. After determining of fresh weight, the plants and fruits were washed once with tap water and twice in deionized water. Four cluster of plants and fruits were combined with each other, seperately. Unripened fruits were omitted after weighing while concentrations of elements were determined in only ripening fruits. Plant and fruit samples, which are expressed as homogenized leaf and stem and fruit samples, were dried in a drying oven at 65°C and then dry weight recorded. All samples were grounded. Before the determination of elemental concentrations by PEDXRF elemental analysis, samples were pelleted with press machine.

Determination of Mineral Element Concentraions of Soil, Plant and Fruit

Homogenied plants and fruit samples were sieved (200 µm) to determine of the essential and non-essential element concentraitions by PEDXRF (Spectro XLAB2000) as reported by Gunes et al., (2009) at the Earth Sciences Application and Research Centre (YEBIM) of Ankara University.

Statistical Analysis

Analysis of variance was performed on the data with one-way ANOVA using MINITAB 17 and significant differences among treatment means were calculated by LSD test (LSD; $P < 0.05$) and compared by descriptive statistics [±standart error (SE)].

Table 1. Some physical and chemical properties of soil

Properties	Method	Amount/ Quantification	
Texture	-	Loamy	
$CaCO_3$	Scheibler	59.60 g kg^{-1}	
pH	1/2.5 water	7.80	
EC	1/2.5 water	0.35 dS m^{-1}	
Organic Matter	Walkley Black	18.20 g kg^{-1}	
N	Kjeldahl	3.52 g kg^{-1}	
Concentration of elements (NH_4OAc-extractable, g kg^{-1})			
K	0.79	Mg	1.86
Ca	5.10	Na	0.25
Concentration of elements (DTPA-extractable, mg kg^{-1})			
Fe	8.73	Cu	2.06
Zn	4.02	Mn	22.6
Total concentrations of elements (XRF, g kg^{-1})			
P	0.97	Na	0.37
K	13.9	Cl	0.05
Ca	47.7	Si	155
Mg	9.23	Al	42.68
S	1.01	-	-
Total concentrations of elements (mg kg^{-1})			
I	2.30	Ba	452
Fe	32570	Sb	1.60
Zn	123	Sn	7.20
Cu	41.20	Rb	58.04
Mn	703	Cr	72.12
Mo	2.70	Ga	13.60
Se	0.30	Ge	1.00
Cd	0.80	Cs	3.80
Co	37.50	Ta	4.20
Br	3.90	Te	1.20
Ti	3288	Ce	60.30
Ni	54.70	Hf	4.00
Sr	256	-	-

RESULTS AND DISCUSSION

Dry and Fresh Weight of Plant and Fruit

Plant and fruit weight of the tomatoes was presented in Table 2. Biofortification with I-Fe-Zn had positive effect on plant and fruit weight. Effect of combined I-Fe-Zn treatments on dry and fresh weight of plant and fruit were statistically important. The highest fresh and dry weight of plant were determined by 10 mg I-Fe-Zn kg^{-1} of soil, respectively 472, 79.50. There was a relationship between the levels of I-Fe-Zn and fruit weight. Fruit fresh weight increased by the combined I-Fe-Zn treatments and the highest fruit weight were determined by the highest level of combined I-Fe-Zn treatment (40 mg I-Fe-Zn kg^{-1} of soil). Especially, fruit weights were increased by the combined I-Fe-Zn treatments, respectively 16%, 47%, 74% when compared the control treatment. This result is accordance with the study of Weng et al. (2013) who explained that I had positive effect on biomass of 10 different vegetables cultivars. Weng et al. (2008) in their study showed that I treatmant is effective on growth rate of spinach. Blasco et al., (2008), concentration of I and antioxidant compounds of lettuce were increased due to the treatment of I. Gioia et al. (2019) suggested that growth rate with Fe and Zn concentrations of microgreen plants were increased by the Fe and Zn treatments.

Table 2. Effect of combined iodine, iron and zinc treatment on plant fresh and dry weight, and fruit weight

Treatments	Plant Fresh weight g plant^{-1}	Dry weight g plant^{-1}	Fruit Fresh weight g fruit^{-1}
Control	254±11.41 c	53.22±4.22 c	522±22.62 d
10 I-Fe-Zn	472±12.48 a	79.50±4.52 a	607±19.00 c
20 I-Fe-Zn	360±8.34 b	63.92±1.75 b	766±16.50 b
40 I-Fe-Zn	372±9.55 b	64.28±0.48 b	906±5.87 a
F	71.27	11.68	98.26
LSD	32.50**	9.74**	26.63**

