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Effect of Zinc Sulphate and Organics on Zinc Content and Yield of Spinach Grown in Inceptisol of Varansi, India

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A B S T R A C T

A greenhouse pot experiment was carried out using factorial CRD with Three replications during *Rabi* 2014 to investigate the of applied Zn and organics on Zinc content and dry biomass yield of *Spinacea oleracea*. The experiment consisted of four levels of Zn, *i.e*. 0, 5, 20 and 40 mg kg^{-1} soil and three levels of organics, *i.e*. control, 3% FYM and 3% PM. Dry matter yield of Spinach in first cutting (3.06 g pot^{-1}) marginally increased due to application of Zn at 5 mg kg^{-1} soil over control (2.84 g pot^{-1}), which was statistically increased with applied level of 40 mg Zn kg^{-1} soil (3.26 g pot^{-1}). In case of second cutting, reductions in dry matter yield were observed at applied level of 20 (1.55 g pot^{-1}) and 40 mg Zn kg^{-1} soil (1.47 g pot^{-1}). Zinc content increased significantly with increase in Zn level. Zinc content gets reduced due to application of farmyard manure (FYM) and poultry manure in soil. DTPA extractable Zn increased with increases in Zn level. FYM and PM reduced the DTPA extractable Zn in soil.

Introduction

Zinc (Zn) deficiency in humans is a widespread problem across the globe. Since, vegetables are the important constituent of daily diet; it is very useful to enrich leafy vegetables with Zn in alleviating Zn deficiency in humans. Under the sponsorship of the All India Coordinated Scheme on Micronutrients in Soils and Plants of the Indian Council of Agricultural Research (now re-designated as All India Coordinated Research Project on Secondary, Micronutrients, and Pollutant Elements in Soils and Plants), out of more than 2.5 lakh soil samples analysed so far from 20 states of the country, as high as 49 % of the samples were found zinc (Zn) deficient. Soils having low Zn status produce plants and grains with lower Zn content. Low dietary intake of Zn appears to be the major reason for the widespread prevalence of Zn deficiency in human population. Lower intake of Zn through diets has also become a major risk factor for human death globally.

Increasing world population has created an urgent need to increase food production. Soils are rich in oxides of Zn under reduced condition which are released during the weathering of minerals (Desai and Rao, 2016). High crop yields associated with greater nutrient removal and use of high analysis fertilizers have resulted in the depletion of micronutrient reserves in soils. Crop yield and quality responses to a number of micronutrients have been reported (Gupta *et al.*, 2008). Soils having low Zn status produce plants and grains with lower Zn content. Low dietary intake of Zn appears to be the major reason for the widespread occurrence of Zn deficiency in peoples. Lower intake of Zn through diets has also become a major risk factor for human death globally. In a comprehensive study, Hotz and Brown (2004) reported that Zn deficiency affects (Singh MV 2001) on an average, one third of world's population, ranging from 4 to 73 % in different countries. Zinc deficiency is responsible for many severe health complications, including impairment of physical growth, immune system and learning ability, combined with risk of infections, DNA damage and cancer development. The studies in relation to the effect of Zn fertilization on its content in the edible plant parts were mostly carried out in cereals, followed by legumes, oilseeds and other cash crops revealed by Rattan *et al.,* (2008) and Katyal *et al.,* (2004). Since green vegetables, particularly Spinach are efficient accumulator of metals including Zn reported by Mirshekali *et al.*, (2012), Sharma *et al.,* (2012) and Glowacka (2012), it is relatively easier to enrich these vegetables by soil application of Zn.

Spinach (*Spinacea oleracea* L.) is an edible flowering plant in the family of Chenopodiaceae. It is native to central and Southwestern Asia. It is an annual plant (rarely biennia), which grows to a height of up to 30 cm. Spinach may survive over winter in temperate region. The leaves are alternate, simple ovate to triangular based very variable in size from about 2-30 cm long and 1-15 cm broad, with larger leaves at the base of the plant and small leaves higher on the flowering stem. Spinach is a low growing fleshly leaved annual that forms a heavy rosette of either smooth or wrinkled leaves. Vegetables, especially those of leafy vegetable grown in heavy metals contaminated soils because of the fact they absorb these metals through their leaves. Spinach has a high nutritional value and is extremely rich in antioxidants, especially when fresh, steamed, or quickly boiled it is a rich source of vitamin A (and especially high in lutein), vitamin C, vitamin E, vitamin K, magnesium, manganese, folate, betaine, iron, vitamin B2, calcium, potassium, vitamin B6, folic acid, copper, protein, phosphorus, zinc, niacin, selenium and omega-3 fatty acids Recently, opioid peptides called rubiscolins have also been found in spinach. The effect of high rates of Zn application on the yield of the vegetable crop needs to be assessed. Keeping this in view, the present investigation was carried out to influence of yield and zinc content by organic (Farmyard manure and poultry manure) and inorganic sources (zinc sulphate) of nutrients under different fertility levels on Spinach crop.

