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RESEARCH ARTICLE

IODINE BIO-FORTIFICATION IN LEAFY VEGETABLES CULTIVATED UNDER DIFFERENT AGROCLIMATIC ZONES OF ASSAM

***Indrani P Bora and Arundhati Baruah**

Rain Forest Research Institute, Jorhat-786001, Assam

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ABSTRACT

North eastern region of India is known as iodine deficient area and the population is prone to dreaded and endemic disease like goiter. Plant biofortification with iodine can increase in the concentration of the element in edible part of the plant that efficiently improves the consumer's health. Leafy vegetable was found to have the strongest capability in iodine enrichment through biofortification. An experiment was carried out for two consecutive years to study iodine bio fortification in the form of KI and KIO_3 in spinach and cabbage grown in different agro climatic zones of Assam. Five dosages of KI and KIO_3 *i.e.*1.0 mg kg^{-1}; 2.0 mg kg^{-1} ;3.0 mg kg^{-1};4.0 mg kg^{-1} and 5.0 mg kg^{-1} was applied in foliar and soil along with recommended dosage of organic (Vermicompost) and inorganic(NPK) fertilizer. Foliar application of KI @ 4.0 mg kg^{-1} showed maximum iodine content during initial year of cultivation (12.24 mg kg^{-1}) which was found to be reduced in successive year (11.66 mg kg^{-1}). The same trend was found in cabbage cultivation also. This may be due to the absorption of iodine by the growing crop during initial year of cultivation. Maximum value was recorded @ 3.0 m g kg^{-1} when iodine was applied in the form of KIO_3 in both spinach and cabbage cultivation. However, iodine concentrations in soil solution were generally higher in iodate treatments compared to iodide in both spinach and cabbage. Low iodine concentrations in soil solutions in treatments with iodide may be due to substantial iodine volatilization. Results showed that foliar application is more effective in biofortification than soil application. It was also observed that iodine accumulation in foliar application was comparatively more under agro forestry than in open condition. This concludes that uptake of iodine by plants grown in soils is dependent on the availability of iodine in the soils, which is essentially governed by adsorption– desorption processes in soils.

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INTRODUCTION

Iodine is widely distributed in nature in both organic and inorganic forms. It is present in the soil as soluble form and is quickly absorbed by plants in the form of negatively charged ions of iodide and iodate. The regions with heavy rainfall, forest cover loss and flooding are likely to be iodine deficient as the superficial layer of soil (in which iodine is present) gets washed. The adsorption and desorption behavior of iodine in soil is effected by factors like pH, organic matter, hydrous oxide of iron and aluminum, calcium carbonate, temperature and texture of the soil (Whitehead, 1978). The iodine biofortification with food plant have suggested as a new strategy to address human iodine deficiency. Crops are able to enhance the absorption and accumulation of this trace element when iodine containing salt or iodine rich organic materials incorporated to the soil.

Many workers (Comandini *et al*., 2013 and Weng *et al*., 2014) suggested that vegetable biofortified with iodine by means of foliar spray or soil application has high stability of iodine in crops. Presently in many areas of the world, the surface soil becomes progressively poorer in iodide mainly because of accelerated deforestation, flooding and soil erosion (Singh, 2004). Human body contains very little iodine (0.00004% or 0.4 ppm), yet it is essentially required to be maintained through food and water (WHO, 2002). According to the World Health Organization, Iodine Deficiency Disorder (IDD) are believed to be one of the commonest preventable human health problems in the world today. Plant biofortification with iodine is defined as the increase in the concentration of the element in edible part of the plant that efficiently improves the consumer's health (Zhao and McGrath, 2009). It can be performed through biotechnological techniques as well as traditional agricultural methods *i.e.* soil fertilization or foliar application of this element (Hong *et al.,* 2008, Strzetelski *et al.,* 2009).

