

Impact of iodine biofortification on greenhouse melon growth and production

ABSTRACT

Aims: Evaluate the impact and biofortification of iodide (I^-) and iodate (IO_3^-), on melon morphology and physiology under greenhouse conditions.

Study design: Experimental design was completely randomized with a 2x2x3 factorial arrangement, for a total of 12 treatments and 4 repetitions, giving a total of 48 experimental units. Means were compared using LSD test at 0.05.

Place and Duration of Study: Experiment was established in greenhouses of Universidad Autónoma Agraria Antonio Narro, Saltillo. Coahuila. Mexico, between April and July 2020.

Methodology: Iodine applications were made 15 days after transplant, with a total of 10 applications to substrate and 5 foliar applications during experiment. Treatments consisted of potassium iodide (KI) applications directly to the substrate and foliar at 0.0, 0.5 and 1 mM of iodide (I^-), every week to the substrate and every 15 days in a foliar way. In same way, with potassium iodate (KIO₃) treatments, applying 0.0, 0.5 and 1 mM of iodate (IO_3^-).

Results: Applications of 1 mM iodate to the substrate increased the number of leaves and leaf area. Yields decreased where iodine was applied. Nitrogen uptake improved in almost all applications of iodide and iodate. Potassium decreased with iodide and iodate applications. The concentration of iodine in the stage of flowering and maturity with applications of iodide to the substrate 1 mM. Iodine concentrations in melon fruit were better at low concentrations of foliar applied iodide and 0.5 mM substrate.

Conclusion: Applications of iodide and iodate have a positive effect to melon fruits, without presenting phytotoxic effects.

Keywords: health; iodine; plant nutrition; Cucumis melo

1. INTRODUCTION

Iodine is an essential micronutrient for human health, as it is required for the synthesis of thyroid hormones: thyroxine (T₄) and triiodothyronine (T₃) [1]. An insufficient intake of iodine causes Iodine Deficiency Disorders (IDDs), which affects millions of people in the world [2]. Recommendations from various institutions and organizations for a sufficient daily supply of iodine for adolescents and adults range from 150 to 200 $\mu\text{g I d}^{-1}$ [3]; pregnant and lactating women have higher iodine needs amounting to 230 and 260 $\mu\text{g I d}^{-1}$, respectively [4]. Biofortification programs in crops with micronutrients can be an alternative strategy or approach for the control of mineral malnutrition [5, 6], Agronomic biofortification with iodine in crops is a new strategy to deal with iodine deficiency in humans [7], agronomic biofortification with iodine in crops is a new strategy to make it against iodine deficiency in humans, most of the Horticultural crops can store iodine, the absorption increases with the amount used during fertilization and has been successfully demonstrated in several plant species [8, 9, 10, 11, 12, 13, 14, 15].

Some reports indicate toxic effects on the reduction of plant biomass due to iodine in the form of iodide and iodate when applied at high doses ($>80 \mu\text{M}$) in different plant species [10, 16]. When excessive doses

of iodine are applied, various symptoms of toxicity are manifested, which can be seen from the deterioration of growth, decrease in biomass, chlorotic spots followed by necrosis of the leaves and death of the plant [16]. However, a positive effect on biomass growth and biomass accumulation has been observed in crops where iodine was used previously, such as spinach, barley, beets, celery, turnip, mustard and alfalfa [17,18, 19, 20]. Especially when iodate is applied it has better results on plant growth [21]. There is currently no published literature on biofortification and effect of iodine for melon. The objective of this study was to evaluate the impact and biofortification of iodide (I^-) and iodate (IO_3^-), on melon morphology and physiology under greenhouse conditions.

2. MATERIAL AND METHODS

2.1 Plant material and growth conditions.

The research was carried out in spring-summer 2020 in a greenhouse at Universidad Autónoma Agraria Antonio Narro, Saltillo. Coahuila. Mexico, between April and July 2020. Environmental conditions of greenhouse had an average temperature of 24 °C and a maximum of 29 °C, relative humidity between 20-60% and average PAR radiation of 200 $\mu\text{M m}^{-2} \text{s}^{-1}$ with a maximum of 416 $\mu\text{M m}^{-2} \text{s}^{-1}$. Hybrid melon seeds (*Cucumis melo* L) cv. Cruiser F1, were used and placed in germinating trays with 200 cavities using acid peat as substrate for germination, placing one seed per cavity, 40 days after sowing, the seedlings were transplanted into 10 L polyethylene containers using an acid peat and perlite mixture as a substrate with a ratio of 80:20 v/v. The planting density was 3.75 plants/ m^2 . When plant had six true leaves, a pruning was carried out on main guide. For nutrition of plant, following nutrient solutions were used, in flowering phase and in fruit filling, with the following concentrations of macronutrients (mM L^{-1}): $\text{NO}_3^- = 14.0$, $\text{NH}_4^+ = 0.6$, $\text{H}_2\text{PO}_4^- = 1.34$, $\text{K}^+ = 6.54$, $\text{Ca}^{2+} = 4.58$, $\text{Mg}^{2+} = 3.82$ (flowering). $\text{NO}_3^- = 10.4$, $\text{NH}_4^+ = 0.6$, $\text{H}_2\text{PO}_4^- = 1.34$, $\text{K}^+ = 7.0$, $\text{Ca}^{2+} = 3.7$, $\text{Mg}^{2+} = 1.93$ (fruit filling). pH was adjusted to 5.8 with sulfuric acid and the electrical conductivity (EC) to 2.0 dS m^{-1} . micronutrients were added in form of EDTA-chelate at a concentration (mg L^{-1}) of iron = 1.5, zinc = 3.0, manganese = 0.6, copper = 0.15 and boron = 0.5.

2.2 Iodine application

Iodine applications were made 15 days after transplantation, with a total of 10 applications to the substrate and 5 foliar applications during experiment. Treatments consisted of potassium iodide (KI) applications directly to substrate and foliar at 0.0, 0.5 and 1 mM of iodide (I^-), every week to the substrate and every 15 days in a foliar way. In a same way potassium iodate (KIO_3) treatments, applying 0.0, 0.5 and 1 mM of iodate (IO_3^-).

