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EFFECT OF ZINC ON GROWTH, DRY MATTER YIELD AND NUTRIENT CONTENT OF *VIGNA RADIATA* (L.) WILCZEK

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ABSTRACT

An attempt was made to study the influence of zinc (Zn) on growth, dry matter yield and nutrient content of greengram (*Vigna radiata* (L.) Wilczek) in a glass house earthen pot experiment. Zinc was applied to the soil in the form of zinc sulphate ($ZnSO_4.7H_2O$) in different concentrations (0, 50, 100, 150, 200 and 250 mg kg^{-1}) in which the greengram plants were grown. The plant samples were analysed 45 days after sowing. The results indicated that low level zinc concentrations (50 and 100 mg kg^{-1}) showed a significant increase in the overall growth, dry matter yield and nutrient content, while higher concentrations (150-250 mg kg^{-1}) decreased the growth, dry matter production and nutrient content of greengram.

INTRODUCTION

Zinc is one of the important heavy metals, which is needed as a micronutrient for plants for various metabolic processes. However at excessive levels, zinc has the potential to become toxic to plants. Zinc has been used increasingly in different forms like nutrients, fungicide, pesticide or disinfectant. Zinc is a heavy metal known to occur in higher concentrations in a majority of wastes arising from modern industries (Nriagu, 1980; Boardman and McGuire, 1990; Outridge *et al*., 2011). When present in higher concentrations in the soil and due to its excessive uptake by plants growing on these soils zinc might induce toxicity. Usually zinc toxicity leads to chlorosis of young leaves (Harmens and Gusmao, 1993; Fontes and Cox, 1995; Hermle *et al*., 2007). Plants exhibiting Zn toxicity have smaller leaves (Ren, 1993; Li *et al*., 2009).

In several cases, zinc toxicity has been found to exhibit necrotic lesions on the leaves, eventually leading to entire leaf death (Harmens and Gusmao, 1993; Hermle *et al*., 2007). In roots, zinc toxicity apparently reduces the growth of main root, induces fewer and shorter lateral roots and yellowing of roots (Sagardoy *et al*., 2008; Li *et al*., 2009). Reports are also available on induced deficiency of Mn or Mg or Fe in Zn toxicity (Wallace and Abou-Zam Zam, 1989; Ren, 1993; Ellis *et al*., 2003). The effects of higher concentrations of Zn on plant growth have been studied by many workers (Fontes and Cox 1995; Kupper *et al*., 1996; Castiglione *et al*., 2007; Todeschini *et al*., 2011). Apart from this, the information on plant metabolism is sporadic. Hence efforts have been made to establish the toxic level of zinc on greengram plants in the present study.

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MATERIALS AND METHODS

A greenhouse experiment was conducted in polyethylene lined earthen pots containing 4 kg of well mixed air-dried soil. The pH of the soil was 6.2. Zinc was applied at the rate of 0, 50, 100, 150, 200 and 250 mg kg^{-1} of soil in the form of zinc sulphate ($ZnSO_4$. $7H_2O$). The treatments were replicated five times in a completely randomized block design. Carefully selected uniform sized greengram seeds were directly sowed in each pot and thinned to five plants per pot, seven days after emergence. The plants were sampled 45 days after sowing and the various morphometric growth parameters were employed. Then the samples were kept in hot air oven maintained at 80°C for 48 hours. Dry weight of root and shoot was determined. Oven dried plants were digested in appropriate acid mixtures and the nutrient contents were measured. Using the acid digest, nitrogen was determined by micro-kjeldahl method and phosphorus was determined by molybdovanadate method measuring the absorbance at 460 nm by spectrophotometer. K, Na, Ca and Mg were determined by flame photometer. Cu, Fe, Mn and Zn were determined by atomic absorption spectrophotometer (AAS). The statistical analysis of the experimental data was carried out as per the procedure given by Gomez and Gomez (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Growth response