***Corresponding author:* Indrani P Bora**
Rain Forest Research Institute, Jorhat-786001, Assam
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Iodine accumulation can be increased in plants through soil application (Dai *et al*., 2004a and b; Zhu *et al.,* 2003). Recent studies demonstrated that a number of vegetables such as spinach, cabbage, lettuce, tomato, alfalfa, pepper, radish, celery can store iodine in their edible tissues, making them good candidates for iodine biofortification programs. It was reported that incorporation of iodine as iodate or iodide form in soil enhanced the iodine content in edible parts of vegetable. Moreover, leafy vegetable was found to the strongest capability in iodine enrichment than stem vegetable (Huan Xin *et al.,* 2013). On the other hand, fruit vegetable was the most weak to the iodine accumulation. Iodine in edible parts of vegetable decreased with the grow time (Mayer *et al.,* 2008). Appropriate dosage of iodine application/addition can accelerate vegetable growth, but excessive iodine is toxic to them. North eastern regions with heavy rainfall, forest cover loss and flooding are likely to be iodine deficient and thus the food grown in this areas cannot provide enough iodine for the people. This situations lead to visible and invisible spectrum of health consequences collectively called iodine deficiency disorders, one of the commonest preventable human health problems in the world today. Bio fortification of vegetables with this element could play an important role in supplementing iodine deficiency as iodine included in plant tissues can be relatively easily absorbed by humans. Keeping this in mind an experiment was conducted to increase the iodine level in leafy vegetables through bio fortification with potassium iodide (KI) and potassium iodate (KIO_3). Trials were conducted under open field as well as agro forestry to determine the influence in different concentration of iodine with inorganic and organic fertilizers. Efficiency of soil and foliar application of iodine is studied in different study sites. This approach will be helpful for people depending on agro forestry system and also on an impact assessment of integrated nutrient management under existed agro forestry models in terms of increasing the yield, productivity of soil and quality of food produce for the human population as a whole in the region and to a certain extent of health of the farming community specially.

MATERIALS AND METHODS

Assam, situated in the North Eastern parts of India, lies between 24^0 and 28^0 18 N latitudes and 89^0 4 and 96.0^0 E longitudes, having boundaries with Bhutan and Arunachal Pradesh in the north, Nagaland, Manipur and a part of Arunachal Pradesh in the east, Mizoram, Tripura and Meghalaya in the south and West Bengal in the west. The total geographical area of 78438 sq. km. and the mighty Brahmaputra and the Barak with their 121 small and tiny tributaries and branches flow through the two valleys. It falls under humid subtropical zone of North east India. The average annual rainfall in the state was 2300 mm, of which nearly 60 to 70% received within a span of 3 to 4 months (May to August). The mean annual maximum temperature varies from 23.6°-31.7°C and minimum temperature varies from 10.0° -25.2C. Extensive survey were conducted to Lakhimpur, Dhemaji, Tezpur in North Bank Plains zone; Sarupather, Jorhat and Dibrugarh in Upper Brahmaputra valley zone; Nowgong, Morigaon and Roha in Central Brahmaputra Valley zone; Boko, Nalbari and Kokorajar in Lower Brahmaputra valley zone; Badarpur Ghat, Karimgang, Udharbund in Barak valley zone and Deohari Rongpi, Phumen Ingti and Bey Killing village of Karbi Anglong district under Hill Zone.

Different area under agro forestry has been identified during the survey and the following sites were selected for layout of the field experiment viz. Pub Majir village (Kamrup), Khanikar village (Golaghat), Chenimari village (Dibrugarh), Nakachari village (Jorhat) and Phumen Ingti (Karbi Anglong). Seeds of cabbage and spinach were procured from M/S agricultural services, Jorhat, Assam. Cabbage seeds were treated with 1% $KMnO_4$ solution for 15 minutes followed by thorough rinse with filter water and then sowed on nicely prepared seedbed. Seeds were kept in seedbed until it grew up to 4-6 leaves and transferred to the experimental plots. Seeds of spinach were soaked in cold water for 24 hours and kept closed tight with muslin cloth for 2-3 days. After sprouting, the seeds were sown in the plot and thinning was carried out as when required. Experiment was conducted in the selected sites with plot size 2mx2m and spacing 50 cm. Different doses of iodine fertilizer in the form of KI and KIO_3 were applied both in soil and foliar. The experiments were conducted for two successive years Treatment details were as follows:

T_0= NPK +Vermicompost
T_1= 1.0mg kg^{-1}KI+NPK +Vermicompost
T_2=2.0mg kg^{-1}KI+NPK +Vermicompost
T_3=3.0mg kg^{-1}KI+NPK +Vermicompost
T_4=4.0mg kg^{-1}KI+NPK +Vermicompost
T_5=5.0mg kg^{-1}KI+NPK +Vermicompost
T_6=1.0mg $kg^{-1}$$KI0_3$+NPK +Vermicompost
T_7=2.0mg $kg^{-1}$$KI0_3$+NPK +Vermicompost
T_8=3.0mg $kg^{-1}$$KI0_3$+NPK +Vermicompost
T_9=4.0mg $kg^{-1}$$KI0_3$+NPK +Vermicompost
T_{10}=5.0mg $kg^{-1}$$KI0_3$+NPK +Vermicompost

(Recommended doses of N (80kg^{-1}ha); P (50kg^{-1}ha); K (50 kg^{-1}ha) and Vermicompost (100kg^{-1}ha.)

Experiment was conducted both in open and under agro forestry. Initial soil physico-chemical properties under different experimental sites were done following the method of Jackson (1973). Iodine was determined with alkaline dry ash technique (Fisher *et al.,* 1986). Results were statistically verified using standard design and analyzed using the analysis of variance (ANOVA). Significant difference between the data was determined at $p < 0.05$.

RESULTS AND DISCUSSION

Estimation of physico chemical properties of soil: Table 1(a and b) represent the initial value of physico-chemical properties of soil under different experimental sites. p^H value was more or less similar in all experimental sites except Phumen Ingti village (5.29) under Karbi Anglong district that showed high p^H value while the other physical properties were low (Table 1a). Likewise water holding capacity (46.55%) and particle density (2.54%) of Chenimari Gaon (Dibrugarh) was found comparatively more than the other study sites. Similar results were also reported by Chaphale and Badole (1999). Highest value of bulk density was recorded in Nakachari village under Jorhat district ans least was observed in Phumen Ingti village of Karbi Anglong hill district. Percentage of organic carbon of soil before sowing of crops was more in PubMajir Village (1.84%) in Kamrup district followed by Chenimari Gaon (Dibrugarh), Khanikar Gaon (Golaghat) and Nakachari village (Jorhat) respectively (Table 1b). Highest value of NPK content was recorded in Chenimari village (Dibrugarh district) followed by PubMajir Village (Kamrup),

Table 1a. Physical properties of soil under different experimental plot

Experimental plot	pH	Particle density ($Mg\ m^{-3}$)	Water holding capacity (%)	Bulk density ($Mg\ m^{-3}$)	Soil texture		
					Sand (%)	Silt (%)	Clay (%)
PubMajir Village (Kamrup)	4.78	2.43	46.12	1.22	67.2	22.7	10.1
Khanikar Gaon (Golaghat)	4.82	2.40	45.38	1.26	69.1	17.8	13.1
Nakachari village(Jorhat)	4.88	2.36	42.65	1.33	70.4	15.9	13.7
ChenimariGaon(Dibrugarh)	4.90	2.54	46.55	1.23	74.2	10.9	14.9
Phumen Ingti village (Karbi Anglong)	5.29	2.21	42.24	1.18	63.8	25.0	11.2

Table 1b. Chemical properties of soil under different experimental plot

Experimental plot	Electrical Conductivity (dSm^{-1})	Organic Carbon (%)	Total N ($kg\ ha^{-1}$)	Avail P ($kg\ ha^{-1}$)	Ex. K ($kg\ ha^{-1}$)
PubMajir Village (Kamrup)	16.8	1.84	1605	13.56	641.56
Khanikar Gaon (Golaghat)	17.7	1.76	1578	12.69	622.22
Nakachari village (Jorhat)	19.4	1.67	1574	13.14	611.07
Chenimari Gaon (Dibrugarh)	18.5	1.82	1671	14.62	661.2
Phumen Ingti village (Karbi Anglong)	15.2	1.08	1515	11.60	484.6