Materials and Methods

A greenhouse pot experiment was conducted to assess the impact of soil application of Zn on its uptake by spinach (*Spinacea oleracea* L) var. All green grown on alkaline soil amended with farmyard manure (FYM) and poultry manure. For the soil, the treatments consist of four levels of Zn, *i.e*. 0, 5, 20 and 40 mg kg^{-1} soil and three levels of organics, *i.e*. control, 3% FYM and 3% PM. All twelve treatment combinations (3 organics × 4 levels of Zn) for each crop were replicated thrice and experiments were laid out in completely randomized design. For the greenhouse study, plastic pots of 5 kg capacity were filled with four kg of soil. A uniform basal dose of N: P_2O_5: K_2O at 11.1: 11.1: 22.2 mg kg^{-1} soil was added in solution form to the soil of each pot through urea, diammonium phosphate and muriate of potash, respectively. Farmyard manure (FYM) and PM at 3% on weight basis were added in powder form and thoroughly mixed with soil. Zinc was applied as $ZnSO_4.7H_2O$ in solution form. The soil in each pot was irrigated with tap water and the pots were maintained at field capacity moisture for one month. After one month, seeds of the crop (10-12 Palak) were sown and a uniform plant population (Palak: 8) was maintained in each pot after a fortnight of germination. Pots were watered daily with required amount of water on weight basis to maintain field capacity. First cutting of Palak (above ground edible portion) was taken at 30 days after sowing (DAS). After first cutting, Palak was top-dressed with N at 11.1 mg kg^{-1} (approx.), and irrigation water. The second cutting of Palak was taken at 55 DAS. Fresh weight of the plant samples was recorded and plant samples were washed with tap water followed by dilute HCl (0.1 N) and finally rinsed with distilled water. Plant samples were first air-dried and then oven-dried in hot air oven at 60 ± 5 $^{\circ}$C until the attainment of constant weight. The dry biomass yield was recorded. Plant samples were ground and digested in a di-acid mixture of $HNO_3/HClO_4$ (9:4) on an electric hot plate and analysed for Zn content using atomic absorption spectrophotometer. Post-harvest soil was taken out of each pot, air-dried, ground and passed through 2 mm sieve and analysed for pH (Jackson, 1973) and DTPA extractable Zn content (Lindsay and Norvell 1978).

To conduct the pot experiment, bulk surface (0-15 cm) soil was collected from the Agricultural Research Farm of Banaras Hindu University, Varanasi. Soil was air-dried, ground and sieved to pass through 2 mm sieve. The pH (8.20) was measured in 1:2.5 soil-water suspension using pH meter (Jackson, 1973). The clear supernatant solution of the above suspension was used for EC (0.26 dS m^{-1}) measurement using Conductivity Bridge (Jackson, 1973). Easily oxidizable organic carbon (0.37 %) of soil was determined by wet oxidation method (Walkley and Black, 1934). DTPA extractable Zn, Cu, Fe and Mn in soil were measured by atomic absorption spectrophotometer (AAS-7000) and found 0.78, 2.16, 34.0 and 7.13 mg kg^{-1}, respectively which were extracted with 0.005 M DTPA—0.01 M $CaCl_2$ (Lindsay and Norvell 1978). Texture of soil was sandy clay loam. FYM and poultry manure were collected from Agricultural Research Farm of Banaras Hindu University, Varanasi and air-dried, ground and sieved to pass through 2 mm sieve. The processed FYM and poultry manure were used for greenhouse experiment. Total organic carbon content in FYM and poultry manure was found 40.9 and 8.13 %, respectively, determined by wet oxidation method (Snyder *et al.,* 1984). FYM and poultry manure samples were digested in di-acid mixture (HNO_3: $HClO_4$: 9:4) and determination for Zn, Cu, Fe and Mn by using Atomic Absorption Spectrophotometer (AAS-7000). The contents of total Zn, Cu, Fe and Mn in FYM were found 154, 62.0, 5200 and 264 mg kg^{-1}, respectively. Total Zn, Cu, Fe and Mn in poultry manure were found 305, 34, 1905 and 412 mg kg^{-1}, respectively.

