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Effect of Zinc on Physico-chemical Parameters of Hydroponically grown Rice Varieties

[1]Humaira, [1]Tayyeba Samreen, [2]Muhammad Javid, [3]Muhammad Amin, [4]Hamid U. Shah, [4]Saleem Ullah, [4]Sahib Alam

[1]Soil Science Directorate, Agricultural Research Institute Tarnab, Peshawar, Khyber Pukhtunkhwa, Pakistan.
[2]Chemistry Division PAEC PO Box # 1331, Islamabad, Pakistan
[3]Department of Environmental Sciences, Shaheed Benazir Bhutto University, Sheringal Dir Upper, Khyber Pukhtunkhwa, Pakistan
[4]Department of Agricultural Chemistry, The University of Agriculture Peshawar, Khyber Pukhtunkhwa, Pakistan

ABSTRACT

Rice varieties JP-05, Swat-I, IRRI-6 and Basmati-2000 were germinated and grown hydroponically. The effect of the Zn dosages (0, 1 and 2µM) on the plant height, crude protein, chlorophyll "a" and "b", zinc and iron contents of plant were evaluated. IRRI-6 and Basmati-2000 attained greater height relative to JP-05 and Swat-I at 1µM of Zn dosage whereas crude proteins were maximum in JP-05 and Basmathi 2000 at 1µM Zn dosage and Swat-1 and IRRI-6 at 2µM Zn dosage. A similar pattern for the leaf chlorophyll "a" and "b" was also observed. Basmati 2000 and JP-5 had got maximum amount of chlorophyll "a" and "b" at 1 µM Zn dosage while Swat-1 showed higher contents of chlorophyll at 2 µM Zn dosage. The Zn contents of the plants increased upto 1µM Zn dosage, however, the Fe contents was observed to decrease. This signified the competitive behavior of Fe with Zn. The Zn dosage 1 µM was found potentially effective in obtaining maximum plant height, crude protein, and chlorophyll "a" and "b" for the rice plants.

Key words: Chlorophyll, hydroponic, plant height, protein, rice, zinc

Introduction

Rice (*Oryza sativa* L.), a member of the graminae family is an important cereal crops (Stone, 2008) feed more than half of the World population (David, 1989). About 92 % of the world's rice is produced and consumed in Asia (IRRI, 1997 and, Luh, 1991). In Pakistan, rice is the third largest crop in terms of sowing after wheat and cotton (Abedullah *et al*., 2007). It accounts for 6.1 percent of the total value added in agriculture and about 1.3 percent to GDP (Government of Pakistan, 2006). Micronutrients (Boron (B), Copper (Cu), Manganese (Mn) and Zinc (Zn)) form an important part of the soil. The deficiency of any of the micronutrients could limit the crop growth. Among these micronutrients, Zn is the most important and commonly deficient micronutrient Worldwide (Welch *et al*., 1991). About 50 % of the soils used for cereal production in the World, have been estimated to have low levels of plant available zinc (Graham and Welch, 1996). Pakistan also lists among those countries, which had the lowest levels of Zn in soils (Sillenpaa and Vlek, 1985). Zn deficiency is the major micronutrient disorder of rice due to the alkaline and calcareous nature of soil in Pakistan. In Pakistan, Zn deficiency spreads over 0.7 million hectares of the rice grown soils (Ponnamperuma, 1981). Zn is adsorbed by the soil and very little is available to the plants (Khan *et al*., 2007). Zn plays multiple roles in the plant biochemical and physiological processes (Broadley *et al*., 2007). It is co-factor in over 300 enzymatic reactions. It also has its role in plant chlorophyll formation. A slight deficiency of Zn will cause a decrease in growth, yield, and Zn content of edible parts (Graham *et al*., 1999). On the other hand, elevated concentrations can also lead to toxicity symptoms and growth inhibition in rice (Song *et al*., 2011). Zn deficiency of the rice field is treated in various ways. In general, the fertilization of rice crops is supplemented with $ZnCl_2$ or $ZnSO_4$, but it might lead to Zn accumulation in soil, which is detrimental to next growing crop. Hence, appropriate amount of Zn is needed in soil to avoid unnecessary accumulation of Zn in the field. In contrast to soil, hydroponic growing system may be employed to avoid accumulation of Zn and minimize environmental contamination, which stems from fertigation runoff (Savvas, 2002; Rouphael *et al*., 2006). Growing plant in solution culture is an easy technique that provide a controlled environment of nutrients to the roots (Sonneveld, 2002) thereby avoiding complexities and interferences induced by soils, which results in saving of irrigation water, fertilizers and high yields of crops (Rouphael *et al*., 2005). In recent years, focus has been paid towards the hydroponic growth of wheat and rice in Asia (Brian *et al*., 2009; Nemati *et al*., 2011).