2.3 Morphological characteristics

Sampling of the morphological characteristics was carried out at flowering and maturity stage (46 and 96 dat), taking one plant as the experimental unit. Were determined: number of leaves (NH), number of female flowers (NFH), number of male flowers (NFM) and leaf area (AF, cm^2), using a leaf area integrator (LICOR LI-3100C).

2.4 Stomatal conductance

It was made at 49 and 81 days after transplanting, taking one leaf per experimental unit. Between 10:00 and 13:00 hrs, stomatal conductance readings (COND , $\text{mol}\cdot\text{m}^{-2} \text{s}^{-1}$) were taken, using an SC-1 Decagon Devices, Inc. porometer.

2.5 Yield, total soluble solids, and oxidation-reduction potential

Harvest was carried out from 87 days after transplanting and a total of 6 cuts were made. Following variables were taken: number of fruits per plant (NF), average fruit weight (AFW, Kg) and yield (YIELD, kg/m²). The total soluble solids of the fruit pulp (TSS, %) were measured with a refractometer (ATAGO model 1018). The oxidation-reduction potential of the fruit pulp (ORP, mV) was determined with an ORP potentiometer (HI98185-01, HANNA, Inc., USA) [22].

2.6 Mineral analysis of aerial part in stages of flowering, maturity and in melon fruits

For determination of mineral content (N, P, K, Ca, Mg, Fe, Cu, Zn and Mn) same plants used to measure the morphological characteristics were used, they were sampled at 46 days after transplantation (flowering), 96 days after transplantation (maturity) and the analysis in the fruits was carried out with three fruits per treatment, they were placed in a continuous circulation oven at 75 °C for two days and proceeded to grind with a mortar, weighing 1 g of the ground and homogenized samples, were dry incinerated at 550 °C for 3 h, then 10 ml of the mixture of nitric acid and perchloric acid 3:1 (v/v) were added at 200 °C in an oven and proceeded to the filtrate with Whatman 42 paper and brought to a final volume of 100 ml with deionized water. Minerals were determined using a Varian AA-1275 atomic absorption spectrophotometer [23]. For the determination of total nitrogen, the micro-Kjeldhal method was used [24]. Phosphorus was determined by colorimetry using a UV-Vis spectrophotometer (Thermo Scientific Modelo G10S) [25].

2.7 Iodine content analysis

Iodine was determined with alkaline digestion technique [26]. 500 mg of dry leaf sample and previously ground fruits with a mortar were weighed. To sample was added 2 ml of 2M KOH and 1 ml of 2M KNO₃. Predigestion was carried out in an oven at 100 °C for 2 hours under an extractor hood, then a muffle was used at a temperature of 580 °C for 3 hours. Ashes were placed in conical tubes where 2 mL of KOH at 2 mM were added. Tube sample was centrifuged at 12,000 rpm for 15 min. Finally, 1 ml of supernatant was taken and filled to 10 ml with KOH at 2M [27], the reading was taken at 178.215 nm with an ICP optical emission spectrometer (Varian 725, ES).

2.8 Statistic analysis

The experimental design was completely randomized with a 2x2x3 factorial arrangement, for a total of 12 treatments and 4 repetitions, with a total of 48 experimental units. Data was analyzed using an analysis of variance and comparison between means was made with Fisher's least significant difference (MSD) ($P=.05$). All statistical analyzes were performed with Statistica 10.0 software (Statsoft Inc, Tulsa, OK).

3 RESULTS AND DISCUSSION

3.1 Morphological characteristics

Applications of iodide and iodate in melon crops did not show statistical differences between treatments in number of female and male flowers (NFF, NMF) (Table 1). Number of leaves (NL) and leaf area (LA) were statistically significant ($P=.05$). Number of leaves and leaf area increased with application of 1 mM iodate to substrate [28]. Iodide applied to substrate at 1 mM, had a negative effect on NL and LA, possibly due to chemical form of iodine and its concentration [29].

3.2 Stomatal conductance

The decrease in leaf area, along with a reduction in stomatal conductance, can limit both interception of solar energy and the rate of photosynthesis and finally the production of biomass in the plant [30, 31]. In Table 1 with a significance ($P = .05$), applications of iodide to substrate 1 mM, limited the number of leaves and leaf area, and stomatal conductance also decreased drastically in measurements made at 49 and 81 days after transplanting. This is because stomatal conductance is influenced by abiotic stress [32], as is CO₂ fixation, which is reduced after stomata closure through abscisic acid (ABA) [33], due to a possible oxidative effect of iodine [34, 35]. In our results, stomatal conductance at low concentrations of 0.5 mM iodide via foliar route and 0.5 mM iodate via substrate maintained the highest values.

3.3 Yield, total soluble solids and oxidation-reduction potential

Regarding to yield and number of fruits (YIELD and NF), (Table 2) ($P = .05$), this presented a decrease where iodine was applied. Best treatments treated with iodine were the applications of 0.5 and 1 mM iodide and iodate to the substrate, respectively, yields obtained experimentally are competitive according to other studies carried out on melon under greenhouse conditions [36, 37]. Average fruit weight (AFW) did not show statistical differences.

In Table 2, concentration of total soluble solids (TSS) in melon fruits, did not show statistical differences between treatments, however high concentrations of iodide and iodate to 1 mM substrate, begin to show a downward trend of TSS [38], in the oxidation-reduction potential, maintains the reduced values in negative range, without alter antioxidant capacity of fruit [22, 39].

Table 1. Effect of the form of application of iodide and iodate by substrate or foliar route, on morphological characteristics and stomatal conductance.

