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Toxicity of Nickel on growth and biochemical changes in *Oryza sativa* L. (rice) plants

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Abstract: The toxicity of nickel (Ni) on growth and biochemical changes in *Oryza sativa L.* (rice) plants were investigated by evaluating the activities of enzymes such as catalase and peroxidase and the content of proteins, sugars and chlorophyll and carotenoid pigments along with the certain growth parameters. Different concentrations of Ni (0.25,0.50 and 1.0 mM) were supplied to the plants. The increasing concentration of Ni caused reduction in root length (6.750, 6.700, 6.550 cm) shoot length (13.150, 12.850, 9.200 cm), fresh weight of roots(0.795, 0.565, 0.420 g) and shoots (0.660, 0.430, 0.365 g) and dry weight of roots (0.115, 0.0650, 0.0350 g) and shoots (0.160, 0.110, 0.0900 g) of the plant. Chlorophyll a (1.045, 0.785, 0.680 mg g^{-1} FW), b (0.535, 0.315, 0.220 mg g^{-1} FW) and carotenoids (0.235, 0.200, 0.200 mg g^{-1} FW) were also decreased as the concentration of Ni was increased,however, the enzyme activity of catalase (26.895, 33.710, 40.425 μ mole H_2O_2 split mg^{-1} protein) and peroxidase (6.955, 9.140, 12.090 units mg^{-1} protein) and the contents of protein(3.160, 4.155, 4.945 % FW) and sugar (14.000, 24.000, 31.000 mg g^{-1} FW) were increased with high concentrations of Ni as compared to the control plants.

Key words: Nickel, rice, growth, chlorophyll, catalase, peroxidase

Introduction

The heavy metals have received special attention at the global level because of their adverse effects on plants growth and metabolism. Accumulation of metals and their toxic effects through food chain can lead to serious ecological and health problems (Malik, 2004). Heavy metals like As, Cd, Co, Cu, Ni, Zn, and Cr are phytotoxic either at all concentrations or above certain threshold levels.

Nickel, being one of the important metal pollutants is of considerable concern, because its concentration is rapidly increasing in soils of different parts of the world (Faryal *et al*., 2007; Rehman and Iqbal, 2008). It is emitted in the environment from a variety of natural and anthropogenic processes and consequently its concentration becomes considerably high in the environment. Nickel is considered as an essential micronutrient (Welch,1995) but it becomes toxic for majority of plant species at high concentration. The findings of Sinha *et al.* (2011) indicated that initially the growth and yield increases with the slight increase in nickel concentration but shows decreasing trend at its higher concentration. The higher concentration of nickel have been shown to bring the toxicity effects on growth (Gerendas *et al.*, 1999) and metabolic disorders in plants (Baccouch *et al.* , 1998). The inhibition of growth, chlorosis, necrosis and wilting were commonly observed in plants exposed to toxic amounts of nickel (Gajewska *et al.*, 2006). It was perhaps because of negative effects of this heavy metal on the photosynthesis (Tripathy, *et al.,* 1981), mineral nutrition (Parida *et al.*, 2003) and water retention (Pandey and Sharma,2002). In the presence of Ni, the contents of mineral nutrients in plant organs may increase, decrease, or stay even. The effects of Ni on nutrient uptake depend in many aspects on Ni concentration in the environment. The decline in nutrient uptake may also result from the Ni-induced metabolic disorders that affect the structure and enzyme activities of cell membranes (Seregin and Ivanov, 2001). Thus, Ni^{2+} affected the sterol and phospholipid composition of the plasma membrane in *Oryza sativa* shoots, with concomitant changes in the ATPase activity. Apparently, these changes affected the membrane permeability and in this way changed the ion balance in the cytoplasm. In view of all this, an attempt have been made to find out the effects of different doses of nickel on the growth and metabolism of rice plants.

