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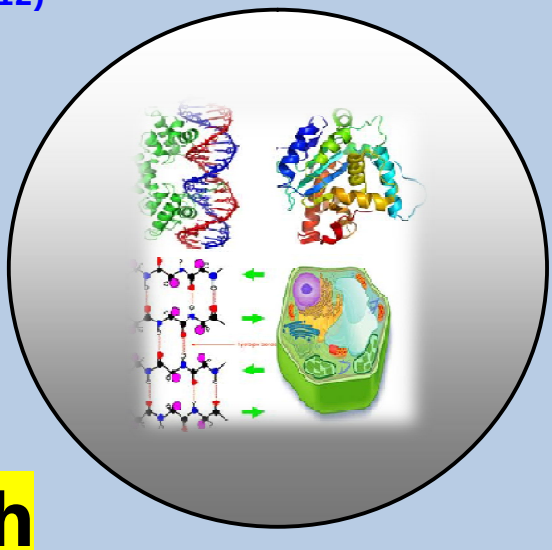

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Effect of Zn and Cu Application on Growth, Yield and Biochemical Responses of Wheat Plants (*Triticum aestivum* L. cv-PBW-343)

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ABSTRACT

A pot experiment was conducted during winter (rabi) season in warehouse condition to study the response of zinc application on wheat reproductive yield, Zn uptake, sugar, proline and protein contents and activity of amylase, catalase and peroxidase. Zn were added to soil @ 0, 5, 25, 50 and 100 mg kg^{-1} as ZnSO4 at the time of sowing in all the treatments while another set of pots supplied with 100 mg Zn+5mg Cu kg^{-1} soil. Least significant difference analysis of data revealed that yield parameters, Zn uptake, sugar, proline and protein contents of wheat plants were significantly increased grown in 50 mg kg^{-1} treated soil. Content of Zn were significantly ($p<0.01$) increased in root and shoot of wheat plants grown at 50, 100 and 100 mg Zn+5mg Cu kg^{-1}. As comparison to control, value of yield parameters of wheat plants grown in soil supplied with 100 mg kg^{-1} Zn were found to be decreased, but supplementation of this level with 5mg Cu increased the value of all the yield parameters. Activity of catalase, peroxidase and amylase were found to be significantly increased by 54.9%, 47% and 75% respectively in the leaves of wheat plants grown in soil amended with 50 mg kg^{-1} compared to control.

Key words: Wheat, Zn, Reproductive Yield, Catalase and Protein

INTRODUCTION

Zinc deficiency is now recognized as one of the most critical micronutrient deficiencies in plants. It is estimated that about 50% of soil used for plantations in the world have low levels of plant-available Zn (Graham and Welch, 1996).

Zinc is an essential micronutrient required for optimum plant growth. Zn is considered to play a critical physiological role in the structure and function of membrane lipids especially under salt stress (Aktas *et al*., 2006). Zinc also plays an important role in the production of biomass (Cakmak, 2008) and in controlling the generation and detoxicification of free oxygen radicals which can damage membrane lipids and sulphydryl groups (Alloway, 2004). Zinc as a micronutrient in wheat production has been clearly proved. Effects of Zinc Deficiency and response to wheat growth stages have been reported from various parts of the plants (Shaheen *et at*., 2007), also zinc shortage has a worldwide problem in human nutrition (Ranjbar and Bahmaniar, 2007).The studies have been shown that one of the effective and productive way to improvement in cereal grains is application of Zn fertilizer either to the soil or foliar application (Jiang *et al*., 2008). A nutrients imbalance may also arise by the presence of an excessive amount of a nutrient element that hinders another nutrient in performing its normal metabolic functions (Malewar, 2005; Zengin and Kirbag, 2007). The higher concentration of micronutrient cation like Zn in culture medium limits the growth of the plants (Sharma and Bapat, 2000). Many workers (Kumar *et al*., 2009) previously have reported that addition of some micronutrients in soil reduced the toxicity of metal ion which is present in excesses. Studies have shown that Cu and Zn interact with each other due to antagonistic relationship as Cu-Zn antagonism has been suggested by many workers (Dangarwala, 2001). Hence, the present study was undertaken to evaluate the effect of soil application of Zn and Zn+Cu interaction on the growth, yield, and Zn tissue concentration and biochemical parameters of wheat plants.