Table 2a. Effect of iodine biofortification (Foliar application) in iodine content of Spinach ($mg\ kg^{-1}$) during 1st year cultivation

Treatment	Under open condition					Under Agro forestry				
	A	B	C	D	E	A	B	C	D	E
T_1	11.46	11.58	11.43	11.52	10.78	11.51	11.68	11.56	11.37	11.22
T_2	11.54	11.79	11.48	11.10	11.36	15.75	16.21	14.79	11.69	11.56
T_3	12.13	12.35	11.63	11.59	11.46	21.17	21.67	15.34	11.62	14.45
T_4	12.37	12.24	11.66	11.68	11.57	24.29	24.38	16.28	11.51	10.17
T_5	12.19	12.13	11.53	11.42	11.48	23.16	23.27	13.69	11.33	10.07
T_6	12.02	12.12	11.35	11.53	11.39	21.46	21.78	14.26	11.67	10.23
T_7	12.38	12.52	12.37	11.44	11.44	22.37	22.54	16.17	11.83	10.46
T_8	12.56	12.65	11.46	11.59	11.36	23.15	23.62	14.95	12.35	12.37
T_9	12.74	12.85	11.88	11.65	11.53	25.81	26.12	21.35	12.47	12.35
T_{10}	12.68	12.26	11.58	11.53	11.29	22.43	21.24	14.56	11.56	11.24
T_0	5.470	7.52	8.23	5.07	4.54	6.023	6.113	5.129	4.534	3.482
SEm±	0.014	0.027	0.016	0.018	0.012	0.014	0.026	0.031	0.026	0.035
CD (P=0.05)	0.024	0.071	0.047	0.063	0.046	0.038	1.02	1.164	0.071	1.16

Table 2b. Effect of iodine biofortification (Foliar application) in iodine content of Spinach ($mg\ kg^{-1}$) during 2nd year cultivation

Treatment	Under open condition					Under Agro forestry				
	A	B	C	D	E	A	B	C	D	E
T_1	10.42	11.36	11.12	10.23	8.82	11.23	11.47	11.36	10.26	10.18
T_2	11.23	11.84	11.26	10.38	10.45	13.35	15.56	13.68	10.78	10.58
T_3	11.46	11.78	11.38	11.68	10.56	15.46	17.38	15.34	11.46	11.23
T_4	12.37	12.87	11.86	11.72	11.27	20.29	22.55	17.36	12.52	11.37
T_5	12.49	12.28	12.06	11.85	11.51	18.36	21.36	13.58	11.12	11.24
T_6	11.22	11.68	11.08	11.68	10.48	15.26	14.42	13.46	11.25	10.56
T_7	11.38	12.52	11.23	11.34	11.08	16.67	16.54	15.38	11.78	10.85
T_8	12.34	12.47	11.65	11.48	11.25	18.62	19.42	17.65	12.27	11.58
T_9	12.42	12.68	11.78	11.56	11.46	22.23	21.74	20.56	12.58	12.67
T_{10}	11.78	11.81	11.26	11.23	11.22	18.48	18.68	15.56	11.23	11.32
T_0	5.71	7.48	5.46	4.37	4.24	4.26	5.145	4.229	4.624	3.512
SEm±	0.02	0.06	0.02	0.02	0.04	0.02	0.04	0.031	0.04	0.05
CD (P=0.05)	0.056	0.042	0.14	0.06	0.16	0.08	0.08	1.164	0.03	0.16

A=PubMajir Village (Kamrup); B= Khanikar Gaon (Golaghat); C= Nakachari village (Jorhat); D= Chenimari Gaon (Dibrugarh) and E= Phumen Ingti village (Karbi Anglong)

Khanikar Gaon (Golaghat), Nakachari village (Jorhat). Least amount of organic carbon and NPK was recorded in Phumen Ingti village of Karbi Anglong district Assam. This may be due to erosion of soil due to the practice of shifting agriculture, a traditional cultivation practice among the hill tribes. Electrical conductivity ranges from 15.2 dSm^{-1} to 19.4 dSm^{-1} and the highest value was recorded in soil of Nakachari village, Jorhat.