Materials and Methods

Effect of nickel was investigated in rice plants using petri dish culture technique. The experiment was performed initially by washing the petri dishes with some common detergent followed by tap water and dilute hydrochloric acid (HCl) then again washed by tap water and rinsed by distilled water. All these washings made the petri dishes free from possible contaminations. Experimental seeds were soaked in controlled nutrient solution and solution of different treatments of heavy metal. High quality Whatman filter papers were used in each petri dish for growing seeds. These filter papers were then soaked with controlled solution or solution of various levels of treatment in replicates and in the temperature range of 20 to 30^0 C. Twenty five seeds were

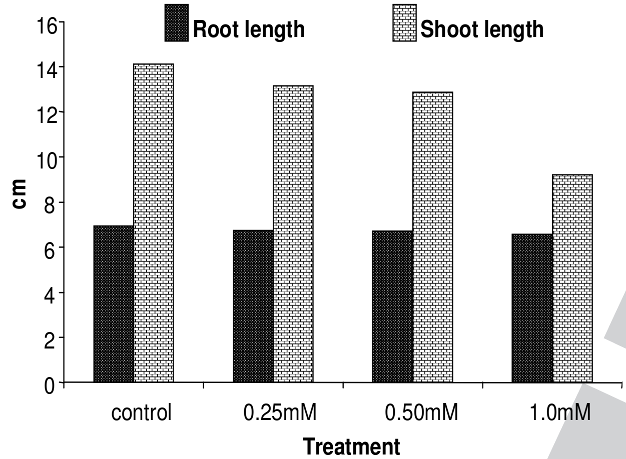

Fig. 1: Effect of different doses of nickel on the length of roots and shoots

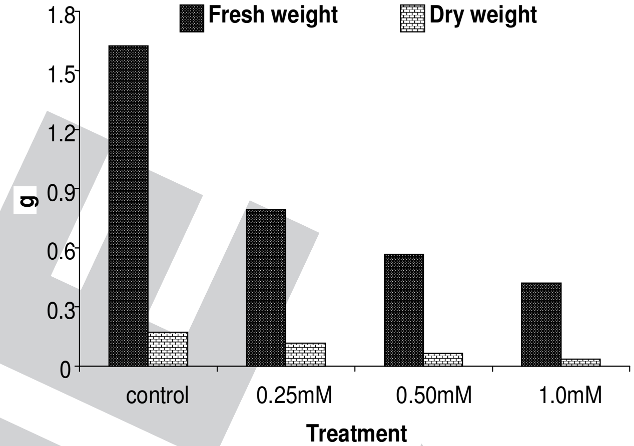

Fig. 2: Effect of different doses of nickel on fresh and dry weight of root

mg g^{-1} FW

Fig. 3: Effect of different doses of nickel on fresh and dry weight of shoot

Fig. 4: Effect of different doses of nickel on chlorophyll(a,b) and carotenoid content

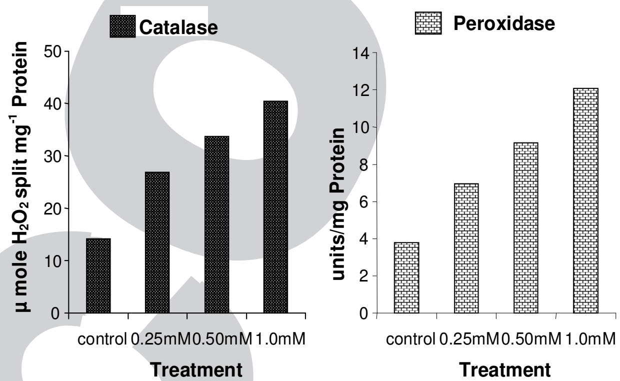

Fig. 5: Effect of different doses of nickel on protein and sugar contents.

Fig. 6: Effect of different doses of nickel on the activity of enzymes

sown in each petri dish and after every twenty four hours, nutrient solution and treatment solution were changed. The level with nutrient solution without any nickel dose served as control. The composition of nutrient solution was same as that was used by Hewitt (1966). Various concentrations of nickel (0.25, 0.50 and 1.0mM) from the stock solution of nickel (0.5M) were made using Nickel Sulphate. Different treatment levels were superimposed over the complete nutrient solution.