Corresponding Author: Humaira, Tayyeba Samreen, Soil Science Directorate, Agricultural Research Institute Tarnab, Peshawar, Khyber Pukhtunkhwa, Pakistan. Tel # +92 91 2964031, Fax # +92 91 2964097
E-mail: tayyeba.samreen@yahoo.com - sarwarhumaira@yahoo.com

Keeping in view the above considerations, a solution culture experiment is designed to grow rice varieties and monitor their growth, biochemical response and nutrients contents under the influence of different levels of Zn doses.

Material and Methods

Rice varieties and growth conditions

Samples (1kg) of four registered rice varieties (JP-05, Swat-I, IRRI-6 and Basmati-2000) were obtained from the different research centers in Pakistan. JP-05 and Swat-I were collected from agricultural research center Swat. IRRI-6 and Basmati-2000 were obtained from national agricultural research Council (NARC) Pakistan and agricultural research institute Dera Ismael Khan respectively. These samples were tested for the effect of Zn concentrations (0, 1 and, 2 µM) on their physicochemical parameters viz. height, crude protein, chlorophylls and mineral constituents. The experiment was performed under the two factors (concentrations × varieties) factorial design in pots containing solution with Hoagland's formula.

Healthy grains of each rice variety were disinfected in 1% sodium hypochlorite solution for 10 minutes. After sterilization the grains were thoroughly washed with sterilized distilled water. About 100 grains were germinated on filter papers in beakers (100mL) containing ¼th sterilized distilled water (Baiyeri and Mbah, 2006). The grains were incubated at 38 ^{o}C and upto 99% germination was completed about in seven days (Sokal and Rohlf, 1997). The germinated seeds were transferred in medium size plastic pots with sterilized sand as a support for seedlings. Pots were perforated from beneath and were placed alternatively in plastic tubs (2L) containing half strength Hoagland's solution (Hoagland and Arnon, 1938). The Pots were finally artificial heated (1000W rod) and lighted (three tube lights per four pots). The solution was renewed regularly after three days period. Iron solution was renewed every alternate day. After eight week, plant sample were taken and were analyzed fresh or dried for the following parameters.

Measurements of plant parameters

Height was measured from sand surface to leaf apex with measuring tape. An average of five leaves was recorded as height data for a pot.

Crude protein

Crude protein (CP) was determined by Kjeldahl method (AOAC, 1990). Half gram sample was taken in digestion flask and 8 gram of digestion mixture (1:7 $CuSO_4$+K_2SO_4) and 10mL of H_2SO_4 was added to the flask. Clear residue was obtained after boiling at 300 ^{o}C for 6 to 8 hours. The residue was diluted upto 100 mL with distilled water in volumetric flask. 10 mL of each of the residue and NaOH (40%) was introduced in Markham Still distillation apparatus, where ammonia produced were collected in 4% boric acid solution containing modified methyl red as indicator. The produced NH_4OH was titrated against HCl (0.01N). Crude protein was calculated as follows.

$$\% \text{ Nitrogen} = \frac{(S-B) * N * 0.014 * D * 100}{Sample\ wt * V} \qquad (1)$$

While crude protein= % N * 6.25 (2)

Where S denotes sample reading, B is blank reading, N normality of HCl, D is the dilution factor, V is the volume of titrant consumed.