** *P* < 0.01.

Concentrations of the Elements

Plant I concentrations were increased by the combined treatment of I, Fe and Zn. The highest concentrations of I and Zn in the plants were determined by the 40 mg kg^{-1} of soil. However, concentration of Fe in plant were not change statististically important. The highest concentrations of I, Fe and Zn in the fruits were determined by highest combine I-Fe-Zn treatment (40 mg kg^{-1}) as 19.9, 39.7 and 39.6 mg kg^{-1} in fruit, respectively (Table 3). Iodine concentrations of plants and fruits were increased due to the increases treatment levels that the highest I concentration were determined by the highest combine treatment, but there was no statistically important difference between the other treatment. Zinc and Fe concentrations of fruits were increased by the the treatments. Some studies demonstrated that some vegetables and fruits such as spinach (Zhu et al., 2003; Weng et al., 2003; Dai et al., 2006; Humphrey et al., 2019), lettuce (Blasco et al., 2008; Voogt et al., 2010), radish and Chinese cabbage (Weng et al., 2003), strawberry (Li et al., 2017a), pepper (Li et al., 2017b) can store I by levels of I treatment. According LandiNİ et al., (2011) fresh weight and I concentration of tomato (*Solanum lycopersicum* L.) were increased by I treatment (5, 10 and 20 mM) and as a result, 5 mM I treatment was enough to uptake a daily human I requirement. Hong et al. (2008) reported that higher than 50 mg I kg^{-1} of soil treatment was shown chlorosis effect on tomato. In the other study by Weng et al. (2013) reported that biofortification of I can be changed due to the different genotypes within the same type of vegetables and levels of treatments. All of these studies results like our results. Iron and Zn concentration of tomato fruits were increased by the I, Fe and Zn treatments. Especially, effect of the highest combined I, Fe and Zn treatment on Fe and Zn concentraions of fruits were remarkable than the other treatment. Researches conducted by Cakmak (2008), Prasad et al. (2014), White and Broadley (2009, 2011), Shahzad et al. (2014), Zaman et al. (2018), Patel et al. (2018), Giardono et al. (2019) shows that combined or separately application of Zn and Fe was reason of the increases of Fe and Zn concentrations as in this research.

Table 3. Effect of combined iodine, iron and zinc treatment on plant and fruit I, Fe and Zn concentrations

Treatments	Plant (mg kg^{-1} DW) I	Fe	Zn
Control	2.50±0.24 c	210±26.20	16.90±1.30 d
10 I-Fe-Zn	68.50±13.0 bc	188±11.10	30.80±0.85 c
20 I-Fe-Zn	162±27.02 b	221±20.10	40.90±1.94 b
40 I-Fe-Zn	308±60.62 a	197±15.50	55.30±4.48 a
F	15.4	0.57	26.2
LSD	104**	ns	7.89**
	Fruit (mg kg-1 DW)		
Control	2.73±0.30 b	28.75±1.11 c	21.05±1.34 c
10 I-Fe-Zn	2.74±0.29 b	30.83±1.57 bc	28.52±1.28 b
20 I-Fe-Zn	5.24±1.77 b	32.39±0.91 b	29.70±0.94 b
40 I-Fe-Zn	19.85±2.71 a	39.64±1.03 a	39.58±1.54 a
F	25.37	16.10	35.54
LSD	5.03**	3.64**	3.99**

ns, non-significant; ** *P* < 0.01.

Treatments of combined I-Fe-Zn had no statistically important effect on the concentrations of P, K, Mg, Na, S, Ca, Si and Al of plants. On the other hand, effect of treatments (40 mg I-Fe-Zn kg^{-1} of soil) on Ca, Na and Si concentrations of fruit were statistically important while P, K, S and Mg of fruit were not statistically important (Table 4). Essential and non-essential elements concentrations of plant and fruit were not signifcantly changed by combined I, Fe and Zn treatments. In the study conducted by Smolen and Sandy (2012) effect of I treatment on P, K, Mg, S, B, Cu, Mn, Mo and Cd concentrations in spinach were not signinificant. On the contrary, concentrations of N, Ca, Na, Fe and Zn of plant were significantly increased by I treatment. Islam et al. (2018) were examined the effect of Fe and I treatment on cherry tomato genotypes. For this purpose, 1 mg Fe L^{-1} and 1 I L^{-1} were applied, seperately. Treatments of these elements were not significant effect on Fe, Mn, Cu and Zn concentrations. In an another study by Krezpilko et al. (2016), 0.5 µM KI L^{-1} treatment was sufficient to enrich seedlings with I and K; however, effect of this treatment was not significant effect on Ca, Zn, Fe and Cu concentration. Krzepiłko et al. (2015) who reported I positive affected the uptake of Mg, Na, Ca and Fe but negative affected Cr uptake in the spinach plant. Smolen and Sady (2011), I treatment was increased the concentrations of Na, Fe, Zn and Al and reduced concentrations of P, S, Cu and Ba concentrations. All of these results show that levels of mineral element concentrations can be change due to the level of I, and Zn treatment and plant genotypes. All of these results of different researcher were showed that concentrations of some essential and non-essential element can change by the levels of treatments and plant cultivars.

