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Original Research Article

Iodine Fortification of Vegetables

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A B S T R A C T

Iodine, one of the most important essential nutrient elements for human health and is an essential component of the thyroid hormones (thyroxin and triiodothyronin). Human body cannot synthesize iodine itself. Thus, the people depend on food materials for their iodine requirement. Its deficiency in human leads to several iodine deficiency disorder (IDD) and is a significant public health problems in the world. An attempt was taken to fortification of iodine in vegetables. A greenhouse pot experiment was carried out to bio-fortify spinach (*Spinacea oleracea* L.) and carrot (*Daucus carota* L.). Four iodine concentrations (0, 1.0, 2.5 and 5.0 mg kg^{-1}) of iodide (I^-) and iodate (IO_3^-) were used. The yield and iodine content of vegetables were significantly improved by the addition of iodide and iodate to the soil; response of leafy vegetable spinach was comparatively higher than carrot. The yield and iodine concentrations in edible parts of vegetables were significantly higher for plants grown with iodate than iodide treatment in soil. The residual iodine concentration in soil was comparatively higher in iodine treated soil than control, irrespective of levels and species. According to these results, iodate at 2.5 mg iodine kg^{-1} of soil can be considered as potential iodine supplementation dosage to increase iodine content in vegetables.

Keywords

Fortification, iodide, iodate, spinach and carrot

Introduction

Iodine is an essential component of the thyroid hormones (thyroxin and triiodothyronin), which plays an important role in the growth of the human body. Thyroxin promotes protein synthesis, regulates energy conversion, preserves the composition of central nervous system, and maintains normal metabolism. Therefore, once the human body is deficient in iodine, it can lead to physiological malfunctioning and health problems generally regarded as Iodine Deficiency Disorders (IDD) (Liao, 1992). The human body needs 50 – 200 µg iodine every day as per World Health Organization (WHO) recommendation for infants to adults (Hetzel, 1983), about 75 – 80% of which comes from vegetable sources (Hetzel and Stanbury, 1980; Liao, 1992). The concentration of iodine in vegetables is mainly determined by two factors: background content of iodine in soil and their biological assimilation. The background content of iodine in soil largely depends on the parent material, texture and geographic distribution of soil. The content of iodine in soil is relatively lower in interior and mountainous areas due to precipitation eluviations, which results in the deficiency of iodine in vegetables and the prevalence of IDD. Human population and livestock that only depend on the food grown in iodine deficient soil cannot obtain sufficient iodine for their body requirement (Hetzel, 1997). Thus, IDD in human and animals are predominant in the areas where vegetation is poor in iodine. IDD constitutes a major nutrition deficiency disorders in world. There are about 1.6 billion people living in iodine deficient area in 118 countries. In India, a nationwide goitre survey revealed that out of 283 studied districts of 29 states and four union territories, 235 districts have prevalence of endemic goitre (National Iodine Deficiency Disorders Control Program, 2003). In addition, endemicity of goitre has also been reported from many new regions of India, including Manipur (Chandra *et al.,* 2006), Tripura (Chandra and Ray, 2001) and Delhi (Pandav *et al.,* 1996). The total number of affected people is estimated to be more than 63 million in India and about 740 million in the world (Benoist and Delonge, 2002).

To prevent and control IDD, iodized salt has been commonly used most economically practical method for supplementing iodine to human needs. Salt is iodized by the addition of fixed amounts of potassium iodide or iodate, as either dry solid or an aqueous solution. However, iodized salt as a daily supplement have some problems. The inorganic iodine is too volatile to be measured and thus difficult to evaluate its validity to the diet (Longvah and Deosthale, 1998). Loss of iodine from iodized salt is mainly due to exposure to humidity and sunlight and also upon short term heating (dry and in solution) as may be encountered in cooking. High humidity results in rapid loss of iodine from salt iodized with potassium iodate, ranging from 30-98% of the original iodine content (Diosady *et al.*, 1998). Due to 30-45% relative humidity and 24 hour exposure to sunlight, the losses were 58.5 and 41%, respectively (Biber *et al.*, 2002). The loss of iodine in iodized salt during cooking of food was 14-63% (Wang *et al.*, 1999). The boiling of 5% iodized salt solution for 5 minutes; the loss of iodine was estimated to 21-68% (Das Gupta *et al.*, 2008).