MATERIALS AND METHODS

A pot culture experiment was conducted to study the effect of different levels of Zn application on reproductive yield and biochemical responses of wheat (*Triticum aestivum* L. cv-PBW-343) plants grown in Gomti-upland alluvial soil (entisol). Some physico-chemical characteristics of the soil were: texture-sandy loam, pH (1:2.5 soil water extract) 8.6, EC2 0.20 dSm^{-1}, organic matter 0.67%, CaCO3 0.80%. The seeds were surface sterilized with 0.01% $HgCl_2$ for 5 min and rinsed extensively in running distilled water (H_2O). Healthy and equal-sized seeds were chosen and soaked in distilled H_2O for 24hr and germinated in dark at (25^0C). Treatments consisted of various levels of Zn (0, 5, 25, 50 and 100 mg kg^{-1}) and 100 mg Zn+5 mg kg^{-1} Cu which were added to soil as a basal dose. The plants were irrigated with tap water as and when required. Biochemical parameters were studied in 50 days old plants; however yield parameters were recorded at maturity. For tissue Zn analysis, oven-dried samples were digested in a nitric and perchloric acid mixture (10:1). Zinc concentrations were estimated in clear digests by atomic absorption spectrophotometry (ElemantAS AAS4141; Electronics Corporation of India Limited, Hyderabad, India). Metal concentrations were expressed as µg g^{-1}dw tissue. Pigments content was measured by the method of (Arnon, 1949) and was expressed in mg g^{-1} FW of tissue. Protein content was estimated by the method of Lowry *et al.* (1951) and contents were expressed as µg g^{-1} FW tissue. Sugar content was determined by the method of Dubois *et al.* (1956).

The quantity of sugar was expressed as mg g^{-1} FW of tissue. Extraction and determination of proline was performed according to the method of bates *et al*. (1973) and calculated as µmol g^{-1} FW against standard proline. Catalase activity was assayed following to the method prescribed by Euller and Josephson (1927) with some recent modifications for the estimation of catalase activity by Bisht (1978). Catalase activity was calculated expressed in terms of µmol H_2O_2 decomposed mg^{-100} FW tissue. Peroxidase activity was estimated by using the method of Luck (1963). Peroxidase activity was expressed in terms of ml ΔOD mg^{-100} FW of tissue. Amylase activity was assayed by the method of Katsuni and Fekuhara (1969). Amylase activity was expressed in terms of mg starch hydrolyzed g^{-1} FW tissue.

RESULTS AND DISCUSSION

Plant height and plant dry weight of wheat (*Triticum aestivum* L. cv-PBW-343) were increased as result of treatment with different concentrations of Zn as soil application (Figure 1). At 50 days, plant height increased by 42.8% at 50 mg kg^{-1} Zn amendment but it was decreased by 3% at 100 mg kg^{-1} but soil amended with 100mg Zn+5mg Cu showed 41% increment in plant height. Compared to control, dry weight of plants increased by 67% at 50mg kg^{-1} Zn amendment which was decreased by 7% at 100 mg kg^{-1} (Figure 1).

Table 1.1 Effect of various levels of Zn and Zn+Cu amendment in soil on biochemical parameters of wheat (*Triticum aestivum* L.) plants observed at 50 days.