Form	Application	Concentration (mM I ⁻)	NFF	NMF	NL	LA (cm ²)	Stomatal conductance (mmol m ⁻² s ⁻¹)	
							49	81
							days after transplanting	
Iodide	Substrate	0	49.50a ^Z	374.5a	129.83ab	17961.21ab	232.13ab	248.68ab
Iodide	Foliar	0	62.17a	430.75a	141.08a	18097.93ab	195.03b	186.18bc
Iodide	Substrate	0.5	72.00a	403a	139.33a	17938.68ab	201.90b	202.15abc
Iodide	Foliar	0.5	59.67a	396a	137.00a	14484.24bc	250.90a	253.38a
Iodide	Substrate	1	66.67a	382.00a	102.58b	12405.61c	134.03c	184.93c
Iodide	Foliar	1	62.33a	296.00a	103.67b	13730.98bc	211ab	198.9abc
Iodate	Substrate	0	49.50a	374.50a	129.83ab	17961.21ab	232.13ab	248.68ab
Iodate	Foliar	0	62.17a	430.75a	141.08a	18097.93ab	195.03b	186.18bc
Iodate	Substrate	0.5	70.67a	343.00a	119.33ab	15156.43bc	252.47a	238.4abc
Iodate	Foliar	0.5	66.33a	398.00a	125.17ab	17554.97ab	198.80b	220.33abc
Iodate	Substrate	1	54.00a	423.00a	146.00a	21218.28a	217.60ab	184.48c
Iodate	Foliar	1	69.33a	428.00a	127.67ab	18380.28ab	187.80b	201.73abc
LSD			23.24	181.91	32.35*	4839.40*	45.86*	63.43*

^z= values with the same letter are statistically equal in accordance with the LSD to a test $P= .05$, *= significant difference to a $P= .05$, NFF= number of female flowers, NMF= number of male flowers, NL= number of leaves, LA= leaf area.

Table 2. Yield, total soluble solids and oxidation-reduction potential with iodide and iodate applications in melon fruits.

Form	Application	Concentration (mM I ⁻)	NF	AFW (Kg)	YIELD (Kg/m ²)	TSS (%)	ORP (mV)
Iodide	Substrate	0	2.00a	0.89a	6.63ab	8.78a	-58.96a
Iodide	Foliar	0	1.75ab	1.14a	7.23a	8.40a	-52.48a
Iodide	Substrate	0.5	1.75ab	0.85a	5.43abc	8.50a	-42.75a
Iodide	Foliar	0.5	1.25ab	1.01a	4.40bc	8.95a	-34.45a
Iodide	Substrate	1	1.33ab	0.88a	3.93c	7.47a	-38.13a
Iodide	Foliar	1	1.25ab	0.94a	4.22bc	8.55a	-42.68a
Iodate	Substrate	0	2.00a	0.89a	6.63ab	8.78a	-58.96a
Iodate	Foliar	0	1.75ab	1.14a	7.23a	8.40a	-52.48a
Iodate	Substrate	0.5	1.25ab	1.22a	5.16abc	8.60a	-36.63a
Iodate	Foliar	0.5	1.50ab	0.97a	4.81abc	8.25a	-53.28a
Iodate	Substrate	1	1.50ab	1.01a	5.62abc	7.55a	-33.20a
Iodate	Foliar	1	1.00b	1.20a	4.50bc	8.40a	-30.98a
	DMS		0.87*	0.4	2.66*	1.86	42.34

^z= values with the same letter are statistically equal according to LSD at a $P= .05$, *= significant difference at a $P= .05$, NF= number of fruits per plant, AFW= average fruit weight, TSS= total soluble solids, ORP = oxidation-reduction potential.

3.4 Mineral analysis in the stages of flowering, maturity and in melon fruits

Results of concentration of minerals in the flowering stage (46 days after transplant, $P= .05$), are observed in Table 3. Concentrations of N in plants treated with iodine increased considerably compared to those where no applications of iodine were made, except for applications of iodate to 0.5 mM substrate [40]. In P, it had better absorption with applications of 0.5 and 1 mM iodide to substrate and 0.5 mM foliar iodide [41], compared to foliar iodate and substrate (0, 0.5 and 1 mM). In K, there was a variation in potassium concentrations between treatments, possibly due to competition with Mg [42]. These results describe a normal behavior of Ca in most treatments, lower requirements are observed in flowering stage [43], highlighting a better adsorption of Ca with iodide and iodate applied foliar 1 and 0.5 mM respectively [44]. In Mg, greater absorption efficiency was observed with 0.5 mM foliar applied iodate and 1 mM iodide applied to substrate and foliar, it should be mentioned that in this flowering stage Mg absorption increased considerably [45, 46]. Also, concentrations of Fe increase with applications of iodide and foliar iodate and to the substrate 0.5 mM and iodide applied to substrate 1 mM, compared to rest of treatments. In Cu, with the application of iodate to the substrate 1 mM, it showed a decrease compared to rest of treatments. As for Zn and Mn, their concentration tended to increase with the 0.5 mM iodide applied to substrate.

In maturity stage (96 days after transplant, $P=.05$, Table 3), N concentration was higher in plants treated with 0.5 and 1 mM iodide and iodate to the substrate [47]; it should be noted that iodine applications did not affect N adsorption. In contrast, iodide treatments reduce the nitrogen concentration in lettuce leaves [48]. However, in our study levels of nutritional sufficiency of nitrogen were maintained in flowering and maturity stages (46 and 96 days after transplant) [42]. Accumulation of P in aerial part at maturity stage presented a decrease when applying iodide and iodate 0.5 and 1 mM by foliar route and to substrate, respectively. It is worth mentioning that treatments of iodide applied to substrate 0.5 and 1 mM, and

applied foliar 1 mM, and iodate applied foliar 0.5 and 1 mM, in addition to substrate 0.5 mM, iodide and iodate 0 mM, had a range of P concentration between 0.3-0.5%, which is considered to be ideal requirement for favorable growth [49]. On other hand, in treatments with 0.5 and 1 mM iodide applied to substrate, 0.5 and 1 mM foliar applied, 0.5 and 1 mM iodate applied to substrate, 1 mM foliar-applied iodate, they presented low concentrations of K. interpreted as resulting from some kind of stress [50].

The possible phytotoxic effect of iodine on plant growth may have been caused by excessive accumulation of this element in plant tissues or by intracellular oxidation to I_2 after uptake [51, 52]. However, 0.5 mM foliar iodate presented best concentration of K. In case of Ca, 0.5 mM iodide applied to substrate presented best accumulation of Ca, and when 0.5 and 1 mM iodate was applied foliar and substrate, 1 mM, iodide and iodate applied to foliar and 0 mM substrate, presented low values. Compared to flowering stage (46 days after transplant), where plant is growing, there was a higher absorption of Mg, however, in maturity stage (96 days after transplant) there was a decrease in absorption of Mg in most of treatments, being iodide applied to substrate 0.5 mM, treatment that presented highest concentration of Mg. Concentrations of Fe toxicity are above 500 mg kg^{-1} in dry weight [45],

in our results highest concentrations of Fe were obtained with the applications of 0.5 mM iodate applied to substrate with values of 446 mg kg^{-1} in dry weight, it was close to toxicity limits; on the other hand, minimum values of Fe are $\leq 40 \text{ mg kg}^{-1}$ in dry weight [42], in most of our results they were in the normal ranges. In Cu and Zn they were not affected by the treatments in maturity stage (96 days after transplantation). In case of Mn, highest concentrations were obtained with applications of iodide applied to substrate 1 mM.