Effect of Foliar application of iodine biofortification: Results represent the effect of foliar application of iodine in the form of KI and KIO_3 of spinach (Table 2a and 2b) for two successive year of cultivation. Foliar application of KI and KIO_3 @ 4.0mg/kg along with full dose of NPK and vermicompost showed maximum iodine content in both the year studied.

Table 3a. Effect of iodine biofortification (Foliar application) in iodine content of Cabbage (mg kg^{-1}) during 1st year cultivation

Treatment	Under open condition					Under Agroforestry				
	A	B	C	D	E	A	B	C	D	E
T_1	11.34	11.63	11.37	11.28	10.32	11.52	11.63	11.24	11.37	9.15
T_2	12.46	14.57	12.64	12.43	10.45	14.26	14.42	12.56	11.62	10.73
T_3	20.41	22.35	16.84	15.59	14.53	22.42	26.63	18.66	16.87	14.21
T_4	20.71	22.51	15.67	14.76	13.47	15.36	16.23	15.37	14.31	12.39
T_5	21.44	22.38	14.54	14.38	12.39	15.47	15.57	14.67	14.17	13.12
T_6	15.67	16.79	14.38	13.44	11.58	15.41	16.22	13.29	12.86	10.19
T_7	24.18	23.22	22.83	13.57	12.39	17.28	24.45	14.68	13.47	11.78
T_8	24.52	26.58	25.35	21.78	18.64	26.74	27.37	23.61	15.29	12.56
T_9	24.35	25.64	23.42	15.34	13.56	21.89	25.37	24.37	14.66	12.35
T_{10}	23.28	23.48	20.56	14.48	12.57	21.76	23.44	22.44	14.51	12.22
T_0	6.526	11.73	13.46	13.56	11.36	9.41	14.46	13.53	13.57	11.24
SEm±	0.014	0.27	0.171	0.014	0.027	0.131	0.138	0.016	0.021	0.017
CD (P=0.05)	0.036	0.39	0.36	0.037	0.051	0.281	0.324	0.123	0.057	0.046

Table 3b. Effect of iodine biofortification (Foliar application) in iodine content of Cabbage(mg kg^{-1}) during 2nd year cultivation

Treatment	Under open condition					Under Agroforestry				
	A	B	C	D	E	A	B	C	D	E
T_1	10.45	10.86	9.56	9.28	8.64	10.32	11.72	11.07	10.45	8.45
T_2	11.38	12.54	11.50	10.55	9.87	12.45	13.59	12.56	11.23	9.68
T_3	12.46	15.36	14.66	12.48	11.62	16.38	17.63	14.46	12.64	10.36
T_4	18.82	18.44	14.45	14.34	12.58	20.55	21.56	16.26	15.75	12.58
T_5	18.36	16.38	12.54	13.48	10.45	15.34	16.36	13.44	13.56	11.44
T_6	14.52	11.54	10.38	12.65	10.44	14.55	14.54	10.24	11.46	10.42
T_7	16.34	12.48	14.83	10.34	11.48	16.34	18.66	12.44	12.64	11.34
T_8	18.65	19.37	17.66	16.65	12.55	18.64	19.48	14.27	14.70	12.26
T_9	21.60	22.74	19.52	14.52	12.88	20.85	21.55	16.42	18.66	14.35
T_{10}	15.55	16.36	17.40	13.46	11.45	17.59	19.36	12.65	14.38	12.22
T_0	6.526	10.48	9.65	10.23	9.36	6.82	10.36	5.53	5.89	5.24
SEm±	0.004	0.03	0.07	0.02	0.05	0.02	0.02	0.04	0.011	0.017
CD (P=0.05)	0.036	0.06	0.06	0.05	0.023	0.06	0.04	0.02	0.021	0.026