Thus, *bio-fortification* would be the safer and effective method to supplement iodine and ultimately IDD in different parts of the world. Supplementation of trace elements in the food chain through plant uptake is generally termed as *bio-fortification* and is believed to be a cost effective way to improve human nutrition (Jopke *et al.*, 1996). 99 % of iodine in food stuffs is bio-available and can be easily assimilated by human. Further, the iodine cannot be easily accumulated in the grains of cereal crops, such as rice and wheat (Mackowiak and Grossl, 1999). Vegetables must be considered to have an advantage in concentrating iodine, because iodine accumulation in the edible parts of vegetables is largely dependent on xylem transport (Herrett *et al.,* 1962). In this experiment leafy vegetable, spinach (*Spinacea oleracea* L.) and root vegetable, carrot (*Daucus carota*) were selected to study the variation of biomass, the absorption and enrichment characteristics of iodine in vegetables and residual iodine content in soil of applied iodine at different concentration ranges. This experiment made an attempt to provides the practical solution of IDD of human and the proper selection of vegetable and iodine species/dose for future *bio-fortification* programme.

Materials and Methods

Site and soil

The soil used in the experiment was collected from Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi (25^0 15' 53" North latitude, 82^0 59' 27" East longitude and at an altitude of 80 m mean sea level), India and the soil was characterized by standard method (Jackson, 1973). The soil was sandy loam with pH 7.8, organic carbon 3.8 g kg^{-1}, plant available N, P, K and S 193.2, 19.0, 203.5, and 19.7 kg ha^{-1} respectively, and hot water extractable iodine (Whitehead, 1973) was 2.27 mg kg^{-1}.

Seed of vegetables

Spinach (*var*. All Green) and carrot seeds (*var*. Pusa Kesar) were collected from Indian Institute of Vegetable Research (Indian Council of Agricultural Research), Varanasi, India.

Experimental design and treatments

The experimental layout was a complete randomized design with three replications. The treatments consisted of two species of iodine (iodide and iodate) and three doses of each species. A nontreated control was also included for comparison.

Greenhouse experiment of iodine fortification

In biofortification experiment of vegetables, the pot experiment was carried out in a greenhouse with temperature of 28 ± 3^0 C/ 18 ± 3^0 C day/night. Each pot was filled with one kg of well pulverized soil. The recommended dose of N, P, K for spinach was 80:50:50 kg/ha and for carrot was 80:50:50 kg/ha applied through urea, single super phosphate and potassium chloride. Full dose of phosphorus, potassium and one third dose of nitrogen were applied at the time of pot filling through mixing and rest N was applied into two split doses at 20 and 30 days after seed sowing, respectively. The moisture content in each pot was maintained to 50% field capacity by watering the pot at regular interval. Five-six healthy seeds of spinach and carrot were sown at proper depth in the month of December and after germination 3 plants in each pot was maintained at 15 days after sowing (DAS). Experimental samples were collected into two stages, 45 and 60 DAS for spinach (leaf) and it was at 60 and 75 DAS for carrot (modified root) *i.e.* edible portions of the vegetables. After harvesting the whole plants of spinach in each pot, the upper leafy part was taken in a paper packet for further processing in laboratory. Similarly, the whole plants of carrot in each pot were harvested in specified periods and only the edible part (modified root) was taken in paper packet after proper washing with water. Potassium iodide and potassium iodate were used as the source of iodine treatments. 100 mg I L^{-1} of potassium iodide and iodate were prepared as stock solution then 0.0, 1.0, 2.5 and 5.0 mg kg^{-1} iodine solution was applied at 20 DAS in the spinach and carrot in respective pots.