In concentration of minerals in fruits Table 3 ($P= .05$), in N it maintained the relatively high trend presented in the stages of flowering and maturation, being reflected in fruits mainly in the treatment applied to the substrate 0.5 mM. Highest concentration of P in fruit occurred with iodide treatment applied to substrate at 1 mM. In K there were no significant differences. In Ca, 0.5 mM foliar applied iodide presented best values. In case of Mg, 0.5 and 1 mM foliar applied iodide treatments and 1 mM iodide applied to substrate, presented highest concentrations. Also, iodide applied foliar 1 mM, presented high values in Fe. In contrast, with applications of iodate applied to substrate 1 mM, in Cu it had higher concentration. Respect to Zn, was more concentrated in iodide treatment applied to substrate 0.5 mM and finally concentrations of Mn were higher in foliar iodate treatment 1 mM.

Table 3. Effect of iodide and iodate on concentration of minerals in flowering stage, maturity and melon fruits.

Form	Application	Concentration (mM I ⁻)	N	P	K	Ca	Mg	Fe	Cu	Zn	Mn
			%						mg Kg ⁻¹		
Flowering stage (46 days after transplant)											
Iodide	Substrate	0	5.35e ^z	0.42cde	1.56abc	2.32ab	0.59abc	87.67c	3.00a	27.33b	137.00c
Iodide	Foliar	0	5.51de	0.39ef	2.18a	2.36ab	0.61abc	130.00a	3.00a	30.00b	187.00ab
Iodide	Substrate	0.5	5.96d	0.57a	1.81abc	2.27ab	0.61abc	111.67abc	4.00a	40.33a	206.00a
Iodide	Foliar	0.5	6.87ab	0.53ab	1.34abc	2.31ab	0.52bc	110.00abc	3.67a	39.00a	186.00abc
Iodide	Substrate	1	7.22a	0.50abc	1.52abc	2.37ab	0.71a	110.00abc	2.67ab	28.33b	158.67abc
Iodide	Foliar	1	6.05cd	0.32f	2.02ab	2.54a	0.71a	92.00bc	3.00a	35.33ab	177.67abc
Iodate	Substrate	0	5.35e	0.42cde	1.56abc	2.32ab	0.59abc	87.67c	3.00a	27.33b	137.00c
Iodate	Foliar	0	5.51de	0.39ef	2.18a	2.36ab	0.61abc	130.00a	3.00a	30.00b	187.00ab
Iodate	Substrate	0.5	4.41f	0.38ef	1.11c	2.36ab	0.67ab	120.67ab	3.33a	32.67ab	155.33bc
Iodate	Foliar	0.5	7.23a	0.49abc	1.15bc	2.50a	0.72a	107.00abc	3.67a	29.33b	160.00abc
Iodate	Substrate	1	5.80de	0.47bcd	1.04c	2.29ab	0.49c	89.67bc	1.33b	35.00ab	159.00abc
Iodate	Foliar	1	6.59bc	0.42cde	1.71abc	2.12b	0.61abc	84.67c	3.33a	28.67b	150.67bc
		LSD	0.59*	0.08*	0.89*	0.31*	0.17*	31.16*	1.51*	8.70*	49.60*
Maturity stage (96 days after transplant)											
Iodide	Substrate	0	3.78de	0.35a	2.25ab	0.10c	0.14de	388.67ab	8.00a	59.33a	81.33d
Iodide	Foliar	0	3.93cde	0.33a	2.21ab	0.09c	0.17de	354.33abc	7.67a	55.33a	94.00d
Iodide	Substrate	0.5	5.69a	0.32ab	0.53d	2.05a	0.30a	358.00ab	7.67a	53.67a	60.33e
Iodide	Foliar	0.5	4.97b	0.28b	0.73d	1.91ab	0.25b	379.67ab	7.00a	74.00a	92.33d
Iodide	Substrate	1	4.37bcd	0.33a	0.63d	1.89ab	0.26ab	144.67d	7.33a	69.67a	174.00a
Iodide	Foliar	1	3.99cde	0.32ab	1.22cd	1.40ab	0.22bc	334.67abc	8.33a	60.33a	98.67cd
Iodate	Substrate	0	3.78de	0.35a	2.25ab	0.10c	0.14de	388.67ab	8.00a	59.33a	81.33d
Iodate	Foliar	0	3.93cde	0.33a	2.21ab	0.09c	0.17de	354.33abc	7.67a	55.33a	94.00d
Iodate	Substrate	0.5	3.69e	0.31ab	1.08cd	1.38b	0.26ab	446.00a	7.33a	59.33a	115.33c
Iodate	Foliar	0.5	4.49bc	0.31ab	2.91a	0.14c	0.13e	280.00bcd	7.67a	66.33a	94.33d
Iodate	Substrate	1	5.00b	0.28b	1.54bc	0.60c	0.18cd	308.00abc	8.00a	60.20a	93.00d
Iodate	Foliar	1	4.14cde	0.32ab	1.85bc	0.12c	0.14de	210.00cd	7.33a	62.00a	151.00b
		LSD	0.66*	0.05*	0.77*	0.65*	0.04*	144.35*	1.57*	21.07	20.47*