Plants treated with low level of zinc (50 and 100 mg kg^{-1}) showed a significant increase in root length, shoot length and leaf area, when compared to control (Table 1). Higher concentrations showed a decrease in the root and shoot length and leaf area. These changes are also in consonance with the previous observations (Godbold *et al*., 1983; Kopponen *et al*., 2001; Todeschini *et al*., 2011) that at high concentrations of zinc, the root formation was poor with a concomitant decrease in root length, girth and volume. Reduction of leaf area due to zinc was also observed by previous findings (Ajay and Rathore 1995; Di Baccio *et al*., 2009). Similarly, Vijayarengan and Lakshmanachary (1995) reported the reduction in leaf area of greengram under nickel stress. Significant increase in the growth, possibly due to zinc is required by plants in trace amount. Under lower zinc application, improved root system helped the plant in better absorption of water and other nutrients dissolved in it and consequently improved the growth of different organs and the entire plant (Sindhu and Tiwari 1990; Kaya and Higgs, 2002). The inhibitory action of excess zinc in root and shoot length and leaf area may be due to reduction in cell division, toxic effect of heavy metal on photosynthesis, respiration and protein synthesis. These obviously contribute to the retardation of normal growth (Kupper *et al*., 1996; Hermle *et al*., 2007; Cherif *et al*., 2010).

Nodule number

A large number of nodules were recorded at lower concentrations of zinc (50 and 100 mg kg^{-1}). Further, the number of nodules decreased with a gradual increased in zinc level (150, 200 and 250 mg kg^{-1}) (Table 2). Similar reduction in nodule number under various metal treatments was reported for aluminium on *Trifolium repens* (Wood *et al*., 1984) and lead, cadmium, mercury, copper and nickel on *Vigna radiata* (Jain *et al*., 1994). A decrease in the number of nodules in greengram plants due to elevated level of zinc would be attributed to the reduction in the development of root system as well as the direct toxicity of zinc on soil microbes. This would be evident that most metal ions are toxic to soil microorganisms from several studies (Angle and Chaney, 1991; Vig *et al*., 2003; Ore *et al*., 2010), even in small quantities. Angle and Chaney (1991) also reported that lowest rhizobial population was found in the soil with the highest extractable metal concentration.

Dry matter yield

Plants treated with zinc at low concentrations (50 and 100 mg kg^{-1}) showed a significant increase in dry matter production of root and shoot. But in higher concentrations (150, 200 and 250 mg kg^{-1}), it showed a gradual decline in the dry matter production (Table 1). This view was supported by previous reports (Lal and Maurya 1981; Dube *et al*., 2003; Di Baccio *et al*., 2009) which indicated that application of zinc, significantly increased the dry weight at lower concentration, while excess of zinc reduced the biomass. Similar reduction in dry matter yield of greengram at higher concentration of heavy metals was observed by Vijayarengan and Lakshmanachary (1995) and Wani *et al*., (2007a,b) due to nickel and Madhavi and Rao (1999) due to cadmium. The decrease in biomass in excess zinc treated greengram might be due to low protein formation, resulting in inhibition of photosynthesis, as well as hampered carbohydrate translocation (Samarakoon and Rauser, 1979; Cherif *et al*., 2010).

Macronutrient content

The effect of zinc on various macronutrient contents (N, P, K, Na, Ca and Mg) of greengram leaves indicated that the nutrient contents increased at low zinc levels (50 and 100 mg kg^{-1}) and decreased at high zinc levels (150-250 mg kg^{-1}). From this data it was also observed that the nitrogen content of greengram showed a progressive decline with the increase in zinc level. However, 50 and 100 mg kg^{-1} of zinc level produced positive effect on the N content of greengram leaves. The results are in close confirmation with the findings of Misra (2001) that the uptake of nitrogen was significantly increased at low level of zinc, while higher concentration shows a declining trend of nitrogen. Similarly, Paivoke (1983; 2003) observed that the uptake of nitrogen from the soil is inhibited by the presence of zinc in pea (*Pisum sativum* L.). Sawhney *et al*., (1990) noticed that higher concentration of heavy metals affect not only the development of root nodules, growth and survival of rhizobia, but also nitrogen fixation and the activity of nitrogenase enzyme. Decrease in nitrogen content of greengram leaf due to zinc could be attributed to poor development of nodules, reduced rate of nitrogen fixation and decreased uptake of nitrogen from the soil.