Analysis of iodine in plant samples

After harvesting the vegetables in specified period, fresh weight of edible parts of spinach and carrot were measured immediately. To determine the iodine content in plant samples, the collected edible parts of plant samples were dried at 65 ^{0}C in hot air oven, ground into powder by grinder and the concentration of iodine was analyzed by the colorimetric technique (Bedi 1999). This method is sensitive at lower level of iodine content and the detection limit is 0.04 µg /g. A 0.5 g of powdered dry plant samples were taken in nickel crucible. The material was moistened with 1.0 mL distilled water, there after about 30 minutes, 1.0 mL of 2N Na_2CO_3 solution was added and dried in oven at 100^0C. The oven dried plant materials were placed in muffle furnace at 600^0C ± 10^0C for 90 minutes. The ashed material in the crucible was dissolved with 5 mL 0.7 N HCl and then after 15 minutes diluted with 5 mL distilled water and centrifuged at 8000 rpm for 5 minutes. The colourless supernatant fluid was transferred exactly 3.0 mL in a 25 mL clean graduated test tube. Solutions of ceric ammonium sulphate (i.e. 0.815% dissolved in water and 8.0 mL concentrated H_2SO_4 / 100 mL) and arsenious acid (i.e. 0.315% arsenious oxide + 0.165% NaOH + 7.0 mL concentrated H_2SO_4 / 100 mL) were taken in separate test tubes on water both at 37^0C for 10 minutes. Then 1.0 mL arsenious acid was added in each test sample tube and again all the tubes were left on water bath for another 30 minutes. There after 11 mL ceric ammonium sulphate was added and optical density was measured at 415 nm in spectrophotometer. The iodides have catalytic effect on the reduction of ceric sulphate by arsenious acid in the presence of sulfuric acid solution. The graded progress of the reaction can be followed by the steady disappearance of the yellow colour of the ceric ions which was measured in the spectrophotometer. The concentration of iodine was directly measured from the standard graph. The iodine concentrations in leaves of spinach and roots of carrot were calculated both as dry weight basis.

Analysis of iodine in soil samples

Soil samples of pot experiment collected after harvesting were air dried at room temperature and grinded and then passed through a 2 mm sieve. A 2.0 g sample of well-prepared dry soil was refluxed with 30.0 mL distilled water at 140 ^{0}C for 45 minutes in (Whitehead, 1973) digestion system. After cooling, the mixture was filtered by Whatman No. 42 filter paper with two washing of residues in 100 mL volumetric flask and make up the volume with double distilled water. There after the mixture was centrifuged at 9000 rpm for 5 minutes and 3 mL supernatant was used for estimation (Bedi, 1999) of iodine content by spectrometric techniques.

Statistical analysis of data

The data obtained for each character were analysed by applying the techniques of analysis of variance (Gomez and Gomez, 1984). The significance of the treatments was tested by F test and at 5% error probability was calculated to compare the means of different treatment.

Results and Discussion

Yield of vegetables

The yield of leafy vegetable, spinach (*Spinacea oleracea* L.) and root crop, carrot (*Daucus carota* L.) on fresh weight basis, grown with different species and levels of iodine are shown in Table 1. The yield of edible parts of both, spinach and carrot were significantly influenced by applied iodine through iodide and iodate in soil. Iodine at 2.5 mg kg^{-1} as both iodide and iodate were produced the highest yield of spinach and carrot. Due to applied iodine at 2.5 mg kg^{-1}, yield of spinach ranged between 22.1-27.0 g /pot and yield of carrot was ranged between 78.1-86.4 g/pot, respectively. The significant impact on yield was comparatively much higher in spinach than carrot. Irrespective of species, doses and days of harvesting, the yield of spinach was increased by the application of iodine and the range of %

increased in yield was 5.01 – 38.46. The yield response of carrot due to iodine treatments was significant at second stage of harvesting (75 DAS) and highest increment was obtained at 75 DAS with iodate at 2.5 mg I kg^{-1} of soil and range of % increased in yield was 0.14 – 13.12. However, yield response of carrot at first stage of harvesting (60 DAS) was not significant. The yield of both the vegetables at second stage of harvesting in all levels and species of iodine were comparatively higher than first stage of harvesting.