Table 4. Effect of combined iodine, iron and zinc treatment on plant and fruit P, K, S, Ca, Mg, Na, Si and Al concentrations

Treatments	Plant (g kg^{-1} DW)							
	P	**K**	**S**	**Ca**	**Mg**	**Na**	**Si**	**Al**
Control	1.85±0.16	27.80±1.43	7.20±0.97	34.7±5.62	5.90±1.27	3.60±0.88	2.20±0.17	0.58±0.06
10 I-Fe-Zn	2.16±0.11	28.83±1.74	8.35±0.60	36.9±3.09	6.52±0.63	3.98±0.67	2.08±0.13	0.48±0.02
20 I-Fe-Zn	2.05±0.27	27.98±1.51	8.48±0.54	39.2±1.60	6.45±0.34	3.40±0.65	2.09±0.11	0.53±0.02
40 I-Fe-Zn	2.02±0.05	24.59±0.32	8.77±0.51	41.4±1.78	6.80±0.38	3.60±0.31	2.17±0.11	0.48±0.02
F	0.60	1.86	1.04	0.71	0.25	0.39	0.20	1.84
LSD	ns	ns	ns	ns	ns	ns	ns	ns
	Fruit (g kg^{-1} DW)							
Control	3.24±0.17	39.29±1.68	1.61±0.09	0.76±0.05 b	1.17±0.11	0.25±0.00 b	0.69±0.02 b	0.32±0.02
10 I-Fe-Zn	3.52±0.15	42.77±2.13	1.79±0.12	0.68± 0.02 b	1.18±0.17	0.24±0.00 b	0.68± 0.01 b	0.32±0.00
20 I-Fe-Zn	3.33±0.12	43.39±2.47	1.75±0.07	0.81±0.06 b	1.01±0.08	0.32±0.06 b	0.79±0.03 a	0.37±0.01
40 I-Fe-Zn	3.89±0.32	49.88±4.21	2.10±0.16	1.08±0.11 a	1.29±0.14	0.97±0.38 a	0.75±0.02 a	0.32±0.02
F	1.94	2.50	3.22	6.23	0.83	3.29	5.81	2.43
LSD	ns	ns	ns	0.21**	ns	0.60*	0.06**	ns

ns non-significant; * $P < 0.05$; ** $P < 0.01$

Levels of treatment had no statistically significant effect on the Cu, Mn, Mo, Cl, Al, Ni, Co and Ce concentrations of the plant and fruit (Table 5).

Table 5. Effect of combined iodine, iron and zinc treatment on plant and fruit Cu, Mn, Mo, Cl, Al, Ni, Co and Ce concentrations

Treatments	Plant (g kg^{-1} DW)						
	Cu	**Mn**	**Mo**	**Cl**	**Ni**	**Co**	**Ce**
Control	7.08±0.69	55.0±6.50	1.43±0.09	16.3±3.17	3.48±0.77	1.93±0.47	14.55±1.27
10 I-Fe-Zn	7.58±0.21	58.1±6.15	2.58±0.32	17.4±0.60	3.95±0.45	1.93±0.40	15.00±2.00
20 I-Fe-Zn	6.73±0.98	54.3±3.78	2.25±0.66	16.9±1.06	3.75±0.47	2.70±0.34	14.57±1.57
40 I-Fe-Zn	6.10±0.57	51.5±1.94	1.93±0.27	16.7±0.65	3.33±0.36	2.35±0.79	15.88±1.07
F	0.85	0.30	1.55	0.08	0.27	0.50	0.17
LSD	ns	ns	ns	ns	ns	ns	ns
	Fruit (g kg^{-1} DW)						
Control	7.15±0.58	11.20±0.35	1.43±0.08	4.16±0.25	1.58±0.15	0.73±0.06	16.25±2.16
10 I-Fe-Zn	7.80±0.65	12.40±0.84	1.75±0.10	4.95±1.16	2.50±0.35	0.95±0.25	14.57±1.92
20 I-Fe-Zn	7.60±0.22	14.15±1.54	2.45±0.56	4.28±0.41	2.10±0.24	0.88±0.28	18.32±1.93
40 I-Fe-Zn	9.10±0.88	19.57±3.98	1.68±0.37	5.09±0.54	3.65±1.48	1.92±1.04	16.40±1.51
F	1.79	2.88	1.67	0.47	1.29	0.97	0.66
LSD	ns	ns	ns	ns	ns	ns	ns

ns, non-significant.