Parameters	Treatments						
	Control	5 mg kg^{-1} Zn	25 mg kg^{-1} Zn	50 mg kg^{-1} Zn	100 mg kg^{-1} Zn	100 mg Zn+5mg Cu kg^{-1}	LSD
Catalase(µmol H_2O_2 decomposed mg^{-100} FW)	242±13.2	237±5.50	360±6.35*	375±14.4*	210±6.06	424±14.1**	91.6146
Amylase(ml H_2O_2 hydrolysed mg^{-1} FW)	10.48±3.4	13.8±1.0*	16.35±0.87**	18.40±1.2**	17.0±2.88**	15.3±0.9**	2.874.60
Peroxidase ΔOD mg^{-100} FW	6.23±0.60	7.80±0.46*	8.60±0.34*	9.16±0.66**	5.60±0.34	8.96±0.5**	1.532.45
Sugar mg g^{-1} FW	4.97±0.57	5.60±0.34	7.89±0.51*	8.28±0.17**	3.96±0.57	9.46±0.3**	2.213.54
Protein (mg g^{-1} FW)	81.0±5.77	86.6±3.75	88.6±4.91	98.2±4.61*	65.3±3.71*	112±7.2**	16.225.9
Proline (mM g^{-1} FW)	4.2±0.40	4.8±0.46	5.26±0.34*	5.60±0.17**	6.02±0.31**	5.62±0.4**	0.671.07

*-value significant at P<0.05 and **- value significant at P<0.0 levels; ± (standard error among triplicate).