A=PubMajir Village (Kamrup); B= Khanikar Gaon (Golaghat); C= Nakachari village(Jorhat); D= Chenimari Gaon (Dibrugarh) and E= Phumen Ingti village(Karbi Anglong)

Table 4a. Effect of iodine biofortification (Soil application) in iodine content of Spinach (mg kg^{-1}) during 1st year of cultivation

Treatment	**Under open condition**					**Under Agroforestry**				
	A	**B**	**C**	**D**	**E**	**A**	**B**	**C**	**D**	**E**
T_1	9.88	9.51	8.21	9.96	6.41	10.62	10.14	9.12	10.56	8.14
T_2	10.12	9.66	9.05	10.56	7.12	11.52	10.62	9.56	11.57	8.11
T_3	10.85	10.11	9.63	11.12	8.56	13.12	11.66	10.62	13.11	8.50
T_4	10.95	10.12	9.83	11.88	9.21	13.76	12.12	11.22	13.21	9.26
T_5	11.28	11.05	10.76	12.02	9.12	14.21	13.91	12.56	14.66	10.56
T_6	10.12	9.56	9.06	11.72	9.08	11.72	11.62	10.12	11.56	9.21
T_7	10.08	10.96	9.12	12.06	9.11	11.62	12.12	10.52	12.66	9.56
T_8	10.25	10.92	9.56	13.12	9.56	11.09	11.56	11.66	13.96	10.76
T_9	12.61	11.62	11.02	13.72	11.22	12.88	12.12	12.18	14.12	11.56
T_{10}	13.66	12.88	11.62	14.02	10.56	15.92	14.97	12.76	14.82	10.63
T_0	6.81	7.82	6.12	6.41	5.05	6.81	6.81	6.85	7.25	5.43
SEm±	0.06	0.02	0.03	0.04	0.02	0.06	0.04	0.12	0.08	0.04
CD(P=0.05)	0.12	0.55	0.5	0.34	0.3	0.02	0.024	0.24	0.08	0.18

Table 4b. Effect of iodine biofortification (Soil application) in iodine content of Spinach (mg kg^{-1}) during 2nd year of cultivation

Treatment	**Under open condition**					**Under Agroforestry**				
	A	**B**	**C**	**D**	**E**	**A**	**B**	**C**	**D**	**E**
T_1	8.24	8.16	7.75	8.46	6.01	9.96	9.96	8.86	9.76	7.66
T_2	9.87	8.46	8.52	9.82	6.82	10.44	9.76	8.24	10.12	7.06
T_3	9.94	9.96	8.61	10.67	7.46	12.02	10.26	9.61	12.66	7.12
T_4	10.62	9.46	8.93	10.21	8.39	12.62	11.44	10.44	12.13	8.16
T_5	10.96	10.88	9.66	11.46	8.12	13.41	12.78	11.16	13.41	9.12
T_6	10.05	8.89	8.14	10.62	8.02	10.61	10.41	9.65	10.12	8.61
T_7	9.25	9.96	7.52	11.81	8.14	10.76	11.61	9.52	11.41	8.42
T_8	10.88	9.21	7.56	12.56	8.44	9.72	10.05	10.61	12.12	9.62
T_9	12.11	10.66	10.11	12.40	10.77	11.91	11.64	11.44	13.91	10.26
T_{10}	12.56	11.25	10.42	12.62	10.14	13.66	12.92	11.61	13.64	9.98
T_0	5.71	6.62	5.12	5.44	4.91	5.96	5.61	6.14	6.72	5.61
SEm±	0.018	0.014	0.014	0.012	0.014	0.035	0.012	0.030	0.018	0.035
CD(P=0.05)	0.037	0.035	0.038	0.044	0.024	0.028	0.031	0.24	0.012	1.16