Chlorophyll "a" and "b"

The analysis of chlorophyll was performed on High Performance Liquid Chromatography (HPLC) by the method of Heinonen (1990). Half gram of fresh leaves taken from each treatment was homogenized in 5 mL cold acetone using a pestle and mortar. The homogenate was added anhydrous sodium sulfate and filtered with wattman's paper No.4. The filtrate was evaporated with nitrogen and re-dissolved in 1 mL of HPLC grade acetone. 20 µL of sample was injected in a HPLC system (Perkins Elmer) with C_{18} column. Acetonitrile-dichloromethane-methanol (70:20:10 v/v/v) was used as mobile phase. Chlorophyll "a" and "b" were detected at 472 nm using UV/VIS Universal Detector with CSW32 integration software. Reading was denoted in µg/g as follows.

$$\mu g/g = \frac{Instrumental\ Reading * V * D.F.}{Sample\ Weight} \qquad (3)$$

Minerals assay

Dried and ground leaves samples were wet digested for minerals as stated by Jones and Mills (1991) in 1: 1 mixture of HNO_3-$HClO_4$ by gradually increasing the temperature upto 300 °C. Iron (Fe) and Zn were determined using double beam atomic absorption spectrophotometer Perkins Elmer model 2380.

Statistical analysis

The data were statistically analyzed with the help of two Factors Randomized Complete Block Design using M-StatC software package, where means were compared by range test of the same software mentioned by Steel and Torrie (1980).

Results and Discussion

Plant height

The effect of Zn on plant height of various varieties of rice treated with different Zn concentrations is shown in figures (1 and 2). It is obvious from the figures that maximum height is noted over control in all rice varieties treated with Zn doses. The Zn effect observed here is reported in the literature on a number of plants (Alloway, 2008). Increase in the plant height with Zn dose might be due to the auxin metabolism as reported by Srinivasan and Naidu (1996) "that Zn favorably influenced the shoot length most probably through auxin metabolism". The data taken in figure (3 and 4) shows the variation of plant height with time interval and the varietal effect. Varieties IRRI-6 and Basmati-2000 produced maximum height compared to JP-05 and Swat-I at 1 µM of Zn concentration whereas at 2 µM of Zn concentration Swat-I and IRRI-6 got maximum height. Statistically the plant height was positively correlated with Zn treatments. Alam and Shereen (2002) while studying the effect of different levels of Zn and phosphorous on wheat during water culture experiment found that wheat shoot length was increased in almost all treatments as compared to the control. Since plant grown in solution culture, shift the absorbed Zn comparatively easy to various parts of plant and hence amplify the tolerance limit of rice.