Fruits											
Iodide	Substrate	0	3.29cd	0.29bc	2.72a	0.77cd	0.50ab	63.67bc	2.67bc	38.67b	5.33bcd
Iodide	Foliar	0	2.78d	0.27c	2.45a	0.80bcd	0.39ab	62.33bc	3.00abc	38.67b	7.67ab
Iodide	Substrate	0.5	5.59a	0.31bc	2.50a	0.61ef	0.51ab	49.33c	3.67ab	76.67a	5.83abcd
Iodide	Foliar	0.5	3.53bc	0.28bc	2.40a	0.98a	0.62a	66.00bc	2.00c	49.33ab	5.00cd
Iodide	Substrate	1	3.28cd	0.42a	2.14a	0.89abc	0.55a	87.33ab	3.33abc	41.00b	3.67d
Iodide	Foliar	1	4.08b	0.27c	2.41a	0.90ab	0.56a	99.33a	2.00c	31.00b	4.00cd
Iodate	Substrate	0	3.29cd	0.29bc	2.72a	0.77cd	0.50ab	63.67bc	2.67bc	38.67b	5.33bcd
Iodate	Foliar	0	2.78d	0.27c	2.45a	0.80bcd	0.39ab	62.33bc	3.00abc	38.67b	7.67ab
Iodate	Substrate	0.5	3.81bc	0.31bc	2.30a	0.53fg	0.33ab	59.00c	2.00c	33.33b	4.00cd
Iodate	Foliar	0.5	3.32cd	0.26c	2.69a	0.47g	0.42ab	89.00ab	3.67ab	31.33b	4.67cd
Iodate	Substrate	1	3.64bc	0.34abc	2.43a	0.72de	0.52ab	56.67c	4.33a	35.33b	6.33abc
Iodate	Foliar	1	3.96b	0.37ab	1.94a	0.55fg	0.23b	56.00c	4.00ab	43.67b	8.00a
LSD			0.62*	0.10*	0.81	0.12*	0.29*	23.85*	1.66*	27.67*	2.37*

²= values with same letter are statistically equal accordance to the LSD at a test $P= .05$, * = significant difference at a $P= .05$.

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3.5 Iodine content analysis

Results of the iodine concentration in aerial part in flowering and maturity stage (46 and 96 days after transplanting), are represented in Fig 1 and 2. Highest concentration of iodine in leaves in flowering stage and maturity occurred when 1 mM iodide was applied to substrate, because root absorbs iodide (I^-) at a higher rate than iodate (IO_3^-) [10, 11, 53], in addition, iodine transport is mainly through xylem and only slightly through phloem [54]. Iodate goes through a reduction process to iodine (I^-) before root absorption, therefore, adsorption rate of iodate is slow, it is limited to reduction process [29]. In Fig 3, increase in concentration of iodine in fruits is described using low concentrations of iodide applied foliar and substrate 0.5 mM in comparison with same dose of iodate. Regarding foliar applications of iodine, studies carried out found evidence of transport system and distribution of iodine through the phloem from leaves to fruits [11], although mechanisms of solute absorption by surfaces of leaves are not yet known exactly, stomata may play an important role in absorption of nutrients applied by air [55].

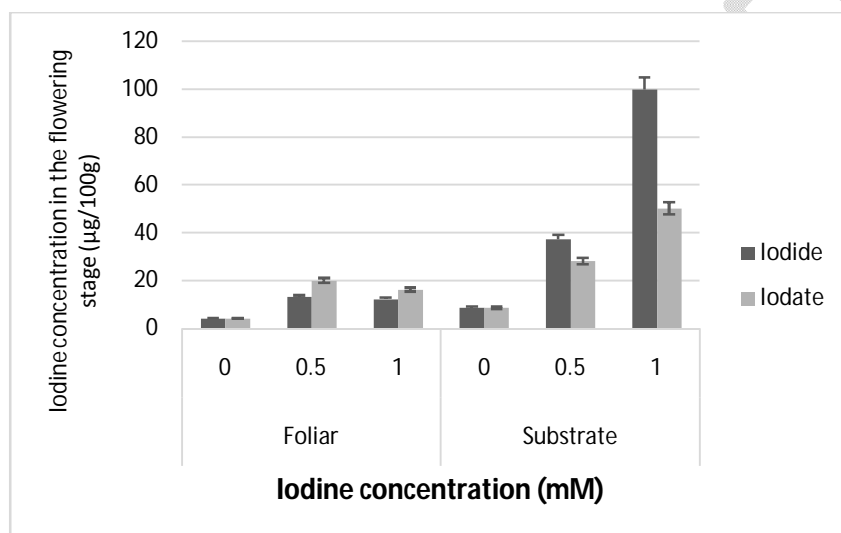


Fig. 1. Iodine concentration in flowering stage with applications of iodide and iodate to substrate and foliar.

Legend: Bar is standard error of means

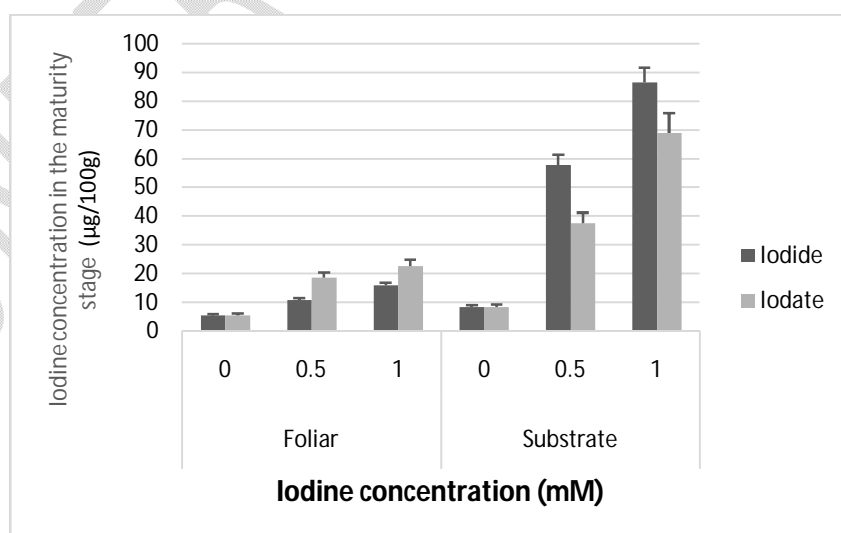


Fig. 2. Iodine concentration in maturity stage, in melon with applications of iodide and iodate to substrate and foliar.