Retarded growth of plants and symptoms due to nickel toxicity were recorded. The physiological parameters such as chlorophyll and carotenoids contents (mg g^{-1} FW) were measured by the method of Petering *et al.* (1940), the total proteins (% FW) was estimated by the method of Lowry *et al.* (1951) while total sugars (mg g^{-1} FW) was estimated by method of Dubais *et al.* (1956). The catalase activity (µ mole H_2O_2 split mg^{-1} protein) was measured by the modified method of Bisht (1972) and the peroxidase activity (units mg^{-1} protein) was measured by the method of Luck (1963).

Results and Discussion

Excess nickel in plant caused chlorosis and reduction in growth of plant. Degree of these symptoms depended on the level of nickel supply. The length of root and shoot and fresh weight,dry weight of root and shoot were found to be decreased at increasing doses of nickel (Fig. 1, 2 and 3). Plants subjected to nickel toxicity showed decrease in the concentration of chlorophyll and carotenoids (Fig. 4). The concentration of both chl a and b and carotenoids were reduced in response to nickel supply. The protein and sugar content were increased with the increasing dose of nickel (Fig. 5). The maximum increase was found at 1.0mM Ni concentration as compared to control plants. The catalase and peroxidase activity were also increased with increasing concentration of nickel (Fig. 6).

Leaf yellowing was observed as the nickel concentration was increased. This might be due to the toxic effect of the metal on chlorophyll pigment. This is in agreement with the reports that nickel concentration caused reduction in pigment contents and biomass yield (Zengin and Kirbag, 2007). Ni exhibited a dose dependant inhibitory effect on chlorophyll content and its biosynthesis in plants. Various nickel concentrations used in this experiment drastically inhibited the photosynthetic area of the plant thus reducing photosynthetic capability leading to retarded growth. Tandon *et al.* (2000) found that increasing doses of nickel caused reduction in the overall growth of shoot and root in Mung plants, nickel at the highest dose of 1.0mM was found to be most toxic for the growth of both shoot and root. According to Seregin *et al.* (2003), the pattern of nickel transport difference includes an arrest of root branching. The other visible phytotoxic symptom was the darkening and stunting of roots which is in agreement with the earlier report of root damage due to nickel toxicity in pigeon pea (Sresty and Rao ,1999). The decrease in these plant pigments might be due to the cellular disorganization under nickel toxicity, which cause agglutination of chloroplasts (Molas, 2001; Gajewska and Sklodowska, 2007). The increase in total protein was related to the concentration of Ni supplied to the studied plants. The majority of studied plants, the total protein content was found to be increased at excess doses of Ni. Earlier Tandon *et al*. (2000) and Tandon and Gupta (2002) reported increased protein content at increasing doses of Ni in Mung plants. When exposed to metal stress condition, there might be the synthesis of new protein. Rai *et al.*, (1995) have earlier reported altered protein profiles at heavy metal stress condition. Saleh (2002) have reported increase protein content in *Chorcorus olitorius* plants treated with Nickel. Kasturi *et al.*, (1992) has also reported increased free protein in sunflower leaves and *Silene vulgaris* in the presence of heavy metals. Total sugar content was found to be increased in this study, Agrawala *et al*. (1977) have reported increase in the concentration of reducing sugar at excess supply of nickel. According to them, excess supply of heavy metal nickel resulted in a significant accumulation of non reducing sugars. Catalase and peroxidase enzymes were increased with increasing dose of Ni. Such increase in the activity of catalase enzyme might be due to the toxic effects of H_2O_2 and Reactive oxygen Species (ROS) produced as a result of membrane damage at higher levels of heavy metal. Catalase changes its activity depending upon the plant species and heavy metal type. In nickel treated plants the activity of another H_2O_2 scavenging enzyme *i.e.* Peroxidase was found to be increased as has been also observed earlier in several species (Tewari *et al.*, 2002). This might be attributed to rapid diffusivity of H_2O_2 produced in the cytosol or might be due to accumulation of high phenols in such conditions.

Hence, it can be concluded that the rice plants subjected to nickel toxicity showed decrease in growth and adverse effects on metabolism and on increasing the concentration of nickel, the decrease in growth, pigments, proteins, sugars and enzymes like catalase and peroxidase were observed.

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