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Growth and metabolism in response to Ni exposure by *Cicer arietinum* L. (gram) plants

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Abstract: Effects of nickel on the growth and metabolism of gram were studied in the seedlings of gram (*Cicer arietinum* L.). The plants were exposed to different concentrations of nickel (0.25,0.50 and 1.0 mM) and selected parameters of growth and metabolism were followed. Excess supply of nickel resulted in the induction of toxicity symptoms and overall reduction in growth of plants. The root length (4.50,4.10 and 3.79 cm), shoot length (16.00,14.00 and 10.16 cm), fresh weight (2.75,2.10 and 1.55 g) and dry weight (0.23,0.20 and 0.16 g) showed reduction at 0.25, 0.50 and 1.0 mM nickel concentration respectively. The chlorophyll content as found decreased (1.52,1.35 and 0.92 mg g^{-1} FW) at 0.25, 0.50 and 1.0 mM nickel concentration compared to the control levels.Total protein (5.14, 3.36 and 2.37 % FW) and sugar (27.50,17.50 and 7.50 mg g^{-1})content were reduced at increasing doses of nickel (0.25, 0.50 and 1.0 mM). However, the activity of enzymes, catalase (10.85, 22.28 and 36.91 μ moleH_2O_2 split mg^{-1} protein) and peroxidase (3.57, 7.28 and 10.97 units mg^{-1} protein) were increased with increasing concentration of nickel (0.25, 0.50 and 1.0 mM) in comparison to the control levels.

Key words: Nickel, Gram, Growth, Metabolism

Introduction

Heavy metal still represent a group of dangerous pollutants, many heavy metals such as nickel, copper, zinc and, are essential as important constituents of pigments and enzymes (Babula *et al.*, 2008). Nickel is considered as an essential micronutrient (Welch,1995) however, it is toxic at supraoptimal concentrations to plants (Schickler and Caspi,1999; Rao and Sresty, 2000; Pandey and Sharma, 2002). Nickel concentration that are toxic to plants vary in magnitude according to plant species. Most common symptom of nickel toxicity in plants is chlorosis, necrosis and reduction in growth. Toxic effects of nickel on plant growth and photosynthesis have been reported in higher plants as well as in algae (Sheoran *et al.*,1990). The higher concentrations 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 growth, nutrient status and photosynthesis showed a distinct decrease strictly related to the period of nickel treatment in wheat seedlings (Ouzounidou *et al.*, 2006). Nickel activity in cowpea was found to cause a reduction in the relative fresh mass of the root and shoots, lateral root formation was inhibited in the highest nickel treatments and the roots growing at the highest nickel activity were short and stubby and brown in colour (Kopittke *et al.*, 2007). The excess supply of nickel interferes with plant water relations and induces oxidative stress.

The aim of the present study is to have better understanding of growth and physiological responses of gram plants in relation to nickel stress.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 gram plants.

Materials and Methods

The experiment was carried out in the petri dishes. Such experiment were performed initially by washing the petridishes and other glasswares with detergent followed by tap water and dilute hydrochloric acid and then again washed by tap water and rinsed by distilled water. All these made the glasswares free from contamination. High quality Whatman filter paper No.1 were used in each sterilized petridishes for growing seeds.The plants were raised in petridishes and supplied with required nutrient solutions. Ten seeds of gram were placed in each petri dish. Experiment was performed in triplicate. The 0.25, 0.50 and 1.0 mM solution of nickel were prepared by using nickel sulphate salt in laboratory using distilled water. After four days of sowing the seeds in petridishes the nutrient solution was applied to control and treatment plants. After four days of sowing the seeds in Petri dishes. However, nickel as nickel sulphate in different doses were superimposed as treatment solutions and were changed after every 24 hr for prevention of possible contamination in growing medium. The seeds were supplied with 5ml of respective solutions.

Retarded growth in plants and symptoms due to nickel toxicity were recorded. Growth parameters such as shoot and root lengths, fresh and dry weight were measured after fifteen days of supply of treatments.

The chlorophyll content (mg g^{-1} F.W.) was measured by the method of Petering *et al.* (1940). The total proteins (% F.W.) was estimated by the method of Lowry *et al*. (1951) while total sugars (mg g^{-1} F.W.) 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). The data presented are the mean of three replicates.