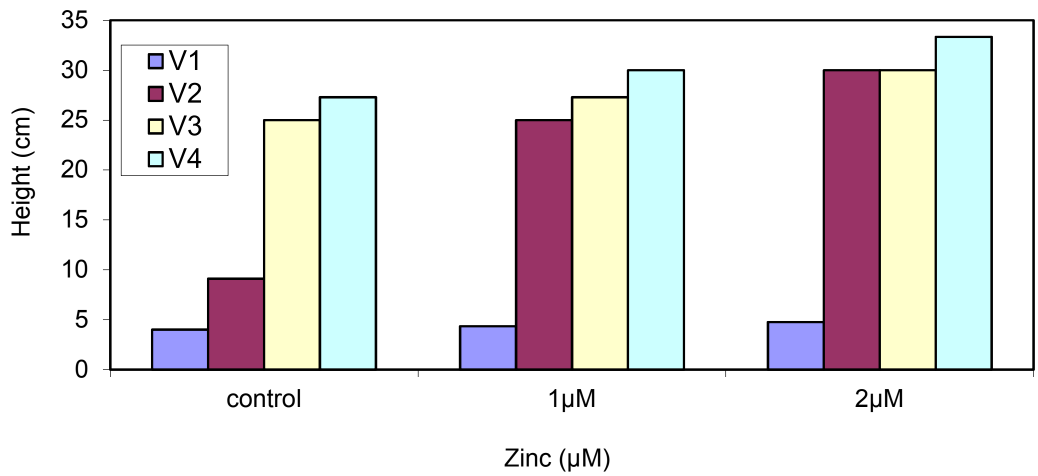

Fig. 1: Zn effect on plant height of various rice cultivars after six weeks.

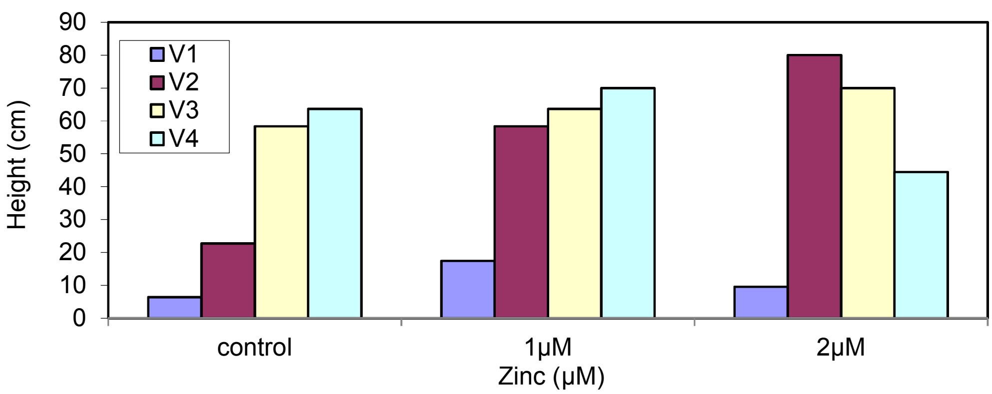

Fig. 2: Zn effect on plant height of various rice cultivars after eighth week.

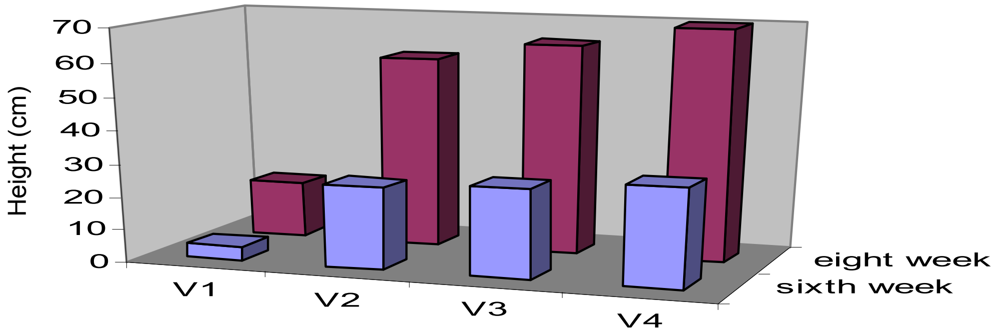

Fig. 3: Effect of weeks on height and varietals difference at 1 µM Zn solution supply.