A=PubMajir Village (Kamrup); B= Khanikar Gaon (Golaghat); C= Nakachari village(Jorhat); D= Chenimari Gaon (Dibrugarh) and E= Phumen Ingti village(Karbi Anglong)

Table 5a. Effect of iodine biofortification (soil application) in iodine content in Cabbage (mg kg^{-1}) during 1st year of cultivation

Treatment	Under open condition					Under Agroforestry				
	A	B	C	D	E	A	B	C	D	E
T_1	7.12	7.32	7.12	7.03	5.62	8.32	7.31	7.12	7.73	5.04
T_2	8.40	8.10	8.32	8.41	6.10	9.12	8.04	7.82	8.13	6.44
T_3	8.93	8.56	8.43	8.61	7.12	9.83	8.81	8.21	9.32	7.22
T_4	9.55	8.71	8.61	8.72	7.81	10.10	9.18	8.52	9.87	7.98
T_5	10.05	8.89	8.76	9.81	8.04	10.40	9.42	9.06	10.05	8.41
T_6	7.52	7.92	7.37	7.31	5.78	8.61	7.66	7.63	7.93	5.14
T_7	8.81	8.12	8.12	7.31	5.60	9.13	8.87	8.11	7.92	6.52
T_8	9.12	8.61	8.53	8.73	6.91	9.83	9.14	8.43	8.66	7.12
T_9	9.67	8.83	8.75	8.81	7.93	10.21	9.48	8.61	9.91	7.99
T_{10}	10.25	9.61	9.59	10.01	8.27	10.89	9.73	9.66	10.39	8.59
T_0	6.87	6.91	6.63	6.23	5.40	7.01	7.89	8.32	9.54	6.66
SEm±	0.016	0.012	0.030	0.171	0.014	0.027	0.131	0.138	0.035	0.030
CD (P=0.05)	0.028	0.031	0.24	0.36	0.037	0.051	0.281	0.324	0.037	0.051

Table 5b. Effect of iodine biofortification (soil application) in iodine content in Cabbage (mg kg^{-1}) during 2nd year of cultivation

Treatment	Under open condition					Under Agroforestry				
	A	B	C	D	E	A	B	C	D	E
T_1	7.61	7.12	6.86	6.92	5.66	8.82	7.81	7.63	7.91	5.90
T_2	8.93	7.81	7.05	7.12	5.89	8.99	7.93	7.51	7.89	5.89
T_3	9.10	8.21	8.13	8.06	6.17	9.11	8.14	7.76	8.77	6.12
T_4	9.41	8.43	8.31	8.27	6.92	9.93	8.78	8.10	9.67	6.95
T_5	9.87	9.63	8.41	9.20	7.84	10.15	10.53	9.71	9.60	7.12
T_6	7.72	8.14	7.72	7.44	5.53	8.81	7.87	7.55	7.93	5.94
T_7	9.11	8.14	7.67	7.66	6.57	9.10	8.11	7.65	8.11	6.11
T_8	9.25	8.35	8.41	8.55	7.00	9.31	8.27	8.12	8.83	6.44
T_9	9.53	8.54	8.63	8.73	7.14	10.05	9.12	8.44	9.13	7.11
T_{10}	10.22	10.14	8.54	8.99	7.94	11.65	10.78	9.12	10.14	6.83
T_0	7.11	6.85	6.80	7.39	5.06	8.77	6.98	7.11	7.64	5.48
SEm±	0.171	0.014	0.030	0.171	0.014	0.014	0.027	0.131	0.138	0.016
CD (P=0.05)	0.36	0.037	0.24	0.36	0.037	0.037	0.051	0.281	0.324	0.028

A=PubMajir Village (Kamrup); B= Khanikar Gaon (Golaghat); C= Nakachari village(Jorhat); D= Chenimari Gaon (Dibrugarh) and E= Phumen Ingti village(Karbi Anglong)

Comparatively more iodine content was recorded during initial year of cultivation (24.38 mg kg^{-1}) which was found to be reduced in successive year (22.55 mg kg^{-1}). This may be due to the absorption of iodine by the growing crop during initial year of cultivation. Bio fortification of iodine under agro forestry was observed high compared to open condition almost all the cases studied. Among the different sites, it was noticed that crops cultivated under Areca nut plantation in Pub Majir village (Kamrup district) and Khanikar village (Golaghat district) showed comparatively high value of iodine content than other sites. Iodine content in spinach leaf was recorded highest in 4 mg kg^{-1} in the form of KI whereas in case of KIO_3 it was recorded comparatively more in 3mg^{-1}kg both in open condition as well as under agro forestry.

