Indian J.L.Sci.3(2) : 47-56, 2014

ISSN : 2277-1743 (Print)
ISSN : 2278-7879 (Online)

FREE RADICAL SCAVENGING ACTIVITIES, GROWTH AND METAL UPTAKE IN CARROT (*Daucus carota* L.) PLANT UNDER HIGHER CONCENTRATION OF ARSENIC

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ABSTRACT

In the present study, growth of carrot (*Daucus carota* L.) plants parts (EDP and Leaf) were significantly influenced under arsenic treated pot after 15, 30, 45 and 60DAS. Fresh weight of EDP was not significantly affected upto 30ppm of arsenic treated plant after 30DAS, while at >30ppm treatments it was significantly ($P<0.05$) affected. Chl-a content was more than Chl-b in leaf of arsenic treated plant after both 30 and 60 DAS. Proline content was found to be increasing trends in leaf under higher arsenic treated plant leaf and it was maximum from T4 to T6 treatment after 60 DAS. Ca, P, K, fructose, sucrose and TSS were found to be more in EDP after 60 DAS followed by 30 DAS. SODRSA and HRSA was more in leaf part upto T2 and T3 treatment after 30 and 45 DAS respectively, as compared with control and other treatments (EDP). No any significant ($P<0.05$) corelation was observed in leaf and EDP of carrot plant for DPPHSRA after 30, 45 and 60DAS. Total phenolic content was more in EDP as compared with leaf after 60DAS followed by 30 and 45days. After 30DAS in EDP showed less amount of arsenic uptake in almost all arsenic treated plant as compared with leaf part, while after 45 and 60DAS, arsenic uptake were maximum in EDP part as compared with leaf.

KEYWORDS : *Daucus carota* L., Arsenic uptake, SODRSA, HRSA and DPPHSRA

Widespread use of arsenicals as pesticides has significantly contributed to the elevation of arsenic concentrations in soils (Adriano, 2001). Arsenic use in 1992 alone was 23,900 metric tons, of which 67% was used for the production of the wood preservative chromate copper- arsenate (CCA). Leaching losses of wood preservatives from CCA-treated wood can potentially contaminate both soil and groundwater. Arsenic is a crystalline metalloid that exists in several forms and oxidation states. Its toxicity and mobility in the environment depend on both its chemical form and species (Adriano, 2001). Total metal concentration alone is insufficient to assess its environmental impact in contaminated soils. Soil available arsenic content is a better indicator of its phytotoxicity than total arsenic concentration (O'Neil, 1990). The consumption of arsenic contaminated water is the main path for its transportation into the environment and biological systems (Zhu et al., 2008; WHO, 2010). Long term use of arsenic contaminated underground water in irrigation may results in the increase of its concentration in agricultural soil and eventually in crop plants (Smith et al., 2009). Arsenic in the environment is causing significant global health problems which have once again brought this element to the attention of the broad public (WHO, 2010). As a non-essential element for living organisms, with propensity to adversely affect the human health and the functioning of ecosystems (Meharg and Hartley 2002), inorganic arsenic calculated as arsenite and arsenate comprise 96% of the total arsenic in vegetables (Smith et al., 2008, 2009). Translocation from roots to shoots unlike phosphorus (P), arsenic (As) has generally low mobility with respect to translocation from roots to shoots in plants except hyperaccumulators (Quaghebeur and Rengel, 2004). The inefficient root to shoot translocation is also reflected in the generally low ratios of shoot As to root As concentrations in plants supplied with inorganic As in tomato (Carbonell-Barrachina et al., 1998). Metal toxicity causes oxidative stress, which can take place possibly by generating reactive oxygen species (ROS) (Sun et al., 2008, 2007). To scavenge ROS and avoid oxidative damage, plants possess the antioxidative enzymes superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD), glutathione peroxidase, ascorbate peroxidase and glutathione reductase, as well as nonenzyme antioxidants such as ascorbic acid and glutathione (Sun et al., 2007, 2008; Upadhyay et al., 2012). More than permissible limit of arsenic (10ppb; WHO 2010) were found in shallow in Ballia district and its main region is geogenic problem (Tripathi 2008). Carrot is common cultivar vegetable in Ballia district, and might be rich source of arsenic for human being through their food intake and causes adverse effects on

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health (Zhu et al., 2008; Smith 2009). Carrots (*Daucus carota* L.) are more than a versatile orange vegetable. Original carrots were purple and yellow, initially described in the 10th century in Iran and northern Arabia (Simon, 2000). Therefore, the present study was based on the effects of arsenic on growth, free radical scavenging activities and arsenic uptake in the carrot plant.