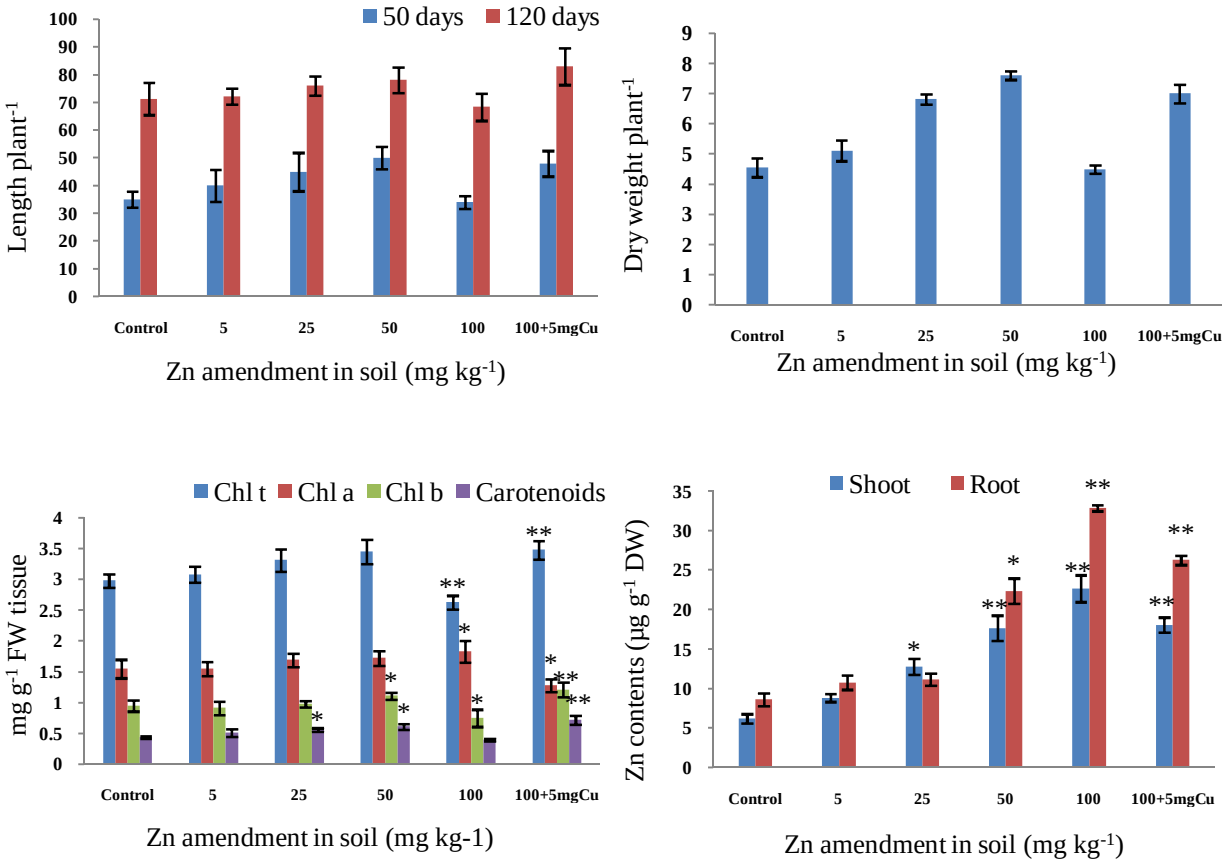
However soil amended with 100mg Zn+5mg Cu kg^{-1} increased the dry weight of plants by 77%. The stimulative effects of Zn on plant growth were also obtained by many workers (Tobbal, 1999; Hemantaranjan *et al*. 2000; Rizk and Abdo, 2001; Wanas, 2002 and Gomaa, 2003). The Zn concentrations in leaves increased significantly with an increase in the level of applied (Figure 1). Compare to control, Zn contents in shoot and root of wheat plants grown at 100 mg kg^{-1} were increased by 264 and 28% respectively. This is in accordance with the reports of many workers. Number of tillers $plant^{-1}$, No. of inflorescence $plant^{-1}$, inflorescence length $plant^{-1}$, Inflorescence weight $plant^{-1}$, No. of grains ear^{-1} and Weight $seeds^{-100}$ significantly ($p<0.01$) increased upto 50 mg kg^{-1}Zn amendment in soil while at 100 mg kg^{-1}, it was slightly decreased below the control levels (Table 1.2). However 5mg kg^{-1} Cu application significantly ($p<0.01$) enhanced the values of these parameter of wheat plants grown in 100 mg kg^{-1} Zn amended soil. Khan (2008) had reported applications of Zn had positive effect on plant growth leading to increased LAI, plant height, number of fertile tillers m^{-2}, and number of filled spikelets $spike^{-1}$, spike length, grains $spike^{-1}$, straw yields and 1000 grain weight culminating in improved grain yield. Other workers have also reported that zinc application improved spike length and effective tillers $plant^{-1}$ (Islam *et al*. 1999) and number of grains $plant^{-1}$ (Genc *et al*. 2006). Genetic improvement of the wheat crop in the past has mostly been obtained from increased number of grain m^{-2} and harvest index (Slafer and Andrade 1991; Reynolds *et al*. 1999; Brancourt-Hulmel *et al*. 2003) and improvement in grain yield also attributable to improved biomass production particularly through fertilizer additions and crop protection (Siddique *et al*. 1989; Donmez *et al*. 2001). In this study, reduction in reproductive yield at 100 mg kg^{-1} Zn could be due imbalance uptake of nutrients which limits the growth of the plants (Sharma and Bapat, 2000). Many workers (Kumar *et al*., 2009) previously have reported that addition of some micronutrients in soil reduced the toxicity of metal ion which is present in excesses. Contents of total chlorophyll, chlorophyll a, chlorophyll b and carotenoids significantly ($p<0.01$) increased up to 50 mg kg^{-1} Zn then decreased by 11, 18, 21 and 9% at 100 mg kg^{-1} Zn respectively (Figure 1). However contents of these pigments significantly enhanced in plants grown in soil amended with 100mg Zn+5mg Cu compared to plants grown in 100 mg kg^{-1} Zn amended soil. These results are in agreement with the results obtained by Rashid and Ahmed (1997) on broad bean plants. They found that, chlorophyll contents were increased by using foliar application with Zn (50 ppm). Hassanein *et al*. (2000) found that, spraying cow pea (*Vigna sinensis*) plants with 10, 50 and 250 mg/L of B or Zn caused high significant increases in the contents of chlorophyll (a) and chlorophyll b. Activity of catalase, peroxidase and amylase was found to be increased with increase in Zn concentration in soil up to 50 mg kg^{-1} Zn level then decreased at 100 mg kg^{-1} Zn. Activity of catalase significantly ($p<0.05$) increased by 54.9% in the leaves of wheat plants grown in soil amended with 50 mg kg^{-1} compared to control (Table 1.1). However value of catalase at 100 mg kg^{-1} Zn treatment was 210 which was increased by 100% in plants grown in 100mg kg^{-1} Zn+5mg kg^{-1} Cu.