Dai *et al.,* (2004) studied six different types of plants classified according to their edible parts (root, tuber, aerial part and leaf) and found that iodate concentration had a significant effect over biomass of plants only in those where the edible part was the leaf but for root vegetable, iodate concentration had non-significant effect.

Zhu *et al.,* (2003) working with spinach plants, observed a direct relationship between foliar biomass and the application of 10 and 100 µm of iodide and iodate. Similarly, Shitou *et al.,* (2002) reported that the concentration of iodine accelerates the growth of pea sprout and iodine fertilized (Shitou, 2003) at lower doses was beneficial to root growth of radish.

Thus, application of iodine as iodide and iodate significantly influenced the biomass of edible part of spinach at both the stages and for carrot at second stage only. It means that both I^- and IO_3^- uptake by the vegetables stimulated their biomass output. Moreover, species of iodine and their doses are most important for stimulation of biomass production. Among the species of iodine, iodate was better responsive than iodide, to increase in yield of leafy vegetable spinach and root vegetable carrot at each level of applied iodine.

Iodine concentration in edible parts of vegetables

The concentration of iodine (Table 2) in the leaves of spinach increased concurrently with the rising in concentration of iodine in soil as iodide upto 5.0 mg I kg^{-1} at both the stages (45 DAS and 60 DAS) of harvesting and for iodate, the highest iodine content was obtained at 2.5 mg I kg^{-1} soil.

For iodide application at 5.0 mg I kg^{-1} soil, the increase in iodine accumulation by spinach grown in iodine enriched soil were 3.3 and 2.3 times higher (Fig 1) than spinach grown in without iodinated soil (control pot) and for iodate at 2.5 mg I kg^{-1} soil, the increasing extent was 3.0 and 2.3 times at 45 and 60 DAS, respectively.

The accumulation of iodine in carrot enhanced significantly (Table 2) due to application of iodine at both the stages of harvesting. The maximum iodine content was noticed for both species of iodine (I^- and IO_3^-) with the application at 2.5 mg I kg^{-1}. Carrot treated with IO_3^- increased the iodine content (Fig. 1) more (3.0 times of control) than I^- (1.9 times of control) at first stage of harvesting. However, at second stage of harvesting, iodine accumulation in carrot was not significantly different in between IO_3^- and I^- treatments. At second stage of harvesting (75 DAS), iodine content were reduced with both the species of iodine and reduction was much greater when iodine applied as iodate in comparison to iodide at each level of iodine.

The iodine content was comparatively higher at 60 DAS than 45 DAS in spinach irrespective of species of iodine source. But reverse trend was observed in case of carrot. Moreover, spinach treated with different species and levels of iodine accumulated much higher iodine in comparison to carrot.

Table.1 Yield (g) of spinach and carrot as fresh weights grown in soil supplied with different forms and levels of iodine

Treatments of iodine (mg I kg^{-1} soil)	Spinach				Carrot			
	45 DAS		60 DAS		60 DAS		75 DAS	
	Iodide	Iodate	Iodide	Iodate	Iodide	Iodate	Iodide	Iodate
0.0	18.76		19.50		74.56		76.38	
1.0	20.68	22.90	22.20	25.60	76.40	78.40	83.20	83.50
2.5	22.16	24.90	24.15	27.00	78.10	80.30	85.70	86.40
5.0	21.00	19.70	22.01	21.20	74.55	77.28	76.49	81.20
SEm ±	0.499		0.538		1.630		1.732	
CD (P= 0.05)	1.514*		1.633*		NS		5.254*	

* Significant at the 0.05 level, NS = Not significant, DAS = Days after sowing

Table.2 Influence of different species and levels of iodine in soil on iodine content in dry weight of edible portions of vegetables (mg kg^{-1})