Results

Plants were raised in petridishes to study the effects of different doses of nickel on the growth and metabolism of gram plants. There was a gradual reduction in growth of plants (Fig.1 A, B) and induction of toxicity symptoms in 0.25, 0.50 and 1.0 mM nickel concentrations. The degree of chlorosis was found to be increased from 0.25 to 1.0 mM nickel. The reduction in the length of roots and shoots, the fresh and dry weight (Fig.2) were also observed at different concentrations of nickel.The chlorophyll concentration was significantly decreased at increasing doses of nickel in comparison to the control plants (Fig.3).The total protein

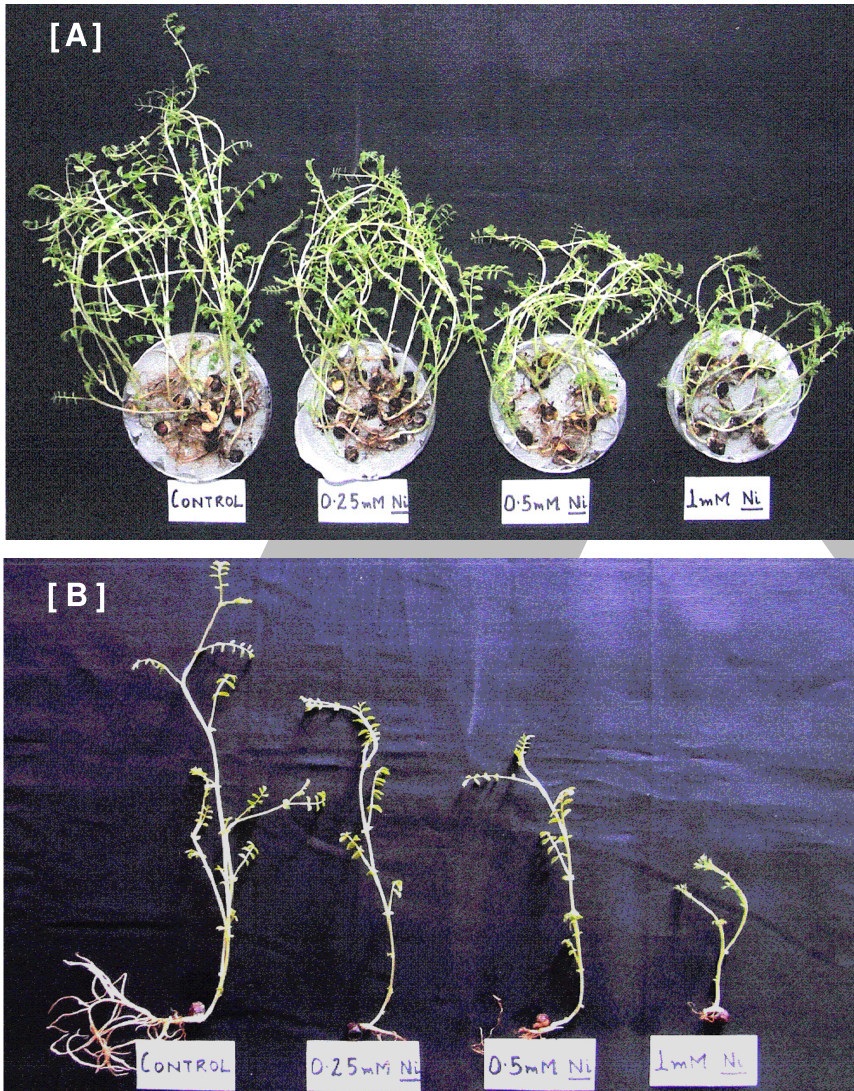

Fig. 1A, B: Effect of different doses of nickel (Ni) on the growth of gram *(Cicer arietinum)* seedlings