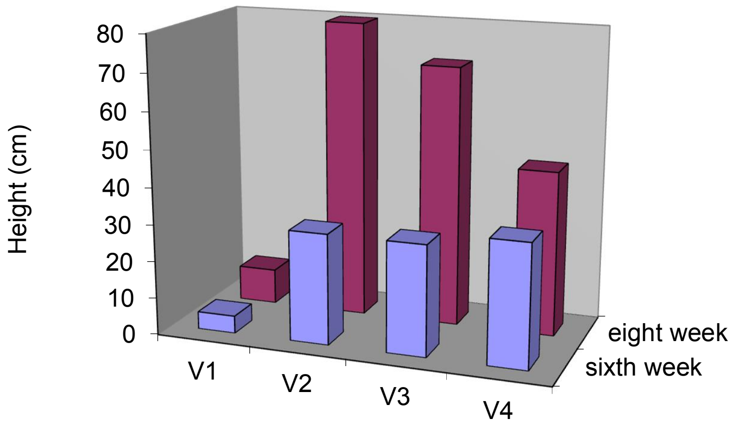

Fig. 4: Effect of weeks on height and varietals difference at 2 µM Zn solution supply.

Protein contents

The average crude protein content presented in Table (1) showed a range of 19.89 to 26.21%. Variety JP-05 contained the lowest crude protein content on average while Basmati-2000 possessed the highest. It was observed that varieties JP-05 and Basmati-2000 exhibited maximum crude protein content at 1µM of Zn concentration Whereas Swat-1 and IRRI-6 at 2 µM Zn. The Zn was positively correlated in case of JP-05, IRRI-6 and Basmati-2000 (r = 0.681*, 0.981**, 0.0801**), respectively (Table 1). It is believed that Zn enhances the activity of zinc-superoxide dismutase, which results in the production of more protein in plant and vice versa in case of low Zn contents. Similar conclusions were reported by Obata *et al.* (1999) in rice cultivation. The behavior observed in the present studies is also analogous to those observed by us in our earlier article on mungbeans grown hydroponically (Samreen *et al.*, 2013).

Table 1: Percent crude protein contents in rice varieties at different concentration of Zn in solution culture

Treatments	V1	V2	V3	V4	Mean	St. dv. ±
Control	17.91	21.21	11.66	27.78	19.64	6.72
1 µM	24.82	16.01	23.38	28.60	23.20	5.28
2 µM	16.94	25.05	29.16	22.24	23.35	5.13
Mean	19.89	20.75	21.40	26.21		
St. dv. ±	4.29	4.54	8.91	3.46		

V_1=JP-05, V_2= Swat-1, V_3= IRRI-6, V_4= Basmati-2000
St. dv. =Standard deviation

Chlorophyll contents

The Chlorophyll "a" contents given in Table (2) showed a range of 158.34 to 504.38 mg/kg. The lowest mean content is observed in Swat-1 which is at par with that of JP-05 whereas the highest mean content is noted in Basmati 2000. The data further illustrate that chlorophyll “a” content increased significantly with the addition of Zn giving an improvement of 31% with 1µM Zn dose. Further addition did not improve chlorophyll “a” content. Maximum chlorophyll “a” content was obtained in Basmati-2000 with 1µM Zn which can be seen in figure (5). The Correlation between Zn treatments and chlorophyll “a” was positive (r = 0.560, 0.737**, 1.00**, 0.504) in all four varieties respectively.

Table 2: Chlorophyll “a” content (mg/kg) in rice varieties at different concentration of Zn in solution culture

Treatments	V1	V2	V3	V4	Mean	St. dv. ±
Control	92.07i	125.67h	153.07g	504.60b	218.85b	192.12
1 µM	302.47d	83.47i	221.07f	543.13a	287.54a	192.88
2 µM	100.67i	265.87e	294.93d	465.40c	281.72a	149.38
Mean	165.07c	158.34c	223.02b	504.38a		
St. dv. ±	119.07	95.49	70.95	38.87		

**Means showing same letter(s) are statistically non-significant at 5% level of probability.*
V_1=JP-05, V_2= Swat-1, V_3= IRRI-6, V_4= Basmati-2000
St. dv. =Standard deviation