Excess of zinc resulted in lowering of phosphorus content in greengram and revealed a close relationship between phosphorus and zinc. Phosphorus deficiency can be induced by zinc toxicity (Adriano *et al*., 1971; Paulsson *et al*., 2002). High concentration of zinc suppresses phosphorus metabolism by lowering the content of inorganic phosphorus. This suggests a negative correlation between zinc and phosphorus (Dube *et al*., 2003; Liua *et al*., 2006). The decreased content of phosphorus in greengram due to zinc treatment could be attributed to P-Zn interaction mechanism. The decrease in potassium content of greengram due to elevated level of zinc is in conformity with the reports of Jalil *et al*., (1994) and Madhavi and Rao (1999). Metal toxicity in general was associated with reduced absorption and accumulation of potassium (Lidon and Henriques 1993). Lindberg and Wingstrand (1985) and Drazi *et al*., (2004) noticed that high concentration of cadmium inhibited the uptake of potassium by inhibiting respiratory rates, ATP levels and ATPase. The decrease in potassium content of greengram due to zinc may be attributed to the toxic effect of zinc on plant growth or competition by other ions, which in turn exercised a regulatory control on potassium uptake.

The reduction of sodium content in greengram at higher zinc concentrations was in close confirmity with the findings (Moral *et al*., 1994; Metwally *et al*., 2005; You-zong *et al*., 2007) suggested that the application of cadmium reduced the uptake of sodium. The decrease in sodium content as a result of heavy metal treatment might be a consequence of deterioration in the physiological state of the plant, which in turn resulted in a reduction in its uptake. Reduction of calcium content of greengram due to high levels of zinc observed in the present study confirms the earlier reports of Lidon and Henriques (1993), Gussarsson (1994) and You-zong *et al*., (2007). Similarly, Ouzounidou (1994) observed a sharp decline in calcium content in the roots and shoots of *Alyssum montanum* plants, when copper was applied in higher concentration. These changes are in consonance with the earlier reports (Jensen and Adalsteinsson 1989; Cui *et al*., 2008) that copper ions tend to displace Ca^{++} ions from exchange sites and are strongly bound in root free space.

Decline in the magnesium content of greengram at high concentration of zinc was in agreement with the earlier report (Woolhouse, 1983) that zinc toxicity induced deficiency of magnesium. In addition, previous report showed that the zinc treatment also decreased magnesium content (Wang *et al*., 2009). Decreased macronutrient content in the greengram

Table 1 Effect of zinc on growth and dry matter yield of greengram

Zinc added in the soil (mg kg^{-})	Root length (cm plant^{-1})	Shoot length (cm plant^{-1})	Root nodules (plant^{-1})	Leaf area (cm^2 plant^{-1})	Dry matter yield (g plant^{-1}) Root	Shoot
0	18.76	28.76	32.8	436.20	0.358	1.556
50	22.56 (+ 20.25)	34.08 (+ 18.49)	41.2 (+ 25.60)	498.06 (+ 14.18)	0.395 (+ 10.33)	1.702 (+ 19.38)
100	19.80 (+ 5.54)	30.55 (+ 6.22)	34.4 (+ 4.87)	463.33 (+ 6.21)	0.364 (+ 1.67)	1.632 (+ 4.88)
150	17.27 (- 7.94)	26.26 (- 8.69)	29.4 (- 10.36)	427.26 (- 2.04)	0.335 (- 6.42)	1.520 (- 2.31)
200	14.35 (- 23.50)	20.84 (- 27.53)	20.4 (- 37.80)	393.60 (- 9.76)	0.318 (- 11.17)	1.470 (- 5.52)
250	12.62 (- 32.72)	16.27 (- 43.42)	16.4 (- 50.00)	357.76 (- 17.98)	0.292 (- 18.43)	1.418 (- 8.86)
CD at 5%	1.28	1.15	3.47	8.42	0.07	0.01
CD at 1%	1.75	1.58	4.73	11.48	0.01	0.01

Each value is the mean of the five replications
Figures in parenthesis – Percentage over to control
All the values are significant at $P < 0.01$