MATERIALS AND METHODS

Set-Up of Pot Experiment

Pot experiment were prepared according to earlier described (Upadhyay et al., 2012), surface (20 cm) soil samples (Agricultural field: As content 0.002 mg kg^{-1}) which were ground to pass through a 4.0mm mesh and were air-dried, then mixed with basal fertilizers, at ratios of 100 mgNkg^{-1} dry weight (DW) soil as NH_4NO_3 and 30 mg P kg^{-1} and 80 mg kg^{-1} as K_2HPO_4 (Wang et al., 2008). Eleven pots were prepared, namely C (control) and treatments (As concentrations: 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 mg kg^{-1} DW soil). Each treatment was carried out in six replicates. The tested topsoil samples were mixed thoroughly with $Na_2HAsO_4.7H_2O$ (Sun and Zhou 2008) at the above-mentioned concentrations, filled into plastic pots (20 cm in diameter, 15 cm in height, 2.5 kg air-dried soil per pot) and equilibrated for 28 days (Sun and Zhou, 2008). Seeds of carrot were surface sterilized in 1% (w/v) sodium hypocloride for one min, washed several times with sterilized distilled water (SDW), and soaked in SDW overnight (Upadhyay et al., 2012). Twenty soaked seeds were sowed directly into the 0.8% agar plate and incubated for three days in dark room (Upadhyay et al., 2012). Three similar sizes of sprouted seed were placed in the each treatment pots in the greenhouse with natural light (10-12h; photoperiod) and temperature (16-30^{0}C). The tested soils were watered to reach 60% of the water-holding capacity and this level was maintained by watering daily throughout the experiment. The plants were harvested after 15, 30, 45 and 60 DAS (days after showing) for further analysis.

Physico-Chemical Analysis of Plant

Edible part (EDP), Leaf (L) of the plant were examine for their length, fresh weight (FW) and dry biomass (DW) were examined after 15, 30, 45 and 60 DAS. Leaf chlorophyll a (Chl-a) and b chlorophyll (Chl-b) were estimated by spectrophotometrically (A663nm and A645nm) by method of (Arnon, 1949). The content of fructose, sucrose was determined by the ferrocyanide method (Furnholmen et al., 1964) and Total soluble sugars by earlier described by Upadhyay et al., 2011. Proline content (µg mg^{-1} FW) was determined according to Bates et al., (1973). Analysis of ions in plant parts, plant were harvested and dried in an oven at 68^{0}C for 72 h and the dried plant parts were ground and digested in an acid mixture (HNO_3:H_2SO_4:$HClO_4$ = 10:1:3) for determination of K^+ and Ca^+ (Richards, 1954). Phosphate content was measured according to Cottenie (1980).

Determination of Superoxide Anion Radical-Scavenging Activity

Superoxide radicals were generated in all solutions was prepared in 0.05 M phosphate buffer (pH 7.8) with some modifications by the method of Giannopolites and Ries (1977). The photo induced reactions were performed in aluminum foil-lined box with two 30W fluorescent lamps. The distance between the reaction solution and the lamp was adjusted until the intensity of illumination reached about 4000 lux. A 30µL aliquot of various concentrations of plant parts extracts was mixed with 3ml of reaction buffer solution (1.3 mm riboflavin, 13 mM methionine, 63 µM nitro blue tetrazolium and 100µM EDTA, pH 7.8). The reaction solution was illuminated for 15 min at 25º C. The reaction mixture, without sample, was used as a control. The scavenging activity was calculated as follows: scavenging activity (%) =

(1-absorbance of the sample/ absorbance) × 100.

Hydroxyl Radical Scavenging Activity

The hydroxyl radical scavenging activity was determined according to the methods described by (Singh et al., 2002). 0.1 ml of carrot plant (*Daucus carota* L.) parts extracts of leaf and stem was taken in different test tubes. 1.0 ml of iron-EDTA solution (0.1% ferrous ammonium sulfate and 0.26% EDTA), 0.5 ml of DMSO (0.85% v/v in 0.1 M Phosphate buffer, pH 7.4) were added to these tubes, and the reaction was initiated by adding 0.5 ml of 0.22% ascorbic acid. Test tubes were capped tightly and heated on a water bath at 80-90^{0}C for 15 min. The reaction was terminated by

the addition of 1 ml of ice cold TCA (17.5 %w/v). 3 ml of Nash reagent (75 g of ammonium acetate, 3 ml of glacial acetic acid, and 2 ml of acetyl acetone were mixed and raised to 1 L with distilled water was added to all of the tubes and left at room temperature for 15 min for the color development. The intensity of the yellow color formed was measured spectrophotometrically at 412 nm against the reagent blank. The percentage of hydroxyl radical scavenging activity is calculated by using the formula: % of hydroxyl radical scavenging activity=1-absorbance of sample/absorbance of blank×100.

Scavenging Activity of DPPH Radical

Scavenging activity in carrot plant (*Daucus carota* L.) against DPPH radicals was assessed according to the method of (Larrauri et al., 1998). 0.1 mM DPPH-methanol solution was mixed with 1 ml of 0.1mM DPPH methanol solution. After the solution was incubated for 30 min at 25ºC in dark, the decrease in the absorbance at 517nm was measured. Control contained methanol instead of antioxidant solution while blanks contained methanol instead of DPPH solution in the experiment. Ascorbic acid and BHT were used as positive controls. The inhibition of DPPH radicals by the samples was calculated according to the following equation: DPPH-scavenging activity (%) = [1-(absorbance of the sample-absorbance of blank)/absorbance of the control] ×100.

Total phenolic content

Total phenolic compounds in *Daucus carota* L. were quantified by using Folin-ciocalteu's method. 50 µl of Folinciocalteu's reagent (50% v/v) were added to 10µl of sample extract. It was incubated for 5 min. After incubation 50µl of 20 % (w/v) sodium carbonate and water was added to final volume of 400 µl, and blank was prepared by replacing the reagent by water to correct for interfering compounds. After 30 min of incubation, the absorbance was measured using spectrophotometer at 760 nm. Total phenol contents were expressed in terms of gallic acid equivalent (gm/100g of dry mass), which is used as a reference compound.