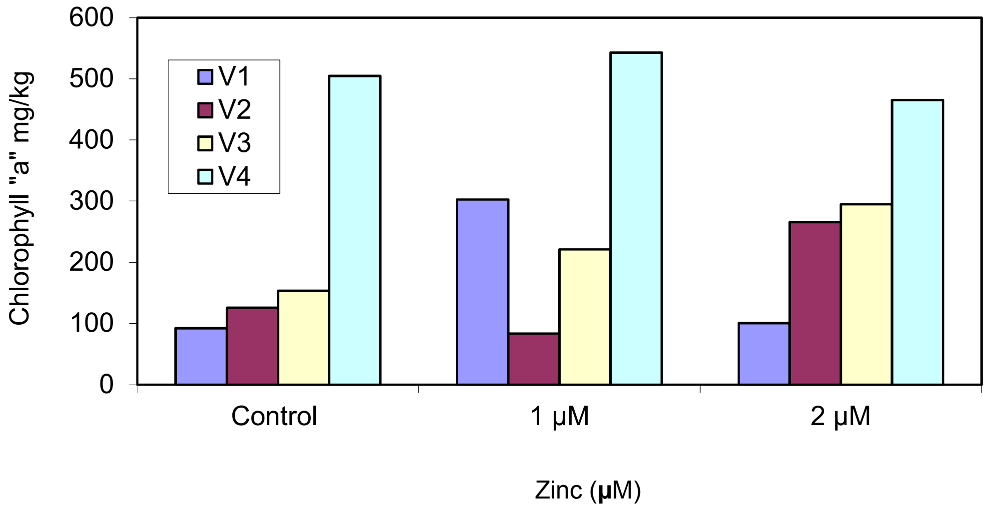

Fig. 5: Chlorophyll “a” of rice varieties as a function of Zn treatments.

Chlorophyll “b” contents given in Table (3) showed a range of 50.91 to 142.98 mg/kg, which is much less than chlorophyll “a” contents of plant. On the contrary, here the lowest mean contents are observed in IRRI-6 whereas the variety possessing highest mean contents Basmati- 2000 is identical in both the cases of chlorophyll “a” and “b”. The data also demonstrate that application of 1µM Zn solution improved Chlorophyll “b” content by 16% as compared to control which is almost 50 % less than the observed in case of chlorophyll “a”. Higher concentration of Zn solution caused a decrease compared to that of the level of control (Figure 6). Chlorophyll “b” content was positively (r = 0.845**, 0.532) dependent on Zn especially in Swat-1 and IRRI-6. Chlorophyll production is related to Zn availability. Reduction in the level of chlorophyll contents may be due to the toxicity of Zn at high dose. This behavior indicates the sensitivity of enzyme of chlorophyll biosynthesis towards Zn (Sharma and Chopra, 1987). Reduction in chlorophyll could also be a result of iron deficiency due to Zn toxicity and competition with iron. Our opinion is that both explanations are justified. High Zn dose is toxic and depress iron contents, which ultimately reduce the chlorophyll a and b contents.

Table 3: Chlorophyll “b” content (mg/kg) in rice varieties at different concentration of Zn in solution culture

Treatments	V1	V2	V3	V4	Mean	St. dv. ±
Control	62.80e	51.33f	48.80f	140.33b	75.82b	43.44
1 µM	82.13d	51.00f	52.93f	165.87a	87.98a	53.84
2 µM	56.07f	58.07ef	51f	122.73c	71.97c	33.97
Mean	67.00b	53.47c	50.91c	142.98a		
St. dv. ±	13.53	3.99	2.07	21.69		

**Means showing same letter(s) are statistically non-significant at 5% level of probability.*
V_1=JP-05, V_2= Swat-1, V_3= IRRI-6, V_4= Basmati-2000
St. dv. =Standard deviation