Biofortification treatments at all levels had no significant effect on Cr, Ti, Ga, Rb and Ba concentrations both plant and fruits out of the Br concentration of plant. Compared to the control, Br concentration in the plants and Sr concentration of fruits significantly increased; however, there were no effect on the other elements (Table 6).

Table 6. Effect of combined iodine, iron and zinc treatment on plant and fruit Cr, Ti, Ga, Br, Rb, Ba and Sr concentrations

Treatments	Plant (mg kg^{-1} DW)						
	Cr	**Ti**	**Ga**	**Br**	**Rb**	**Ba**	**Sr**
Control	4.43±0.96	49.95±6.58	0.50±0.12	20.68±3.29 b	3.03±0.29	50.28±5.51	222±31.70
10 I-Fe-Zn	5.63±1.50	52.17±3.68	0.48±0.12	27.23± 1.07 a	3.45±0.19	51.65±2.36	227±19.30
20 [1] I-Fe-Zn	5.67±1.42	59.55±6.77	0.78±0.30	28.80±0.74 a	3.35±0.16	52.40±3.23	244±7.92
40 I-Fe-Zn	4.33±0.51	67.05±6.48	0.55±0.13	28.72±1.82 a	2.93±0.17	53.95±0.44	260±9.83
F	0.40	1.66	0.56	3.75	1.51	0.20	0.79
LSD	ns	ns	ns	6.13*	ns	ns	ns
	Fruit (mg kg^{-1} DW)						
Control	2.53±0.09	3.58±1.22	0.30±0.07	4.38±0.86	4.38±0.34	12.40±2.16	5.53±0.90 b
10 I-Fe-Zn	3.30±0.80	3.35±1.04	0.45±0.10	4.45±0.16	4.80±0.15	6.63±0.43	4.20±0.25 b
20 I-Fe-Zn	2.75±0.10	1.73±0.13	0.23±0.03	5.30±0.72	4.85±0.23	9.35±1.90	5.15±0.48 b
40 I-Fe-Zn	3.20±0.27	1.87±0.09	0.38±0.11	6.30±0.62	5.93±0.58	11.10±2.94	7.70±0.84 a
F	0.74	1.44	1.30	1.94	3.29	1.45	4.81*
LSD	ns	ns	ns	ns	ns	ns	2.08

ns non-significant; *$P < 0.05$

Levels of combined I-Fe-Zn treatments had no statistically significant effects on the concentrations of Sn, Cs, Ge, Sb, Ta, Te and Hf both plant and fruits (Table 7).

Table 7. Effect of combined iodine, iron and zinc treatment on plant and fruit Sn, Cs, Ge, Sb, Ta, Te and Hf concentrations

Treatments	Plant (mg kg^{-1} DW)						
	Sn	**Cs**	**Ge**	**Sb**	**Ta**	**Te**	**Hf**
Control	0.95±0.16	5.70±0.82	0.23±0.03	0.88±0.03	1.20±0.21	1.08±0.16	1.35±0.16
10 I-Fe-Zn	0.85±0.03	5.63±1.26	0.20±0.01	0.95±0.03	1.45±0.06	1.48±0.11	1.40±0.39
20 I-Fe-Zn	0.90±0.00	4.23±0.31	0.20±0.01	0.95±0.12	1.05±0.25	1.40±0.04	1.58±0.19
40 I-Fe-Zn	0.95±0.03	4.23±0.06	0.20±0.01	0.95±0.09	1.35±0.27	1.53±0.33	1.33±0.18
F	0.35	1.17	1.00	0.24	0.65	1.10	0.21
LSD	n.s	n.s	n.s	n.s	n.s	n.s	n.s
	Fruit (mg kg^{-1} DW)						
Control	1.05±0.32	5.53±0.83	0.15±0.03	0.85±0.03	1.15±0.03	1.53±0.26	1.00±0.11
10 I-Fe-Zn	0.93±0.13	4.15±0.03	0.40±0.20	0.88±0.03	1.20±0.04	1.23±0.19	1.63±0.23
20 I-Fe-Zn	0.83±0.03	5.95±1.18	0.15±0.03	0.85±0.03	1.05±0.16	1.28±0.03	1.10±0.15
40 I-Fe-Zn	0.85±0.05	4.20±0.01	0.23±0.03	0.90±0.06	2.78±1.85	1.25±0.03	3.72±2.33
F	0.34	1.64	1.32	0.41	0.78	0.74	1.17
LSD	ns	ns	ns	ns	ns	ns	ns

ns, non-significant

CONCLUSION

Especially in developing countries, people needs daily intake such as Fe, Zn and I which essential for people. This study is the first proof to determine the effect of combined I-Fe-Zn treatments on concentrations of I, Fe and Zn with yield and it shows that biofortification was an important way to eliminate of these three elements deficiency in plants.

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