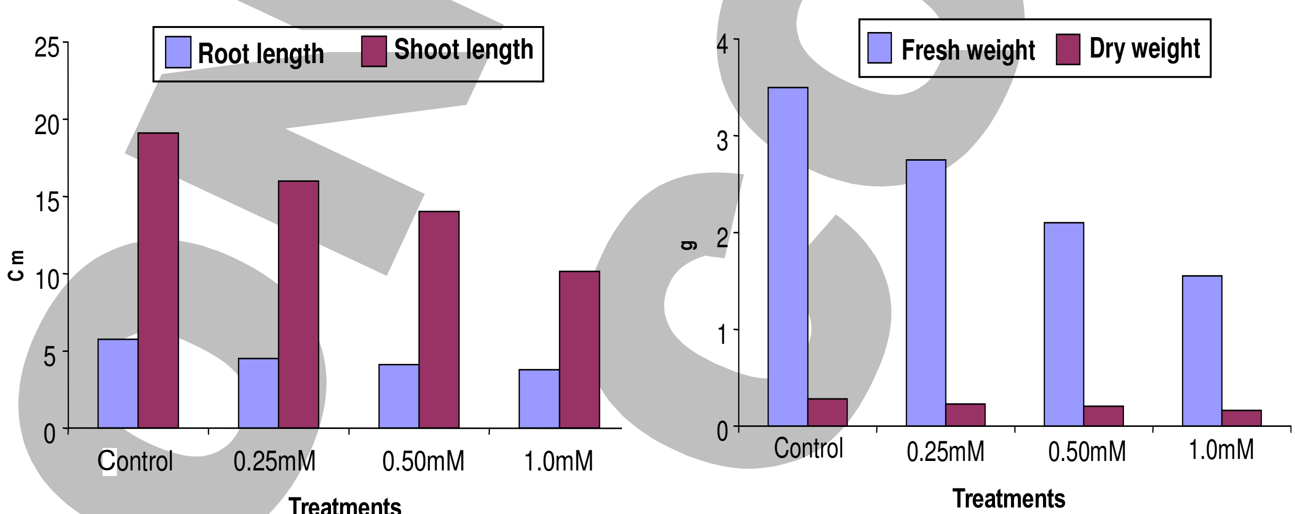

Fig. 2: Effect of different doses of nickel on the length of roots and shoots and fresh and dry weight

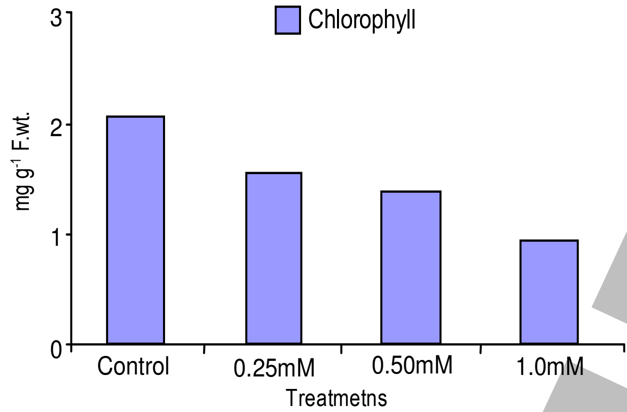

Fig. 3: Effect of different doses of nickel on chlorophyll content

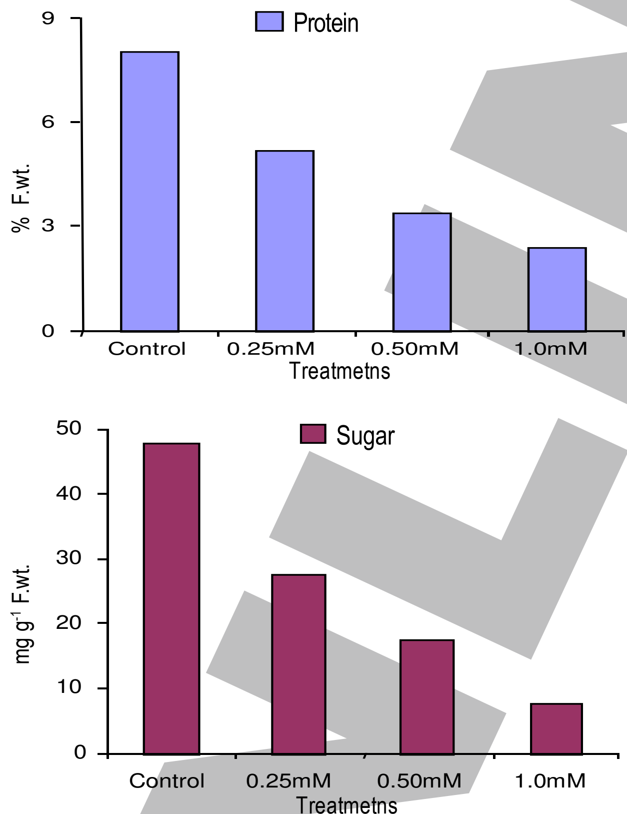

Fig. 4 : Effect of different doses of nickel on protein and sugar contents

and sugar contents were also decreased from 0.25 to 1.0 mM nickel (Fig.4). The catalase and peroxidase activity were found to be significantly increased with an increasing concentration of nickel as compared to the control plants (Fig. 5). Thus, the result showed the toxic effects of nickel on the growth and metabolism of gram plants.

Discussion

Nickel had deleterious effects on the morphology and physiology of gram plants. This element had an inhibitory effects on the root and shoot growth of the said plant. The roots are directly exposed to heavy metals in soil or in culture solution and reduction in root elongation is considered as one of the first toxic effect in plants (Foy,1978). Nickel might also have negative role on auxin