Plant and Soil Metal Analysis

After 60 DAS of the treatment subsequently, the plants were separated into two parts: Edible and leaf. They were dried at 100°C for 10 min, then at 70°C in an oven until completely dry. The plant and soil samples were digested with a solution of 3:1 HNO_3:$HClO_4$ (v/v). The concentration of arsenic was determined using the atomic absorption spectrophotometry method (Sun et al., 2007).

RESULTS

Growth of carrot (*Daucus carota* L.) plants were significantly influence in arsenic treated pot after 15, 30, 45 and 60DAS (Table, 1). Results revealed from observation, length of EDP of the plant was significantly ($P<0.05$)

Table 1: Influence of Arsenic On Growth Parameters of Edible Part (E), leaf (L) and leaf chlorophyll a & b of the Carrot (*Daucus carota* L.) Plant in Pot-Experiment (Green House Experiment) After 30 and 60 DAS

Treatment	Length (c.m.)				Fresh weight (g)				Dry weight (g)				Chl-a		Chl-b	
	30 DAS		60 DAS		30 DAS		60 DAS		30 DAS		60 DAS		30 D	60 D	30 D	60 D
	E	L	E	L	E	L	E	L	E	L	E	L	L	L	L	L
C*	13.0^{a}	10.0^{b}	18.0^{a}	11.2^{b}	67.4^{a}	18.9^{a}	103^{a}	32^{a}	12.2^{a}	6.0^{a}	28^{ab}	11.2^{a}	49^{a}	65^{ab}	30^{a}	41^{a}
10 ppm	13.2^{a}	10.0^{b}	18.0^{a}	9.3^{cb}	65.0^{a}	18.2^{a}	92^{b}	30^{a}	10.2^{ab}	5.5^{ab}	21^{b}	11.6^{a}	44^{ab}	61^{a}	30^{a}	41^{a}
20 ppm	13.0^{a}	8.1^{c}	17.2^{a}	9.1^{c}	65.0^{a}	12.3^{b}	69^{bc}	26^{b}	9.5^{b}	4.1^{b}	14.2^{bc}	7.6^{b}	41^{ab}	60^{a}	22^{ab}	28^{b}
30 ppm	13.1^{a}	7.1^{bc}	17.1^{a}	6.8^{d}	51.5^{b}	11.1^{b}	58^{c}	23^{b}	9.0^{b}	4.1^{b}	12.9^{c}	7.2^{b}	40^{ab}	59^{a}	20^{ab}	28^{b}
40 ppm	9.3^{ab}	5.5^{c}	17.0^{a}	6.3^{d}	50.2^{ab}	10.8^{b}	55^{c}	22^{c}	9.0^{b}	3.2^{bc}	12.0^{c}	6.0^{bc}	36^{b}	53^{ab}	21^{ab}	22^{bc}
50 ppm	8.2^{b}	5.0^{c}	17.0^{a}	5.8^{d}	43.2^{b}	12.1^{b}	53^{c}	20^{c}	8.5^{b}	3.1^{c}	11.7^{c}	6.0^{bc}	22^{c}	42^{b}	18^{b}	19^{c}
60 ppm	7.1^{b}	3.2^{d}	14.2^{b}	5.6^{d}	37.1^{bc}	9.5^{bc}	43^{cd}	19^{cd}	7.2^{bc}	3.0^{c}	10.0^{c}	5.7^{c}	21^{c}	25^{c}	12^{cb}	17^{c}
70 ppm	6.5^{c}	3.1^{dc}	12.5^{bc}	5.3^{d}	36.0^{bc}	5.3^{c}	41^{d}	12^{d}	7.0^{c}	2.5^{c}	10.0^{c}	5.1^{c}	15^{d}	22^{c}	11^{cb}	14^{c}
80 ppm	3.6^{cd}	2.1^{d}	7.0^{d}	4.6^{d}	20.2^{d}	4.6^{c}	38^{de}	11^{d}	6.1^{c}	2.2^{c}	9.7^{c}	4.3^{d}	14^{d}	13^{d}	8^{d}	11^{cd}
90 ppm	3.1^{d}	1.3^{e}	4.2^{de}	2.9^{e}	17.7^{d}	3.2^{d}	23^{e}	9^{de}	5.0^{d}	1.6^{d}	5.5^{cd}	2.7^{de}	10^{de}	12^{d}	8^{d}	8^{de}
100 ppm	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Data are only of 30DAS and 60DAS, Figures followed by different letters in a same line are significantly different at $P < 0.05$, n= 5.
C*= Control (0 ppm Arsenic in pot), DAS or D = Days after showing, E=Edible part of the Plant, L=Leaf of the plant, Chl-a= Chlorophyll-a and Chl-b= Chlorophyll-b in (mg/g).