In all the treatments the value decline in successive year of cultivation that supported the findings of Dai *et al*., (2004a). Foliar application of iodine in cabbage (Table 3a and 3b) was also shows the same trend. Cakmak *et al.,* (2017) in their study clearly showed that foliar application of iodine containing fertilizers is highly effective in increasing grain iodine concentrations in wheat, rice and maize. HuanXin and Yan (2008) studied mechanism of iodine uptake by cabbage by conducting a series of controlled experiments, including solution culture, pot planting, and field experiments. They found that uptake was more sensitive to metabolism inhibitor in lower concentration of iodine. The inorganic iodine fertilizer provided a quicker supply for plant uptake, but the higher level of iodine was toxic to plant growth.

Effect of Soil application of iodine biofortification: Table 4(aandb) and 5(aandb) represents the different concentration of iodine content (KI and KIO_3) applied in soil in spinach and cabbage cultivation plot. From this results revealed that iodine content was more in crops planted under open condition. Iodine concentration was significantly higher at Khanikar goan under open as well as betel nut agro forestry system. Maximum amount of iodine content was obtained at the initial year and it gradually declined in successive year due to heavy absorption by the crops (Dai *et al.,* 2004b). Iodine concentrations in soil increased with increasing amount of iodine concentrations applied to the soil as iodide or iodate. However, iodine concentrations in soil solution were generally higher at iodate treatments than iodide treatments for all crops cultivation. Fuge (1996) suggests that volatilization of iodine from soils plays an important role in the global iodine cycle and its transfer to the biosphere. The production of volatile iodine from soil is thought to be dependent on the amounts of iodide in soils (Keppler *et al*., 2000, 2003). Since the adoption of application of iodine fertilizer to soil as a complementary strategy to supplement dietary iodine intake is relatively new, the fate and behavior of different species of iodine in soil–plant systems is largely unknown. Site specific observation revealed that Khanikar goan soil contained more iodine content in all the seasonal crops followed by Pub Majir goan > Na Kachari > Chenimari goan. A distinct variation was noticed in Phumen Ingti village due to slash and burning operation and heavy soil erosion. The reason for low iodine concentrations in soil solutions in treatments with iodide could be due to substantial iodine volatilization. Muramatsu *et al*., (1996) and Johnson *et al.,* (2002) also found that iodine in the soil was volatilized from the soil–plant system into the atmosphere as organic iodine. Above results concluded that soil of this study was under iodine deficient belt due to faster deforestation that leads to increase soil erosion and leaching. Foliar application of iodine in the dose of KIO_3 (4.0 mg kg^{-1}ha) has allowed to higher accumulation of iodine in leafy vegetables. The iodine content in edible part uptake by spinach was significantly increased with increasing level of iodine. Iodine content was remarkably more during initial year of cultivation and it declined subsequent year. A significant increase of iodine content was recorded under agroforestry compared to open condition. Soil fertilization with iodine resulted in an increased synergistic effect of nutrient uptake. Cultivation of both spinach and cabbage under agroforestry showed better result than in open condition in all the treatment studied. This confirms that uptake of iodine by plants grown in soils is dependent on the availability of iodine in the soils, which is essentially governed by adsorption– desorption processes in soils. Though iodine bio fortification is already a feasible strategy the success seems to largely depend on the correct choice of the system of distribution, doses and timing of application. No general protocols are effective for all species. A detailed study is necessary in this regard to understand the behavior of each individual plant in the environment where the cultivation is carried out.

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