Treatments of iodine (mg I kg^{-1} soil)	Spinach				Carrot			
	45 DAS		60 DAS		60 DAS		75 DAS	
	Iodide	Iodate	Iodide	Iodate	Iodide	Iodate	Iodide	Iodate
0.0	1.49		8.50		7.50		3.07	
1.0	9.60	10.73	12.70	14.00	10.23	20.20	7.97	6.39
2.5	15.07	15.40	17.30	19.60	14.10	22.60	9.17	8.57
5.0	16.77	15.25	19.67	18.30	8.30	17.16	5.87	5.67
SEm ±	0.51		0.71		0.60		0.52	
CD (P= 0.05)	1.55*		2.16*		1.81*		1.60*	

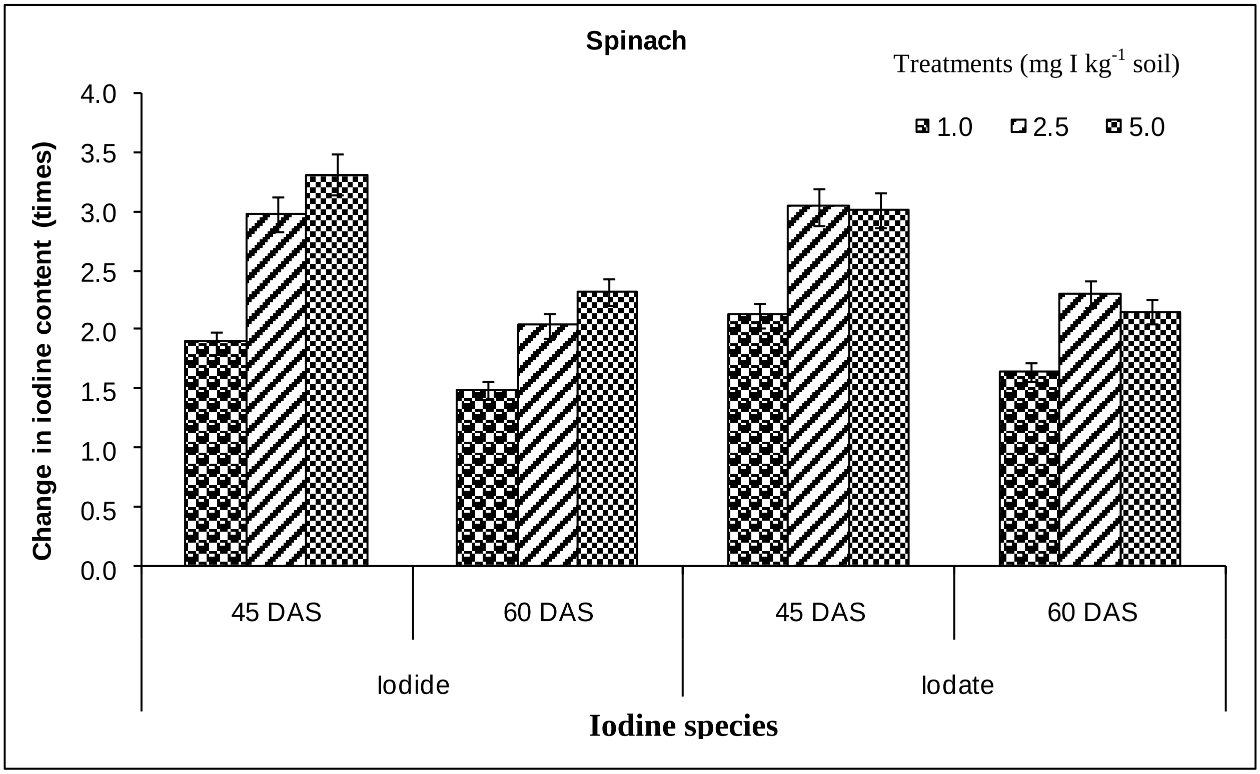
* Significant at the 0.05 level

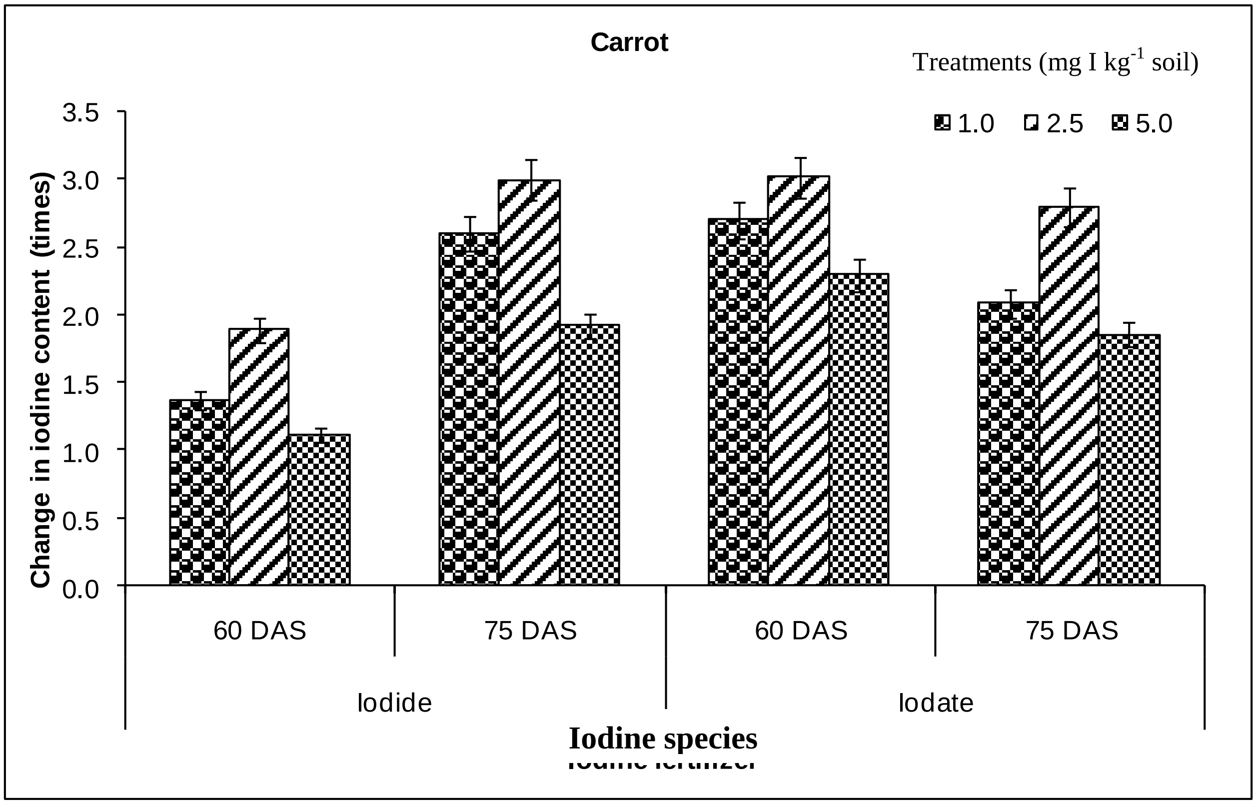
Table.3 Residual hot water-extractable iodine in soil (mg kg^{-1}) with applied iodine fertilizers

Treatments of iodine (mg I kg^{-1} soil)	Spinach				Carrot			
	45 DAS		60 DAS		60 DAS		75 DAS	
	Iodide	Iodate	Iodide	Iodate	Iodide	Iodate	Iodide	Iodate
0.0	0.020		0.015		0.019		0.015	
1.0	0.068	0.139	0.032	0.064	0.031	0.049	0.018	0.021
2.5	0.162	0.174	0.057	0.116	0.057	0.136	0.026	0.056
5.0	0.212	0.243	0.123	0.137	0.143	0.184	0.076	0.105
SEm ±	0.008		0.006		0.005		0.003	
CD (P= 0.05)	0.024*		0.019*		0.016*		0.010*	