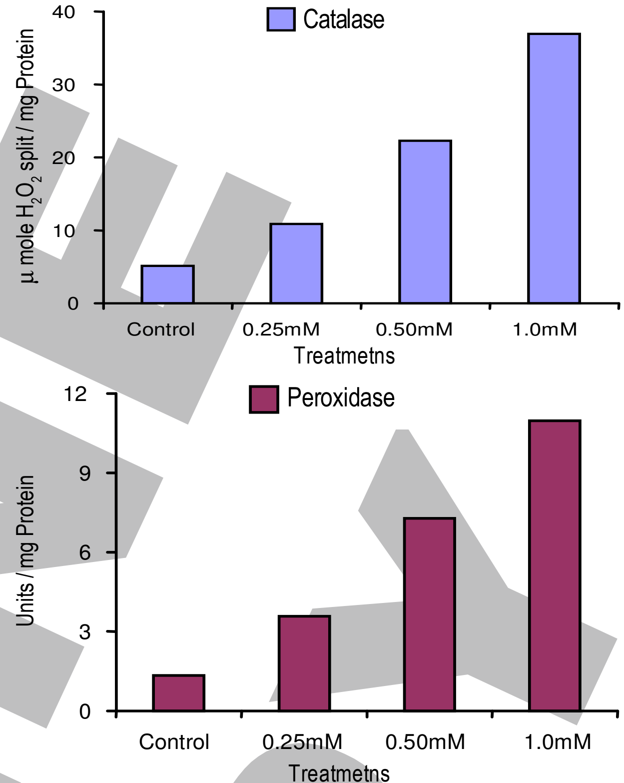

Fig. 5 : Effect of different doses of nickel on the activity of enzymes

synthesis in plants resulting into decreased root and shoot growth. It was found that fresh weight and dry weight of whole plant decreases with increasing concentration of nickel. The decrease in the relative rate of growth indicated the negative effect of this metal on the photosynthetic apparatus.

Nickel exhibited a concentration dependant inhibitory effect on chlorophyll content and chlorophyll biosynthesis process in plants. The decrease in these plant pigments under nickel toxicity might be due to the cellular disorganization, which cause agglutination of chloroplast (Pandey and Pathak, 2006; Gajewska and Sklodowska (2007). The damage in thylakoids structure related with the important disturbance in the metabolic function of organelles effecting chlorophyll biosynthesis, photosynthesis and activities of redox enzymes justifying a decrease in growth (Baryla *et al.*, 2001). Adverse effect of nickel on content of chlorophyll might be due to the toxic effect of this metal on iron metabolism of gram plants. Since chlorophyll content decreased significantly with increasing dose of nickel indicates negative changes on the acceptor side of PS II, which may also result from diminution of calvin cycle (Ouzounidou *et al.*, 2006). Agrawala *et al.* (1977) were of the view that an excess supply of heavy metals reduced iron absorption by plants and affected its distribution in roots and shoots. The decrease in total protein was related to the concentration of nickel supplied to the plants. Changes in protein composition during nickel stress reflect the de-arrangement in protein degradation. Delhaize *et al*. (1989) have reported altered protein profiles at heavy metal stress condition. Possibly the decreased amount of total protein at excess doses of heavy metal nickel might have resulted from toxic effects of this metal on the nitrogen metabolism of plant.

Excess concentration of nickel caused reduction in total sugar content in the plant. The lower sugar level may be due to lowered synthesis or diversion of the metabolites to other synthesis processes (Tripathi and Tripathi,1999). Saleh (2002) also reported reduced carbohydrate content in *Corchorus olitorius* plants. According to him, the carbohydrate formation is inhibited by nickel due to an effect of a reduced photosynthesis. Thus, sugar and carbohydrate content in nickel treated plants might have resulted due to the adverse effect of toxic doses of this metal on the process of photosynthesis.

Activities of enzymes catalase and peroxidase was found to be significantly increased on increasing the concentration of nickel. A greater activity of the enzymatic components of the anti oxidative system indicates the high metal stress condition in plant. Hydrogen peroxide is a well known reactive oxygen species and is generated under the stress conditions. According to Wecks and Clijsters (1996), catalase and peroxidase play an important role in preventing oxidative stress by catalyzing the reduction of hydrogen peroxide. Heavy metal toxicity results in changes in growth and metabolism of plant by invitro uptake of heavy metals resulted secondary responses such as oxidative damage by producing highly reactive oxygen species (Panda *et al.*, 2003).

Nickel decreased dry weights of roots and shoots and reduced chlorophyll content in leaves of plants and changes in the activities of the antioxidant enzymes like catalase and peroxidase, in both roots and leaves indicated that nickel caused an oxidative stress in plants (Sar Saeidi *et al.*, 2007). According to Singh *et al.* (2007) and Nath *et al.* (2008,2009) the supraoptimal level of heavy metals caused considerable reduction in seedling growth, photosynthetic pigments, proteins, sugars and increased levels of catalase and peroxidase.

In the present study, the effects of nickel were investigated. The gram plants subjected to nickel toxicity showed decrease in growth and adverse effects on metabolism. A significant reducing effect was observed in fresh and dry weight, chlorophyll, proteins and sugars content on increasing the concentration of nickel. The excess supply of nickel caused an increase in the activity of anti oxidative enzymes such as catalase and peroxidase. Thus, it may be concluded that nickel in gram plants produces phytotoxic effects as it showed inhibitory effects on the growth and metabolism of the plants.

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