Legend: Bar is standard error of means

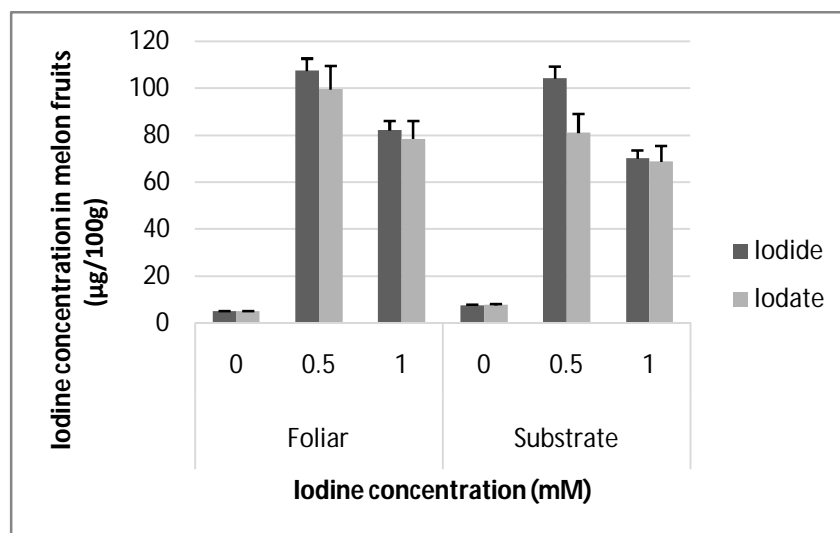


Fig. 3. Iodine concentration in melon fruits with applications of iodide and iodate to the substrate and foliar.

Legend: Bar is standard error of means

4. CONCLUSION

In our results with applications of iodide and iodate is possible accumulation of iodine in fruits. Plants can efficiently absorb and translocate sufficient quantities of iodine with low doses of 0.5 mM iodide and iodate, without presenting phytotoxic effects. Therefore, from two forms of iodine application, iodate could be recommended form due to its slow release characteristics. However, more research is needed under controlled conditions to better understand impact of iodide and iodate.

REFERENCES

1. Babiker A, Alawi A, Al Atawi M, Al Alwan I. The role of micronutrients in thyroid dysfunction. *Sudan J Paediatr.* 2020;20(1):13-19. DOI: 10.24911/SJP.106-1587138942.
2. Caffagni A, Pecchioni N, Meriggi P, Bucci V, Sabatini E, Acciarri N, Ciriaci T, Pulcini L, Felicioni N, Beretta M, Milc J. Iodine uptake and distribution in horticultural and fruit tree species. *Ital J Agronomy.* 2012;7(3):229–236. DOI: <http://dx.doi.org/10.4081/ija.2012.e32>
3. Lawson PG, Daum D, Czauderna R, Meuser H, Härtling JW. Soil versus foliar iodine fertilization as a biofortification strategy for field-grown vegetables. *Front Plant Sci.* 2015; 23;6:450. DOI: 10.3389/fpls.2015.00450. PMID: 26157445.
4. Chittimoju SB, Pearce EN. Iodine Deficiency and Supplementation in Pregnancy. *Clin Obstet Gynecol.* 2019;62(2):330-338. DOI: 10.1097/GRF.0000000000000428. PMID: 30829881.
5. Lyons GH, Stangoulis JC, Graham RD. Exploiting micronutrient interaction to optimize biofortification programs: the case for inclusion of selenium and iodine in the HarvestPlus program. *Nutr Rev.* 2004;62(6):247-52. DOI: 10.1301/nr2004.jun247-252. PMID: 15291398.
6. Nestel P, Bouis HE, Meenakshi JV, Pfeiffer W. Biofortification of staple food crops. *J Nutr.* 2006;136(4):1064-7. DOI: 10.1093/jn/136.4.1064. PMID: 16549478.

7. Lawson PG, Daum D, Czauderna R, Meuser H, Härtling JW. Soil versus foliar iodine fertilization as a biofortification strategy for field-grown vegetables. *Front Plant Sci.* 2015;23;6:450. DOI: 10.3389/fpls.2015.00450. PMID: 26157445; PMCID: PMC4477264.
8. Blasco B, Rios JJ, Cervilla LM, Sánchez-Rodríguez E, Ruiz JM, Romero L. Iodine biofortification and antioxidant capacity of lettuce: potential benefits for cultivation and human health. *Annals of Applied Biology.* 2008;152(3):289–99. DOI: <https://doi.org/10.1111/j.1744-7348.2008.00217.x>
9. Voogt W, Holwerda HT, Khodabaks R. Biofortification of lettuce (*Lactuca sativa* L.) with iodine: the effect of iodine form and concentration in the nutrient solution on growth, development and iodine uptake of lettuce grown in water culture. *J Sci Food Agric.* 2010;15;90(5):906-13. DOI: 10.1002/jsfa.3902. PMID: 20355129.
10. Caffagni A, Arru L, Meriggi P, Milc J, Perata P, Pecchioni N. Iodine Fortification Plant Screening Process and Accumulation in Tomato Fruits and Potato Tubers. *Commun Soil Sci Plant Anal.* 2011; 42(6): 706–718. DOI: <https://doi.org/10.1080/00103624.2011.550372>
11. Landini M, Gonzali S, Perata P. Iodine biofortification in tomato. *J Plant Nutr Soil Sci.* 2011; 174(3): 480-486. DOI: <https://doi.org/10.1002/jpln.201000395>
12. Kiferle C, Gonzali S, Holwerda HT, Ibaceta RR, Perata P. Tomato fruits: a good target for iodine biofortification. *Front Plant Sci.* 2013; 27;4:205. doi: 10.3389/fpls.2013.00205. PMID: 23818889; PMCID: PMC3694224.
13. Smoleń S, Sady W, Ledwożyw-Smoleń I, Strzetelski P, Liszka-Skoczylas M, Rożek S. Quality of fresh and stored carrots depending on iodine and nitrogen fertilization. *Food Chem.* 2014; 15;159:316-22. doi: 10.1016/j.foodchem.2014.03.024. Epub 2014 Mar 15. PMID: 24767061.
14. Cakmak I, Prom-U-Thai C, Guilherme LRG, Rashid A, Hora KH, Yazici A, Savasli E, Kalayci E, Tutus Y, Phuphong P, Rizwan M, Martins FAD, Dinali GS, Ozturk L. Iodine biofortification of wheat, rice and maize through fertilizer strategy. *Plant and Soil.* 2017; 418(1);319-335. DOI: <https://doi.org/10.1007/s11104-017-3295-9>
15. Budke C, thor Straten S, Mühlhng KH, Broll G, Daum D. Iodine biofortification of field-grown strawberries—Approaches and their limitations. *Scientia Horticulturae.* 2020;269:109317. DOI: <https://doi.org/10.1016/j.scienta.2020.109317>.
16. Mackowiak CL, Grossl PR. Iodate and iodide effects on iodine uptake and partitioning in rice (*Oryza sativa* L.) grown in solution culture. *Plant Soil.* 1999;212(2):135-43. DOI: 10.1023/a:1004666607330. PMID: 11762382.
17. Smoleń S, Wierzińska J, Sady W, Kołton A, Wiszniewska A, Liszka-Skoczylas M. Iodine biofortification with additional application of salicylic acid affects yield and selected parameters of chemical composition of tomato fruits (*Solanum lycopersicum* L.). *Scientia Horticulturae.* 2015;188: 89-96. DOI: <https://doi.org/10.1016/j.scienta.2015.03.023>
18. Pauwels GB. Iodine as a micronutrient for plants. *Plant and Soil.* 1961; 14(4):377-392. DOI:10.1007/BF01666295
19. Dai JL, Zhu YG, Zhang M, Huang YZ. Selecting iodine-enriched vegetables and the residual effect of iodate application to soil. *Biol Trace Elem Res.* 2004;101(3):265-76. DOI: 10.1385/BTER:101:3:265. PMID: 15564656.
20. Altınok S, Sozudogru-Ok S, Halilova H. Effect of iodine treatments on forage yields of alfalfa. *Communications in soil science and plant analysis.* 2003;34(1-2):55-64. DOI: <http://dx.doi.org/10.1081/CSS-120017415>
21. Dai JL, Zhu YG, Huang YZ, Zhang M, Song JL. Availability of iodide and iodate to spinach (*Spinacia oleracea* L.) in relation to total iodine in soil solution. *Plant and Soil.* 2006;289(1–2):301–308. DOI:10.1007/s11104-006-9139-7
22. Benavides A, Foroughbakhch R, Verde MJ. Alta correlación de la productividad con los sólidos solubles y redox de peciolas en espinacas. *Ciencia UANL.* 1999;2:373–378. Retrieved from <http://docplayer.es/13247131-Productividad-solidos-solubles-y-redox-de-extractos-de-peciolas-en-espinacas.html>
23. Fick KR, Miller SM, Funk JD, McDowell LR, Houser RH. Methods of mineral analysis for plant and animal tissues. AID publications. 1976.