* Significant at the 0.05 level

Fig.1 Change in iodine content in vegetables at various fortification levels





The spinach is a leafy vegetable plant in which iodine directly transferred to leafy portion from soil. The leaf is the central factory of plant photosynthesis, to which large quantity of potassium cation is transported primarily to maintain the electrical charge balance at the site of adenosine triphosphate production in chloroplasts (Evans and Sorger, 1966). According to Donnan equilibrium theory (Salisbury and Ross, 1992), equal amount of anions should also be transported to leaves at the same time during the growth and development of plant. The iodine anions present in soil can compete with other soluble anions to be transported to leaves, therefore resulting in higher and continuous accumulation of iodine in leaves in spinach. However, the reduction of iodine concentration in carrot at latter stage was mainly due to faster transfer of iodine from root to leafy part and transfer of iodine from root to shoot was comparatively faster in iodate treated plants than iodide. The process of transfer of iodine from soil to plant parts with moisture and mineral matters, root of the plants act as a tunnel and leaf blade is the destination where photosynthesis takes place, which leads to the iodine accumulation in leafy part of plant rather in root portion of carrot. As a result accumulated iodine in root of carrot rather shifted faster in leafy part. It was previously proved that ability and bioaccumulation of iodine in leafy vegetable spinach was greater than root crop of radish (Huanxin *et al.,* 2003).

Residual iodine status of the soil

The hot water extractable iodine (plant available) concentrations in soil were determined after harvesting the vegetables to assess the availability of iodine in soil. The hot water extractable iodine concentrations of soil in both spinach and carrot increased (Table 3) with increasing iodine concentrations applied to the soil as iodide or iodate; but amounts of residual iodine were very low (Hong *et al.,* 2009) in comparison to the applied doses. However, residual iodine concentrations in soil generally higher for iodate treated soils than iodide treated soils in both the crops at both the stages of harvesting. The reason for low iodine concentrations in soil in treatments with iodide could be due to substantial iodine volatilization. Fuge (1996) suggests that volatilization of iodine from soil plays an important role in the global iodine cycle and its transfer to the biosphere. Muramatsu *et al.,* (1995) and Johnson *et al.,* (2002) also found that iodine in the soil was volatilized from the soil plant system into atmosphere as organic iodine (as organo iodides). The production of volatile organo-iodides form soil is thought to be ultimately dependent on the amounts of iodide in soils (Keppler *et al.,* 2003). Moreover, available iodine content in soil was higher at first sampling stage than second sampling stage in both the vegetables. But, the residual iodine content in soil was too low in both the vegetables to create any consequence in soil and ground water contamination/pollution.

In the present study, the technique of bio-fortification of iodine in vegetables through the application of different species of iodine in soil during cultivation was developed. The bio-iodine in vegetables can serve as a safer iodine source for human body as an alternative to the inorganic iodine in iodized salt.

This research concluded that spinach and carrot responded differently to different species and levels of iodine *viz.* iodide and iodate. Considering both the yield and iodine fortification in edible parts of vegetables, spinach can be considered as a relatively efficient vegetable targeted for iodine bio-fortification than carrot. It should be noted that if soil application of iodine is used to bio-fortify vegetable crops, one should avoid the excessive applications of iodine to soils in order to maintain reasonable crop yields. Further, this experiment indicated that the potential for iodine bio-fortification in vegetables was much greater with iodate (Dai *et al.*, 2006) than with iodide and level of iodate was 2.5 mg I kg^{-1} of soil in respect of both yield and iodine content in edible parts of vegetables. Thus, according to these results, iodate can be considered as potential iodine fertilizer to increase iodine content in vegetables. In the long run, this technique can raise the general iodine level in the food chain in those iodine deficient areas that helps to control IDD without any ecosystem problems due to the presence of very low amount of residual iodine after harvesting the vegetable crops.

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