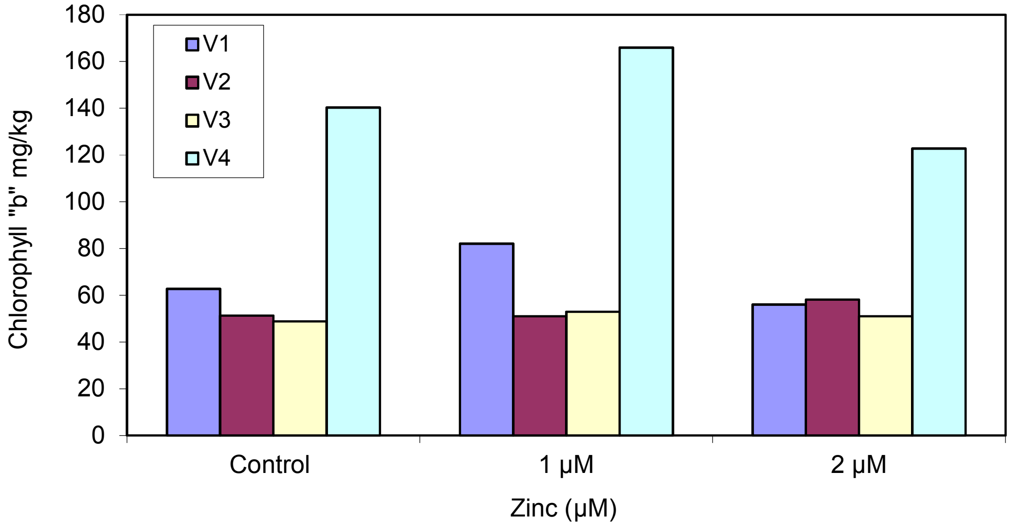

Fig. 6: Chlorophyll “b” of rice varieties as a function of Zn treatments.

Plant zinc contents

Plant mean Zn contents presented in Table (4) showed a range of 37 to 10.8 mg/kg. The data showed that varietal differences occurred with regards to the accumulation of Zn. Basmati-2000 contained the lowest mean content and JP-05, Swat-1, IRRI-6 showed significantly higher mean content. Similarly the application of Zn significantly increased the plant Zn contents over control. However, the dose of the application did not matter significantly above 1 µM Zn (Figure 7). The outcome of the current study is in fair agreement with those of Fernandez and Nicor (1996) who reported that increasing the Zn supply, increased the Zn concentration in the roots and likewise in the whole rice plant. The reduction of plant Zn content with high Zn dose may be due to severe phytotoxic effect on rice. High dose is likely to destroy the metabolic balance in plants. Thus the application of 1 µM Zn solution appeared to be an optimal dose for rice crop in the present studies.

Table 4: Zinc content (mg/kg) in rice varieties at different concentration of Zn in solution culture

Treatments	V1	V2	V3	V4	Mean	St. dv. ±
Control	12.6	12.8	7.8	11.4	11.2b	2.4
1 µM	48.4	33.8	51.4	12.6	36.6a	17.8
2 µM	48	39.8	51.6	8.6	37a	19.6
Mean	36.4a	28.8a	37a	10.8b		
St. dv. ±	20.6	14.2	25.2	2		

**Means showing same letter(s) are statistically non-significant at 5% level of probability.*
V_1=JP-05, V_2= Swat-1, V_3= IRRI-6, V_4= Basmati-2000
St. dv. =Standard deviation

Plant Iron contents

Plant iron contents given in Table (5) showed a range of 56.40 to 66.50 mg/kg and had almost non-significant variation in their mean values. However, the lowest mean contents were observed in Swat-1 and the highest mean content in JP-05. Zn application strongly influenced the plant iron contents and a reduction of 22 % is noted over control with 1 µM dose while application above 1µM dose insignificantly influence iron contents (Figure 7). The correlation of Zn treatments and Fe content showed negative trend (r = -0.788**, -0.992**, -0.995**, -0.726*) in all of the four varieties. The uptake of Fe^{2+} from solution phase to roots and further translocation from roots to top of the plant is accompanied by a competition with Zn^{2+}. If Zn^{2+} is in excess, it might suppress the Fe^{2+} uptake by roots and would thus have an effect on the overall iron contents of the plant. The results of the present study are in agreement with those of Olsen (1972), who reported that antagonistic interactions of an excess of Zn led to a noticeable reduction in plant Fe contents. Tisdale *et al.* (1997), also reported antagonistic effect of Fe^{2+} and Zn uptake in the rice varieties. The observations in hydroponics studies are not in accordance with the behavior of plants grown in soil (Giordano *et al.*, 1971; Rosen *et al.*, 1977) where it was noticed that Zn did not block Fe transport from soil to the growing plants.