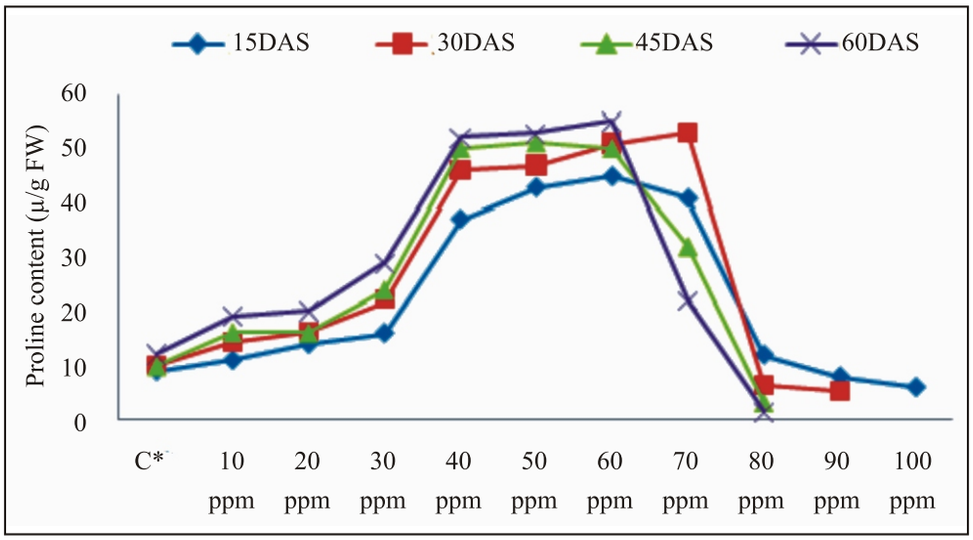

Figure 1: Proline Content (µg/g FW) in Fresh Leaf of Carrot Plant (*Daucus carota* L.) Under Different Concentration of Arsenic (w/w; ppm) in Pot Experiment After 15, 30, 45 and 60DAS. (DAS=Days After Showing, C* =Control)

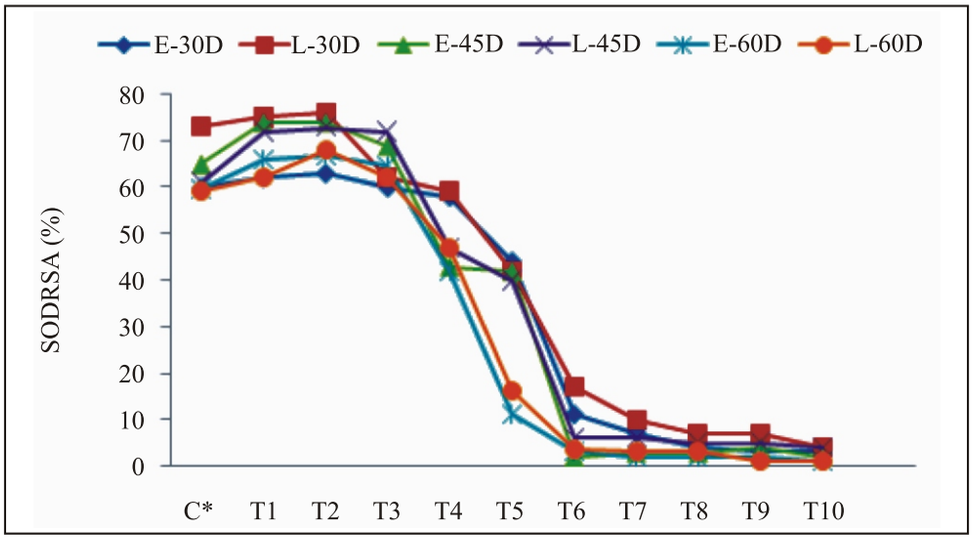

Figure 2: Super Oxidize Dismutase Radical Scavenging Activity (SODRSA) Activity in Carrot Plant (E=Edible Part and L=Leaf) Under Different Concentration of Arsenic Treatment [w/v (C*=Control, T1=10ppm, T2=20ppm, T3==30ppm, T4=40ppm, T5=50ppm, T6=60ppm, T7=70ppm, T8=80ppm, T9=90ppm and T10=100ppm)] After Different Days (D= (DAS=Days After Showing)

unaffected up to 30ppm (treatment T3) and 50 ppm (treatment T5) arsenic treated pot after 30days and 60days respectively, while length of leaf of the plant was significantly affected and reduced on increased concentration of arsenic treatment after 30 and 60 days (Data shown only 30 and 60DAS). Fresh weight of EDP was significantly not affected upto 30ppm treated plant after 30DAS, while at >30ppm treatments it was greatly affected after 60 DAS (table, 1). Dry biomass of EDP was at a par upto T5 treated plant after 30 DAS, while leaf part was significantly affected at >20ppm to 100ppm after both 30 and 60 DAS (Table, 1). Chl-a and Chl-b content of the plant treated leafs showed significant influence after both 30 and 60 DAS (Table, 1), Chl-a content was more than Chl-b after both 30 and 60 DAS and Chl-a was unaffected upto 30ppm

Table 2 : Evaluation of Calcium (Ca), Phosphorus (P), Potassium (K), Fructose, Sucrose and Total Soluble Sugars Contents in Carrot (*Daucus carota* L.) Plant Parts (EDP / Leaf) Under Different Concentrations (ppm) of Arsenic in Pot Experiment, After 30 and 60 DAS (days after showing)