Results and Discussion

Dry matter yield

Dry matter yield of Palak in first cutting marginally increased (7.7%) due to application of Zn at 5 mg kg^{-1} soil over control, which was statistically, increased with applied level of 40 mg Zn kg^{-1} soil (Table 1). The positive response of spinach, radish, pea, and pepper to applied zinc was also reported earlier by various researchers (Georgievea *et al.,* 1997; Talatam and Parida, 2009). Application of FYM at 3% significantly increased the dry matter yield of Palak in first cutting over control, where no organic was added (Table 1). On an average, the dry matter yield was significantly higher in poultry manure added soil (3.62 g pot^{-1}) than FYM applied soil (3.58 g pot^{-1}); although dry matter yield increased in both poultry manure and FYM added soil over control (2.07 g pot^{-1}). Interactive effects of applied Zn and organics dry matter yield of Palak increased in poultry manure and FYM applied soil at all levels of applied Zn (Table 2). The results were in accordance with Ray *et al.,* (2013) dry biomass yield slightly increased due to application of Zn at 5 mg kg^{-1} soil. Dry matter yield of Palak in second cutting revealed that the dry matter yield was increased marginally due to application of zinc at 5 mg kg^{-1} soil (Table 1), findings accordance with Suvarna *et al.*, (2015). Interactive effect on second cutting the dry matter yield was statistically increased with application of zinc at 5 mg kg^{-1} over control, while highest yield was increased (12.92%) with the Zn application at 40 mg kg^{-1}. Effect of FYM at different levels of applied zinc on the dry matter yield of Palak in second cutting was similar to that obtained in first cutting. Dry matter yield decreased (23.61%) significantly at the Zn application at 40 mg kg^{-1} over control. It was observed that dry matter yield of Palak in first cutting was higher as compared to second cutting across the treatments. The results were in accordance with Ray *et al.,* (2013).

Zinc content in shoot of spinach

Zinc content in shoot of Palak (First cutting and second cutting) increase with concomitant increase in applied level of zinc. Zinc content in shoot of Palak First cutting increased to the tune of 1.53, 2.36 and 2.81 fold and second cutting tune of 1.39, 2.02 and 2.26 fold due to application of Zn at 5, 20 and 40 mg kg^{-1}, respectively over control (Table 1).

Table.1 Effect of applied zinc and organic manures on Dry matter yield (g pot^{-1}) and Zn content of Spinach (first and second cutting)

Treatments	Dry Matter yield (g pot^{-1})		Zinc Content (mg kg^{-1})	
	First cutting (30 DAS)	Second cutting (55 DAS)	First cutting (30 DAS)	Second cutting (55 DAS)
Zinc levels (Zn) (mg kg^{-1})				
Zn0	2.84	1.45	68.01	117.01
Zn5	3.06	1.57	80.34	136.02
Zn20	3.19	1.55	105.24	170.51
Zn40	3.26	1.47	146.23	220.45
SEm±	0.06	0.03	1.95	3.04
CD (P=0.05)	0.19	0.09	5.74	8.92
Organic manures (OM)				
No organics (Control)	2.07	1.34	105.37	170.58
PM	3.62	1.60	100.78	165.72
FYM	3.58	1.58	93.72	146.71
SEm±	0.05	0.02	1.70	2.63
CD (P=0.05)	0.16	0.08	4.98	7.73
Zn × OM	S	S	S	S

FYM – Farm yard manure; PM – Poultry manure; OM – Organic manure; S- Significant

Table.2 Interactive effect of applied zinc and organic manures on Dry matter yield (g pot $^{-1}$) of Spinach (first cutting and second cutting)

	First cutting				Second cutting			
Applied Zn (mg kg^{-1})	**Control**	**FYM (3 %)**	**PM (3 %)**	**Mean**	**Control**	**FYM (3%)**	**PM (3%)**	**Mean**
Zn0	1.46	3.53	3.51	2.83	1.44	1.47	1.43	1.45
Zn5	2.02	3.58	3.57	3.06	1.48	1.62	1.60	1.57
Zn20	2.37	3.60	3.59	3.19	1.35	1.65	1.64	1.55
Zn40	2.41	3.72	3.67	3.27	1.1	1.66	1.65	1.47
Mean	2.07	3.61	3.59		1.34	1.60	1.58	
CD(P=0.05)	Zn=0.18,	O= 0.16,	Zn*OM=0.32		Zn=0.09, O= 0.08, Zn*OM=0.16			

Table.3 Interactive effect of applied Zn and organic manures on Zinc content (mg kg^{1}) in Spinach (first cutting and second cutting)

	First cutting				Second cutting			
Treatments	**ontrol**	**PM**	**FYM**	**Mean**	**control**	**PM**	**FYM**	**Mean**
Zn0	66	69.53	68.5	68.01	112.87	135.05	103.11	117.01
Zn5	93.07	76.1	71.85	80.34	148.79	141.13	118.14	136.02
Zn20	109.5	107.99	98.24	105.24	190.85	167.29	153.42	170.52
Zn40	152.92	149.5	136.26	146.23	229.8	219.4	212.17	220.46
Mean	105.37	100.78	93.71		170.58	165.72	146.71	
SEm±	Zn=2.77, OM=2.40, Zn ×OM=4.80			Zn =4.30,OM =3.73,Zn ×OM=7.45				
CD (P=0.05)	Zn=5.74,OM =4.97,Zn ×OM=9.95			Zn =8.92,OM =7.73,Zn ×OM =15.45				