Table 2 Effect of zinc on nutrient content of the leaves of Greengram

Zinc added in the soil (mg kg^{-1})	N	P	K	Na	Ca	Mg	Cu	Fe	Mn	Zn
	mg g^{-1}						mg g^{-1}			
0	54.6	5.34	32.9	1.74	13.6	4.16	25.27	325	86.55	55.16
50	60.2 (+ 10.25)	5.86 (+ 9.73)	37.6 (+ 14.28)	1.86 (+ 6.89)	14.8 (+ 8.82)	4.75 (+14.18)	34.56 (+36.76)	342 (+ 5.23)	95.63 (+ 10.49)	86.11 (+ 56.70)
100	57.6 (+ 5.49)	5.42 (+ 1.49)	33.2 (+ 0.91)	1.78 (+ 2.29)	13.9 (+ 2.20)	4.26 (+ 2.40)	27.59 (+ 9.18)	330 (+ 1.53)	89.80 (+ 3.75)	107.59 (+96.81)
150	51.8 (- 5.12)	5.25 (- 1.68)	31.6 (- 0.39)	1.69 (- 2.87)	13. (- 3.67)	4.02 (- 3.36)	18.61 (- 26.35)	295 (- 9.23)	80.49 (- 7.00)	142.62 (+ 158.55)
200	47.8 (-12.45)	5.07 (- 5.05)	28.8 (- 12.46)	1.61 (- 7.47)	12.5 (- 8.08)	3.96 (- 4.80)	16.45 (- 34.90)	264 (+ 18.76)	72.62 (-16.09)	158.93 (+ 188.12)
250	42. (- 22.16)	4.98 (- 6.74)	26.2 (- 20.36)	1.54 (- 11.49)	12.1 (- 11.02)	3.74 (- 0.09)	15.30 (- 39.45)	223 (- 31.38)	67.55 (- 21.95)	184.27 (+ 234.06)
CD at 5%	3.06	0.11	1.94	0.06	0.39	0.10	1.17	5.58	1.10	2.05
CD at 1%	4.17	0.15	2.64	0.08	0.54	0.14	1.59	7.61	1.50	2.80

Each value is the mean of the five replications
Figures in parenthesis – Percentage over to control
All the values are significant at $P < 0.01$

leaves under high zinc levels observed in the present study justifies the above statements.

Micronutrient content

Results on the effects of zinc on micronutrient (Cu, Fe and Mn) content of greengram leaves is furnished in the Table 2. Increased zinc content of soil significantly decreased the micronutrient content (Cu, Fe and Mn) of greengram leaves. However, low level of zinc (50 and 100 mg kg^{-1}) increased the Cu, Fe and Mn content of greengram plants over the control. The results are in close conformity with previous findings that an elevated zinc application decreased the copper content in rice (Agarwal and Gupta, 1994) and rapeseed seedlings (Wang *et al*., 2009). Aluminium (Smalley *et al*., 1993) and cadmium (Jalil *et al*., 1994; Streeta *et al*., 2010) application also significantly decreased the content of copper, mainly because of the significant decrease in plant biomass. The response of excess zinc have frequently been attributed to an interference with iron metabolism. This view was supported by Smalley *et al*., (1993), Ouzounidou (1994) Moral *et al*., (1994) and Wang *et al*., (2009). Earlier reports (Kim *et al*., 1978; Samarakoon and Rauser, 1979; Ren *et al*., 1993) state that the interference of heavy metals in excess amount with normal iron metabolism was known to induce physiological iron deficiency. The decrease in iron content of greengram with increase in zinc level suggested a heavy metal induced iron deficiency. Apparent zinc induced manganese deficiency has been reported by a number of researchers. Woolhouse (1983) reported induced deficiency of manganese due to zinc toxicity. This view was also supported by previous reports (Ruano *et al*., 1988; Wang *et al*., 2009) that high zinc supply strongly decreased the manganese content of plants. Apart from zinc, cadmium also affects the Mn content of plants (Moral *et al*., 1994; Streeta *et al*., 2010). Reduction in manganese resulted in the reduction of chlorophyll content. Decrease in manganese content may be due to the competition of zinc with manganese for transport sites in plasmalemma.

Uptake and accumulation of zinc

Maximum zinc accumulation in the greengram leaves was recorded at 250 mg kg^{-1} level in the soil. The minimum zinc accumulation of greengram leaves was observed in control. Increase in zinc level in the soil resulted in the higher uptake and accumulation of zinc by the plants. Gladstones and Loneragan (1967) made similar observations that uptake of zinc increased with the increased application of zinc in clove and peas. This view is also supported by Sakal *et al*., (1985); Davis and Parker (1993) and Stoyanova and Doncheva (2002).

CONCLUSION

From these observations it can be concluded that, low zinc concentration (50 and 100 mg kg^{-1}) had stimulatory effect on growth, dry matter yield and mineral nutrient content of greengram. Application beyond these levels (150, 200 and 250 mg kg^{-1}) adversely affected the growth, dry matter yield and nutrient content, and it also indicated that more stimulatory effect could be achieved in 50 mg kg^{-1} than 100 mg kg^{-1} of soil zinc level over the control.

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