Treatment	Nutrient content (ppm) in EDP						Fructose (mg/g)		Sucrose (mg/g)		Total soluble sugars (mg/g)	
	Ca		P		K							
	30 D	60 D	30 D	60 D	30 D	60 D	30 D	60 D	30 D	60 D	30 D	60 D
C*	34.4^{a}	59.8^{a}	34.2^{abc}	31.8^{a}	144.0^{ab}	151.0^{a}	18.4^{a}	22.8^{ab}	23.7^{a}	32.7^{ab}	105^{a}	111^{a}
10 ppm	30.5^{ab}	57.9^{a}	22.1^{a}	21.5^{ab}	132.2^{b}	141.5^{a}	18.2^{a}	20.3^{a}	22.5^{a}	23.2^{a}	102^{a}	112^{a}
20 ppm	32.3^{b}	54.6^{a}	21.0^{b}	20.6^{ab}	131.4^{b}	121.0^{ab}	17.1^{a}	20.6^{a}	22.7^{a}	22.0^{a}	107^{a}	115^{a}
30 ppm	31.0^{b}	43.8^{bc}	17.3^{c}	16.2^{b}	105.6^{bc}	111.5^{ab}	15.7^{ab}	18.2^{b}	21.0^{a}	21.4^{a}	108^{a}	119^{a}
40 ppm	28.5^{bc}	44.0^{c}	15.6^{c}	11.5^{bc}	83.3^{c}	93.3^{b}	14.5^{b}	15.5^{bc}	16.8^{b}	20.7^{a}	93^{a}	98^{a}
50 ppm	24.9^{c}	42.9^{c}	13.9^{d}	10.3^{c}	56.2^{cd}	56.4^{bc}	14.3^{b}	14.8^{c}	13.3^{bc}	15.1^{b}	85^{ab}	91^{ab}
60 ppm	21.3^{cd}	38.6^{d}	12.1^{cd}	10.1	44.7^{d}	34.2^{c}	12.3^{bc}	12.9^{cd}	12.6^{c}	12.5^{c}	71^{b}	84^{b}
70 ppm	10.7^{d}	21.7^{dc}	11.6^{d}	9.8^{c}	40.1^{d}	30.0^{c}	8.2^{c}	11.7^{cd}	7.2^{cd}	10.4^{cd}	22^{c}	52^{bc}
80 ppm	9.5^{d}	18.4^{e}	4.8^{de}	8.2^{c}	13.9^{de}	14.1^{d}	4.5^{c}	10.5^{d}	6.1^{d}	9.7^{d}	18^{c}	22^{c}
90 ppm	4.3^{de}	12.2^{e}	2.7^{e}	ND	6.2^{e}	ND	3.1^{d}	ND	2.7^{e}	ND	16^{c}	20^{c}
100 ppm	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Data are only of 30DAS and 60DAS, data are expressed as mean ± SD. Figures followed by different letters in a same line are significantly different at $P < 0.05$, n= 6. C*= Control (0 ppm Arsenic in pot), D= Days after showing, EDP=Edible part of the Plant and ND=Not detected.

As treatment in plant leaf after 30 DAS. Proline content in leaf of arsenic treated plant were found to be increasing trends from T1upto T7 treatments after 60 DAS followed by 15, 30 and 45 DAS (Figure, 1), while maximum proline content was observed from T4 to T6 treated plant leaf after 60 DAS. Higher concentration of arsenic treated carrot plant showed significant reduction in EDP of Ca, P, K, fructose, sucrose and total soluble sugars (TSS) after 30 and 60 DAS (Table, 2). Ca, P, K, fructose, sucrose and TSS were found to be more in EDP after 60 DAS followed by 30 DAS. Ca, P and K content in arsenic treated plants were showed significant ($P<0.05$) reduction after 30, 20 and 30ppm of treatment respectively. Fructose concentration was found to be at a par upto 50ppm treated plant, sucrose was significantly reduced after 30ppm arsenic treated plant. TSS was increased up to 30ppm treated plant over the control and decrease from 30 to 90ppm treated plant. Antioxidant scavenging activities were examined in carrot plant under different concentration of arsenic treatments after 30, 45 and 60 DAS, result revealed that SODRSA was more in leaf part upto T2 and T3 treatment after 30 and 45 DAS respectively (Figure, 2) as compared with control and other treatments. SODRSA were almost higher in leaf part among all the treatments after 30 DAS. Reductions in SODRSA were observed from T3 to T6 treatments after that from T6 to T9 was almost stabilized (figure, 2). Hydroxyl radical scavenging activities in carrot plant leaf ware significantly higher after 30, 45 and 60 DAS as compared with EDP (Figure, 3), while maximum HRSA was more after 30 DAS as compared with 45 and 60 DAS. No any significant ($P<0.05$) correlation was observed in leaf and EDP of carrot plant for DPPHSRA after 30, 45 and 60 DAS (Figure, 4). Total phenolic content was more in EDP as compared with leaf, after 60 DAS followed by 30 and 45 DAS (Figure, 5A and 5B). Arsenic uptake in plant showed significant accumulation in both parts (EDP and Leaf) of the plant at all treatments after 30 and 60 DAS (Table, 3). During early days (30 DAS) EDP showed less amount of arsenic in all treatment (T1 to T6) as compared with leaf part, while at treatment T7 and T8 showed maximum uptake of arsenic 57 and 54 ppm respectively, after 30 DAS. Result reveled after 45 and 60 DAS, arsenic uptake were maximum in EDP part as compared with leaf (Table, 3). T6 treated plant showed maximum arsenic concentration (EDP: 62ppm; Leaf: 48pppm) among all the treatment after 60 DAS.