24. Bremner JM. Nitrogen-total. *Methods of soil analysis: Part 3 Chemical methods*. 1996;5:1085-1121. DOI: <https://doi.org/10.2136/sssabookser5.3.c37>
25. Association of Official Agricultural Chemists, Horwitz W. *Official methods of analysis* (Vol. 222). Washington, DC: Association of Official Analytical Chemists. 1975. Retrieved from: http://sutlib2.sut.ac.th/sut_contents/H125800.pdf
26. Fischer PW, L'Abbé MR, Giroux A. Colorimetric determination of total iodine in foods by iodide-catalyzed reduction of Ce⁺⁴. *J Assoc Off Anal Chem*. 1986;69(4):687-9. PMID: 3745099.
27. Ujowundu CO, Ukoha AI, Agha CN, Nwachukwu N, Igwe KO, Kalu FN. Effects of potassium iodate application on the biomass and iodine concentration of selected indigenous nigerian vegetables. *Afr J Biotechnol*. 2010;9(42):7141–7147. DOI: 10.5897/AJB09.1268
28. Blasco B, Ríos JJ, Cervilla LM, Sánchez-Rodríguez E, Rubio-Wilhelmi MM, Rosales MA, Romero L, Ruiz JM. Iodine application affects nitrogen-use efficiency of lettuce plants (*Lactuca sativa* L.). *Acta Agric Scand B Soil Plant Sci*. 2011;61(4):378–383. DOI: 10.1080/09064710.2010.492782
29. Zhu YG, Huang YZ, Hu Y, Liu YX. Iodine uptake by spinach (*Spinacia oleracea* L.) plants grown in solution culture: effects of iodine species and solution concentrations. *Environ Int*. 2003;29(1):33-7. DOI: 10.1016/S0160-4120(02)00129-0. PMID: 12605934.
30. Dai Z, Edwards GE, Ku MS. Control of Photosynthesis and Stomatal Conductance in *Ricinus communis* L. (Castor Bean) by Leaf to Air Vapor Pressure Deficit. *Plant Physiol*. 1992;99(4):1426-34. DOI: 10.1104/pp.99.4.1426. PMID: 16669054; PMCID: PMC1080643.
31. Hall AE, Schulze ED. Stomatal response to environment and a possible interrelation between stomatal effects on transpiration and CO₂ assimilation. *Plant Cell Environ*. 1980;3(6):467–474. DOI: 10.1111/1365-3040.ep11587040
32. Ronga D, Lovelli S, Zaccardelli M, Perrone D, Ulrici A, Francia E, Milc J, Pecchioni N. Physiological responses of processing tomato in organic and conventional Mediterranean cropping systems. *Scientia Horticulturae*. 2015;190:161–172. DOI: <http://doi.org/10.1016/j.scienta.2015.04.027>
33. Leyva R, Sánchez-Rodríguez E, Ríos JJ, Rubio-Wilhelmi MM, Romero L, Ruiz JM, Blasco B. Beneficial effects of exogenous iodine in lettuce plants subjected to salinity stress. *Plant Sci*. 2011;181(2):195-202. DOI: 10.1016/j.plantsci.2011.05.007. Epub 2011 May 23. PMID: 21683885.
34. Mynett A, Wain R. Herbicidal action of iodide: effect on chlorophyll content and photosynthesis in dwarf bean *Phaseolus vulgaris*. *Weed Research*. 1973;13(1):101–109. DOI: 10.1111/j.1365-3180.1973.tb01250.x
35. Blasco B, Ríos JJ, Leyva R, Cervilla LM, Sánchez-Rodríguez E, Rubio-Wilhelmi MM, Rosales MA, Ruiz JM, Romero L. Does iodine biofortification affect oxidative metabolism in lettuce plants? *Biol Trace Elem Res*. 2011;142(3):831-42. DOI: 10.1007/s12011-010-8816-9. Epub 2010 Sep 14. PMID: 20838926.
36. Moreno RA, García GL, Cano RP, Martínez CV, Márquez HC, Rodríguez DN. Desarrollo del cultivo de melón (*Cucumis melo*) con vermicompost bajo condiciones de invernadero. *Ecosistemas Y Recursos Agropecuarios*. 2014;1(2):163–173. DOI: <https://doi.org/10.19136/era.a1n2.164>
37. Vásquez MN, Folegatti MV, da Silva Dias N, de Sousa VF. Fertirriego por goteo superficial y enterrado del melón (*Cucumis melo* L.) en invernadero. *Ingeniería del agua*. 2006;13(1):13-23. DOI: <http://dx.doi.org/10.4995/ia.2006.2884>
38. Kiferle C, Gonzali S, Holwerda HT, Ibaceta RR, Perata P. Tomato fruits: a good target for iodine biofortification. *Front Plant Sci*. 2013;27;4:205. DOI: 10.3389/fpls.2013.00205. PMID: 23818889; PMCID: PMC3694224.
39. Juárez MA, Ortega OH, Pérez LF, Cadenas PG, Benavides MA. Cu Nanoparticles absorbed on chitosan hydrogels positively alter morphological, production, and quality characteristics of tomato. *J Appl Bot Food Qual*. 2016;89:183-189. DOI: 10.5073/JABFQ.2016.089.023
40. Smoleń S, Rożek S, Ledwożyw SI, Strzetelski P. Preliminary evaluation of the influence of soil fertilization and foliar nutrition with iodine on the efficiency of iodine biofortification and chemical