FYM – Farm yard manure; PM – Poultry manure; OM – Organic manure

Table.4 Influence of applied Zn on DTPA extractable Zn (mg kg^{-1}) in soils after the Harvest of Spinach

Treatments	DTPA extractable Zn
Zinc levels (Zn) (mg kg^{-1})	
Zn0	2.14
Zn5	3.29
Zn20	6.14
Zn40	12.41
SEm±	0.14
CD (P=0.05)	0.40
Organic manures (OM)	
No organics (Control)	6.10
PM	5.85
FYM	6.04
SEm±	0.12
CD (P=0.05)	0.35
Zn × OM	S

FYM – Farm yard manure; PM – Poultry manure; OM – Organic manure; S- Significant

Table.5 Effect of applied Zn and organics on DTPA extractable Zn (mg kg^{-1}) in post-harvest soil

Treatments	control	PM	FYM	Mean
Zn0	1.8	2.38	2.23	2.14
Zn5	2.92	3.46	3.24	3.21
Zn20	5.49	5.94	6.98	6.14
Zn40	13.95	11.21	11.38	12.18
Mean	6.04	5.75	5.96	
SEm±	Zn=0.25 ,	OM=0.22,	Zn × OM=0.44	
CD (P=0.05)	Zn=0.52 ,	OM=0.45,	Zn × OM=0.90	

FYM – Farm yard manure; PM – Poultry manure; OM – Organic manure; Zn- Zinc

The value of zinc uptake at 5.0 and 7.5 ppm were statistically equal (Choudhary *et al.,* 2014). Zinc content in shoot of Palak (first cutting) significantly reduced due to application of FYM and poultry manure (Table 1) over control (105.37 mg kg^{-1}). The results were in accordance with Ray *et al.,* (2013). FYM was more effective in reducing zinc content in shoot of Palak as compared to Poultry manure. As observed in the first cutting, the Zn content in Palak (second cutting), reduced due to application of both FYM and PM (Table 1). Zinc content was lower (146.71 mg kg^{-1}) in the FYM applied soil as compared to poultry manure treated (165.71 mg kg^{-1}) and control soil (170.58 mg kg^{-1}). Some earlier reports have also shown that addition of organic manures (e.g. FYM) reduced the phytoavailability of heavy metals (Brown *et al.,* 2003). A significant reduction in zinc content in the shoot of Palak grown on waste water irrigated soil due to application of FYM was reported earlier by Singh *et al.,* (2010). Findings of present as well as earlier investigations are in contrary to the findings of Gupta *et al.,* (1989), who reported a significant increase in the concentration of zinc in wheat upon addition of FYM. Lower efficiency of sludge in reducing the phytoavailability of zinc as compared to FYM may be ascribed to the quality (Vacha *et al.,* 2002) of organic matter and zinc content in these two organic amendments.

Interactive effects of applied zinc and organics on Zn content in shoot of Palak was found statistically significant in both cutting (Table 3). Across the treatments, the zinc content in Palak was much higher in second cutting as compared to that in first cutting, which may be attributed to better proliferation and development of roots leading to better exploration of soil by the roots as compared to first cutting. The results were in accordance with Ray *et al.*, (2013).

DTPA extractable Zn in post-harvest soil

DTPA extractable Zn was increased by 0.53, 1.86 and 4.7 fold over control due to application of Zn at 5, 20 and 40 mg kg^{-1}, respectively (Table 4). On an average, application of FYM marginally reduced (6.04 mg kg^{-1}) the DTPA extractable Zn in soil as compared to control (6.10 mg kg^{-1}), whereas poultry manure also reduced the extractable Zn (5.85 mg kg^{-1}) in soil (Table 4). The results were in accordance with Ray (2013). Brown *et al.*, (2003) also reported that organic amendments, like FYM, bio-solids and bio-solid compost may affect the bio-availability of heavy metals in soil due to its high content of organic matter, P and Fe. Ineffectiveness of sludge in altering the extractability of DTPA for Zn might have been related to high inherent Zn content of sludge as compared to that of FYM. The reduction in DTPA extractable Zn in FYM treated soil contradicts the earlier findings of Paulose (2003). The interactive effect of applied Zn and organics on the DTPA extractable Zn was statistically significant (Table 4). The reduction in DTPA extractable Zn in FYM amended soil contradicts the findings of Chaudhary and Narwal (2005).

It can be concluded from the present study that soil application of Zn may prove to be very effective in enriching Spinach with Zn. As far as enrichment is concerned, no additional advantage was obtained in case of Zn application along with FYM or poultry manure. However, it appears that the crop could withstand better to the higher level of applied Zn in presence of organics, particularly FYM.

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