DISCUSSION

Carrot is an economically important horticultural

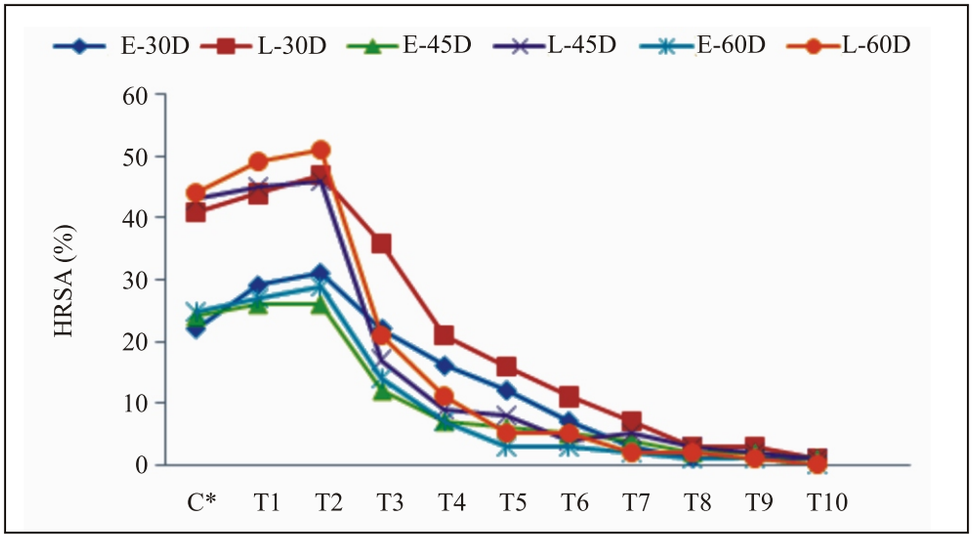

Figure 3 : Hydroxyl Radical Scavenging Activity (HRSA) Activity in Carrot Plant (*Daucus carota* L.) (E=Edible Part and L=Leaf) Under Different Concentration of Arsenic Treatment [w/v (C*=Control, T1=10ppm, T2=20ppm, T3==30ppm, T4=40ppm, T5=50ppm, T6=60ppm, T7=70ppm, T8=80ppm, T9=90ppm and T10=100ppm)] After Different Days (D= (DAS=Days After Showing)

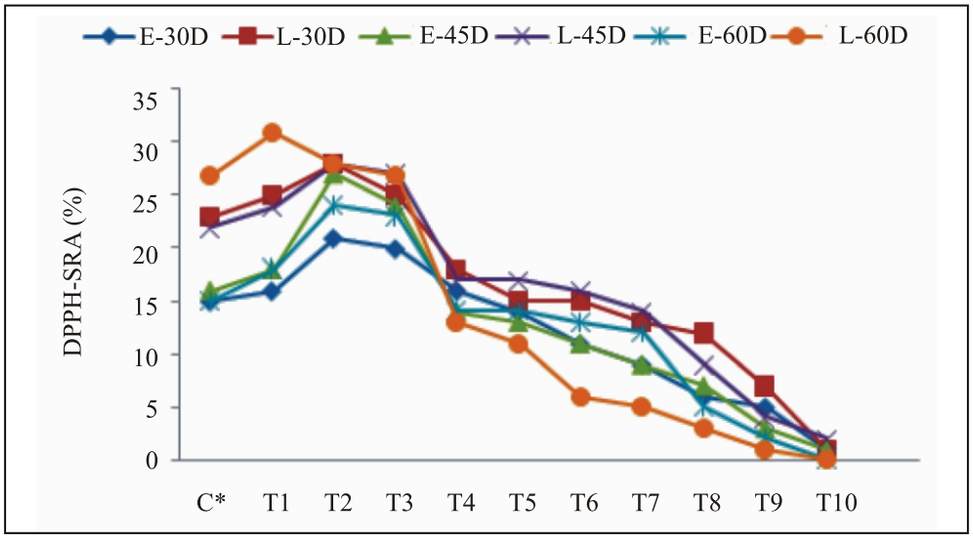

Figure 4 : DPPH-SRA Activity in Carrot Plant (*Daucus carota* L.) (E=Edible Part and L=Leaf) Under Different Concentration of Arsenic Treatment [w/v (c*=control, T1=10ppm, T2=20ppm, T3==30ppm, T4=40ppm, T5=50ppm, T6=60ppm, T7=70ppm, T8=80ppm, T9=90ppm and T10=100ppm)] After Different Days (d= (das=Days After Showing)

crop that has gained popularity in recent decades due to increased awareness of its nutritional value. Presence of arsenic metal in the soil allow to enter in the tropic level and damaging the ecosystem, in this connection the present study, Growth of carrot (*Daucus carota* L.) plants were significantly influence under arsenic treated plants after 15, 30, 45 and 60 DAS. Length of EDP of the plant was significantly ($P<0.05$) unaffected up to 30ppm and 50 ppm arsenic treated pot after 30 DAS and 60 DAS respectively, while length of leaf of the plant was significantly affected and reduced on increased concentration of arsenic treatment after 30 and 60 DAS. Meharg and Hartley., 2002 reported