- composition of lettuce. *Journal of Elementology*. 2011;16(4):613-622. DOI: 10.5601/jelem.2011.16.4.10
41. Osuna HTG, Mendoza AB, Morales CR, Rubio EM, Star JV, Ruvalcaba RM. Iodine application increased ascorbic acid content and modified the vascular tissue in *Opuntia Ficus-Indica* L. *Pak. J. Bot*: 2014;46(1);127-134.
 42. Barker AV, Pilbeam DJ. *Handbook of plant nutrition* (2nd ed.). CRC press. 2015: DOI: <https://doi.org/10.1201/b18458>
 43. Fageria NK. *The use of nutrients in crop plants*. CRC press. 2016. DOI: <https://doi.org/10.1201/9781420075113>
 44. Singh SP, Kumawat N, Kumar V, Singh S. Response of organic manure and inorganic iodine fertilization on minerals content in spinach (*Spinacia oleracea* L.). *J Pharm Phytochem*. 2018;7(1); 1899-1903.
 45. Marschner H. *Marschner's mineral nutrition of higher plants*. Academic press. 2011.
 46. Vargas CJM, Castillo GAM, Pineda PJ, Ramírez AJA, Avitia GE. Extracción nutrimental de jitomate (*Solanum lycopersicum* L.) en mezclas de tezontle con aserrín nuevo y reciclado. *Revista Chapingo. Serie horticultura*. 2014;20(1);71-88. DOI: 10.5154/r.rchsh.2013.02.005
 47. Cortés FC, Rodríguez MN, Benavides MA, García CJL, Tornero CM, Sánchez GP. El yodo aumenta el crecimiento y la concentración de minerales en plántulas de pimiento morrón. *Agrociencia*. 2016;50(6);747-758. Retrieved from: http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S1405-31952016000600747
 48. Blasco B, Ríos JJ, Cervilla LM, Sánchez RE, Rubio WMM, Rosales MA, Ruiz JM, Romero L. Photorespiration process and nitrogen metabolism in lettuce plants (*Lactuca sativa* L.): induced changes in response to iodine biofortification. *J Plant Growth Regul*. 2010;29(4);477-486. DOI: <https://doi.org/10.1007/s00344-010-9159-7>
 49. Lambers H, Brundrett MC, Raven JA, Hopper SD. Plant mineral nutrition in ancient landscapes: high plant species diversity on infertile soils is linked to functional diversity for nutritional strategies. *Plant Soil*. 2010;334(1);11–31. DOI: 10.1007/s11104-010-0444-9
 50. Fageria NK, Baligar VC. Nutrient availability. In: *Encyclopedia of Soils in the Environment*. Hillel, D., Ed.; Elsevier: San Diego, CA, USA. 2005;63–71.
 51. Blasco B, Ríos JJ, Leyva R, Cervilla LM, Sánchez-Rodríguez E, Rubio-Wilhelmi MM, Rosales MA, Ruiz JM, Romero L. Does iodine biofortification affect oxidative metabolism in lettuce plants? *Biol Trace Elem Res*. 2011;142(3):831-42. DOI: 10.1007/s12011-010-8816-9. Epub 2010 Sep 14. PMID: 20838926.
 52. Stevens R, Page D, Gouble B, Garchery C, Zamir D, Causse M. Tomato fruit ascorbic acid content is linked with monodehydroascorbate reductase activity and tolerance to chilling stress. *Plant Cell Environ*. 2008;31(8):1086-96. DOI: 10.1111/j.1365-3040.2008.01824.x. Epub 2008 Apr 21. PMID: 18433441.
 53. Gonnella M, Renna M, D'Imperio M, Santamaria P, Serio F. Iodine Biofortification of Four Brassica Genotypes is Effective Already at Low Rates of Potassium Iodate. *Nutrients*. 2019;11(2):451. DOI: 10.3390/nu11020451. PMID: 30795581; PMCID: PMC6413110.
 54. Herrett RA, Hatfield HH, Crosby DG, Vlitos AJ. Leaf Abscission Induced by the Iodide Ion. *Plant Physiol*. 1962;37(3):358-63. DOI: 10.1104/pp.37.3.358. PMID: 16655658; PMCID: PMC549793.
 55. Fernández V, Brown PH. From plant surface to plant metabolism: the uncertain fate of foliar-applied nutrients. *Front Plant Sci*. 2013;31;4:289. DOI: 10.3389/fpls.2013.00289. PMID: 23914198; PMCID: PMC3728483.