Similarly activity of peroxidase significantly ($p<0.01$) increased by 47% in the leaves of wheat plants grown in soil amended with 50mg kg^{-1} compared to control (Table 1.1). However activity of amylase significantly ($p<0.01$) increased by 75% grown in soil amended with 50 mg kg^{-1} compared to control. Tobbal (2006) and Gamal El-Gebaly *et al*. (2003) reported increased activity of CAT and POX enzymes in plants treated with Zn. Saha and Gupta (1997) and Khan *et al*. (2002) also have reported reduced catalase activity under salt stress. In his experiments, reduced catalase activity at 100 mg kg^{-1} Zn was due to decrease in protein contents. Sandalio *et al*., (2001) showed that the reduction of CAT activity in pea plants was due to a decrease in the protein content. Sugar, protein and proline contents were found to increase in the leaves of wheat grown in Zn amended soil (Table 1.1). Sugar contents was significantly ($p<0.01$) increased in the leaves of wheat plants by 12.8, 58.8, 66.7 and 138% grown in soil amended with 5, 25, 50 and 100 mg kg^{-1}Zn+ 5mg kg-1Cu. While protein contents was significantly ($p<0.01$) increased by 21.0 and 69% in the leaves of wheat plants grown in soil amended with 50 and 100 mg kg^{-1}Zn+ 5mg kg^{-1}Cu. Value of proline in wheat leaves of control plants was 4.20 which were significantly increased by 14, 25, 33 and 43% in the plants grown in soil amended with 5, 25, 50 and 100 mg kg^{-1}.

Table 1.2 Effect of various levels of Zn and Zn+Cu amendment in soil on reproductive yields of wheat (*Triticum aestivum* L.) plants observed at 120 days.

Parameters	Treatments						
	Control	5 mg kg^{-1} Zn	25 mg kg^{-1} Zn	50 mg kg^{-1} Zn	100 mg kg^{-1} Zn	100 mgZn+5mg Cu kg^{-1}	LSD
No. of tillers $plant^{-1}$	5.96±0.31	6.96±0.14	7.43±0.29*	8.21±0.23*	5.20±0.11	8.40±0.8**	1.29 2.10
No. of inflorescence $plant^{-1}$	2.96±0.45	3.70±0.11	4.03±0.60	5.20±0.34**	2.53±0.31	5.63±0.4**	1.25 2.00
inflorescence length $plant^{-1}$	6.53±0.26	7.13±0.29	8.53±0.31	10.2±0.69**	6.53±0.31	11.8±0.5**	2.22 3.56
No. of grains ear^{-1}	28.1±3.95	29.8±1.87	35.4±1.40	40.0±2.89**	25.4±2.3	50.1±5.3**	9.39 15.0
Inflorescence weight $plant^{-1}$ (g)	6.93±0.54	7.58±0.34	8.80±0.46*	9.58±0.34**	6.36±0.23	10.2±0.4**	1.57 2.51
Weight $seeds^{-100}$ (g)	3.85±0.08	4.85±0.20*	4.90±0.23*	5.21±0.24*	3.23±0.14	5.46±0.2**	0.88 1.41

*-value significant at P<0.05 and **- value significant at P<0.0 levels; ± (standard error among triplicate).



*-value significant at P<0.05 and **- value significant at P<0.0 levels; ± (standard error among triplicate).

Figure 1. Effect of various levels of Zn and Zn+Cu amendment in soil on plants length, dry weight, pigments contents and Zn contents of wheat (*Triticum aestivum* L.) plants.

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