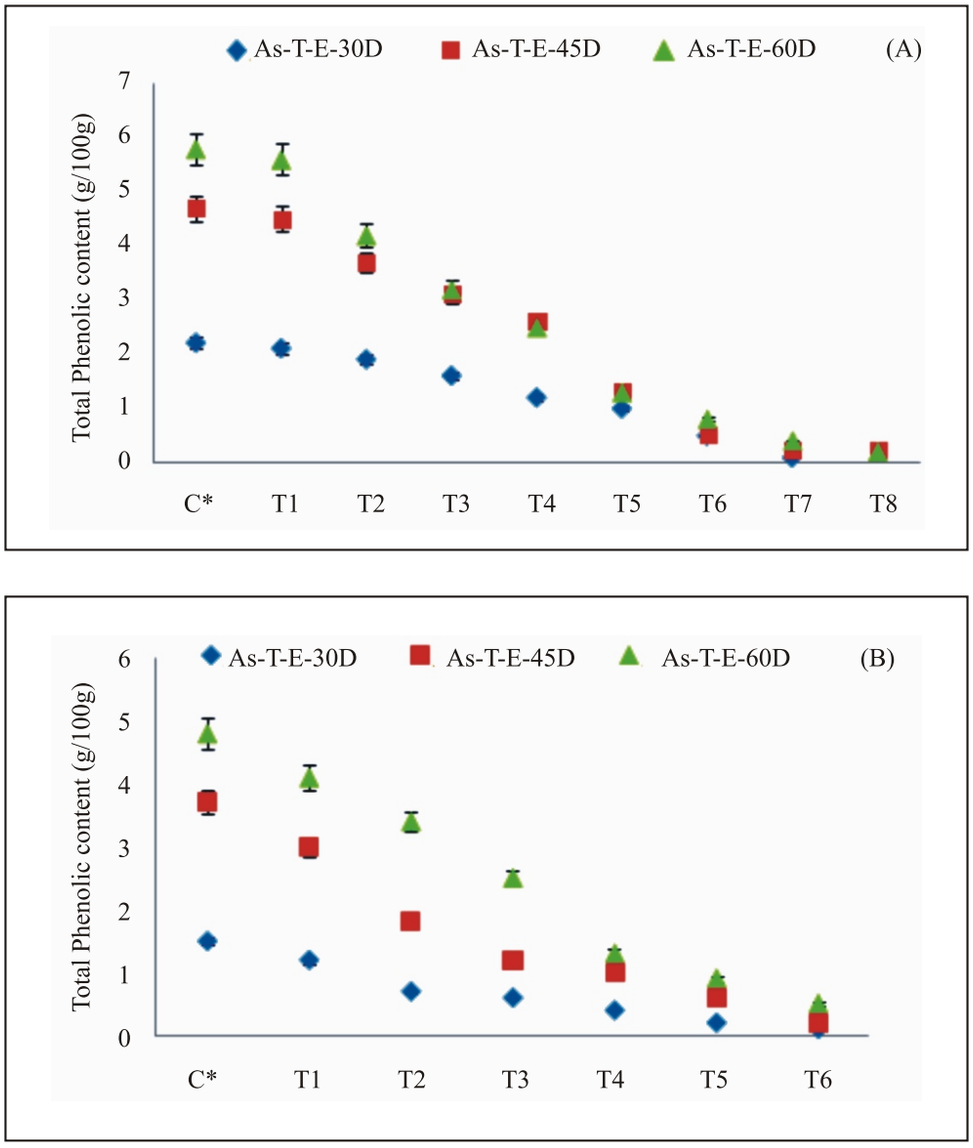

Figure 5 : Total Phenolic Content in Carrot (*Daucus carota* L.) E=Edible part (A) and L=Leaf (B) Under Different Concentration of Arsenic Treatment [w/v (C*=Control, T1=10ppm, T2=20ppm, T3==30ppm, T4=40ppm, T5=50ppm, T6=60ppm, T7=70ppm and T8=80ppm] After Different Days (D= DAS=Days After Showing)

concentration of arsenic play adverse effect on growth of the plant as well as ecosystem. As might be expected, total soil As concentrations are not a good predictor of As phytotoxicity when soils with widely differing properties are compared (Adriano et al., 2001; Smith et al., 2008, 2009). In an anaerobic environment under reducing conditions, arsenite (AsIII) is the dominant then (AsV) and inorganic arsenic species are highly toxic to plants (Zhu et al., 2008). Arsenate is a phosphate analog, and therefore it can compete with phosphate in the cytoplasm, replacing phosphate in ATP, leading to the disruption of energy flows in cells (Meharg and Hartley, 2002;Ramirez et al., 2013). On the other hand, AsIII is highly toxic to plants because it reacts with sulfhydryl groups (-SH) in enzymes, their cofactors and tissue proteins (Tripathi et al., 2007). Chl-a content was more than Chl-b after both 30 and 60 days and Chl-a was unaffected upto 30ppm As treated plant leaf after 30days followed by 60days. Similar report are available in

Table 3 : Uptake of Arsenic (ppm) Metal in Carrot (*Daucus carota* L.) Plant Parts (EDP=Edible Part and L=Leaf Part) Under Different Concentrations of Arsenic Treatment in Pot [w/v (C*=Control, T1=10ppm, T2=20ppm, T3==30ppm, T4=40ppm, T5=50ppm, T6=60ppm, T7=70ppm and T8=80ppm] After 30 and 60DAS

Plant part *vs* Days (D)	Arsenic treatment										
	C*	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
EDP-30D	0.0	8 ± 1.2^{ab}	13 ± 2.4^{a}	20 ± 2.9^{ab}	28 ± 3.4^{a}	50 ± 5.2^{a}	51 ± 4.6^{ab}	57 ± 4.3^{a}	54 ± 2.1^{a}	ND	ND
Leaf-30D	0.0	10 ± 1.5^{a}	16 ± 1.2^{ab}	22 ± 3.1^{b}	32 ± 2.4^{a}	38 ± 4.1^{ab}	40 ± 2.9^{bc}	45 ± 3.5^{a}	27 ± 2.5^{b}	ND	ND
EDP-45D	0.0	14 ± 1.6^{abc}	24 ± 1.9^{b}	39 ± 1.7^{bc}	50±4.4b	58 ± 3.0^{a}	62 ± 4.0^{c}	53 ± 6.1^{a}	63 ± 3.6^{a}	ND	ND
Leaf-45D	0.0	12 ± 1.2^{c}	13 ± 1.5^{bc}	38 ± 2.5^{c}	41 ± 1.5^{ab}	42 ± 2.7^{ab}	46 ± 3.6^{c}	46 ± 2.9^{ab}	25 ± 2.3^{b}	ND	ND
EDP-60D	0.0	26 ± 2.2^{d}	27 ± 2.4^{c}	49 ± 5.2^{cd}	57 ± 1.8^{b}	59 ± 3.7^{bc}	63 ± 2.9^{ac}	52 ± 5.2^{a}	12.3 ± 1.1^{c}	ND	ND
Leaf-60D	0.0	22 ± 2.7^{d}	28 ± 1.3^{c}	22 ± 2.1^{bc}	48 ± 1.9^{ab}	48 ± 2.6^{abc}	48 ± 5.0^{bc}	51 ± 2.1^{ab}	9.3 ± 1.0^{c}	ND	ND