Table 5: Iron content (mg/kg) in rice varieties at different concentration of Zn in solution culture

Treatments	V1	V2	V3	V4	Mean	St. dv. ±
Control	73.90	62.60	70.30	103.40	77.60	17.90
1 µM	61.90	57.40	62.50	61.30	60.80	2.3
2 µM	63.70	49.20	57.10	71.50	60.40	9.5
Mean	66.50	56.40	63.30	66.40		
St. dv. ±	65	68	66	72		

**V1=JP-05, V2= Swat-1, V3= IRRI-6, V4= Basmati-2000*
St. dv. =Standard deviation

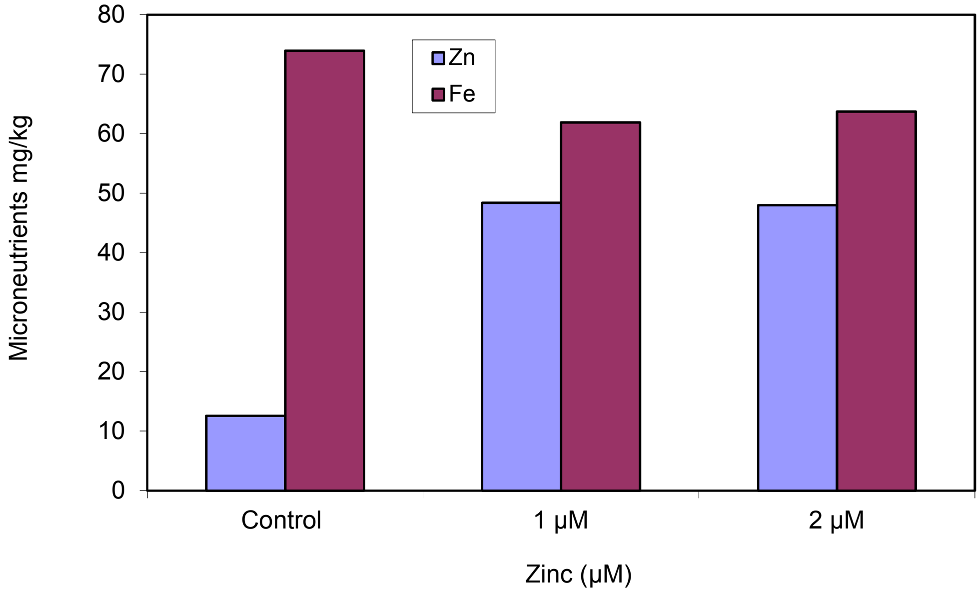

Fig. 7: Plant zinc and iron contents of rice varieties as a function of Zn treatments.

Conclusions

It is concluded that Zn had a profound effect on various parameters of the rice cultivars. Plant height, crude protein and chlorophyll “a” “b” contents improved with zinc dose compared to control and have shown an optimal limit of 1 µM zinc dose. Plant zinc contents increases while iron have shown a competitive behavior with zinc. All the rice cultivars differ in their zinc requirements. JP-05 and Basmati-2000 attained greater plant height at 1 µM dose whereas Swat-I and IRRI-6 achieved more height at 2 µM Zn dosage. Plant protein and chlorophyll contents were maximum in Basmati-2000 than all of the varieties. Zinc dose of 1 and 2 µM are tolerant and toxic levels respectively.

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