Data are only of 30DAS and 60DAS, data are expressed as mean ± SD. Figures followed by different letters in a same line are significantly different at $P < 0.05$, n= 6. C*= Control (0 ppm Arsenic in pot), D= Days after showing, EDP=Edible part of the Plant and ND=Not detected.

the case of NaCl stress in plant, Chl-a and Chl-b contents were influenced on metal stress (Upadhyay et al., 2013). In the present study, proline content in leaf of arsenic treated plants were found to be increasing trends from T1 to T7 treatments after 60days followed by 15, 30 and 45 days (Figure, 1), while maximum proline content was observed from T4 to T6 treated plant leaf after 60 days. Proline content was increased under stress (Upadhyay et al., 2014) and proline may act as a mediator of osmotic adjustment, protects macromolecules during dehydration and serve as a hydroxyl radical scavenger. Ca, P, K, fructose, sucrose and TSS were found to be more in EDP after 60 days followed by 30 days. Ca, P and K content in arsenic treated plants were showed significant ($P<0.05$) reduction after 30, 20 and 30ppm of treatment respectively. Carbonell-Barrachina et al., 1994 showed that the effects of arsenite contamination of tomatoes caused a reduction in the absorption and accumulation of boron (B), copper (Cu), manganese (Mn), and zinc (Zn), while absorption of iron (Fe) increased (Carbonell-Barrachina et al., 1994). Decreased absorption as well as translocation to aerial parts were described to the structural damage caused by the modus operandi of As (III). Effects of As (III) on the absorption and accumulation of certain macronutrients again in tomato plants: Calcium (Ca), Potassium (K), Magnesium (Mg), Nitrogen (N), and Phosphorous (P) (Carbonell-Barrachina et al., 1998). As (III) levels lowered vegetative growth and fruit yield and diminished dry and fresh weights of tomato plants. A reduction of root P, Ca, and Na was observed, while root N and K increased. Foliar concentrations of Ca, K, and P were reduced below deficiency thresholds, but were deemed not important enough to explain the effects of As (III) as a simple nutritional disorder (Carbonell-Barrachina et al., 1998). Fructose concentration was found to be at a par upto 50ppm treated plant, sucrose was significantly reduced after 30ppm arsenic treated plant. Nutrient imbalance is common phenomenon under metal/abiotic stress (Carbonell-Barrachina et al., 1998).Translocation from roots to shoots unlike phosphorus (P), arsenic (As) has generally low mobility with respect to translocation from roots to shoots in plants except hyperaccumulators (Quaghebeur and Rengel, 2004). TSS was more in plant under arsenic stress it might be indicate to maintain cell turgidity as well as osmoprotectants (Upadhyay et al., 2014). Gajewski (2007), reported that carrot plants have good amount of phenolic compound as well as antioxidant activity. In the present study, total phenolic content was more in EDP as compared with leaf after 60days followed by 30 and 45days. In the present study, arsenic uptake in plant showed significant accumulation in both parts (EDP and Leaf) of the plant at all treatments after 30 and 60days (Table, 3). During early days (30days) EDP showed less amount of arsenic in all treatment (T1 to T6) as compared with leaf part, while at treatment T7 and T8 showed maximum uptake of arsenic 57

and 54 ppm respectively, after 30 days. In aerobic soils, arsenate (AsV) is the most stable and dominant species and uptake of arsenate by plants has been studied extensively (Singh et al., 2008, 2009). Ramirez et al., (2013), reported that the arsenic accumulate in vegetables of different families, and arsenic uptake trends were Asteraceae > Brassicaceae > Amaranthaceae> Cucurbitaceae > Liliaceae > Solanaceae> Fabaceae family. Similar findings were earlier reported by Smith et al., 2008,2009 for arsenic accumulated in several crops viz. example beetroot, lettuce, potato and radish. The amount of As in the edible parts of these vegetables can reach relatively high levels of As, for example more than 30 mg As kg^{-1} (DW) in radish, which could pose a problem for heavy consumers of particular vegetables cultivated in As contaminated soils. However, the result revealed from arsenic uptake in EDP of carrot plant was beyond the recommended permissible level of the arsenic (WHO, 2010) under higher concentration of arsenic treated soil. Therefore, we conclude that cultivation of carrot plant could not be beneficial for human food without proper testing of arsenic concentration in soil.

ACKNOWLEDGEMENT

This research is a part of Ph.D. thesis of Shyni Paul, supported by department of Biological Sciences, Sam Higginbottom Institute of Agriculture, Technology & Science, Allahabad, U.P, India.

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