

# Effect of Nickel Sulphate Toxicity On Radish Plant

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**Abstract-** To check and analyze the effect of nickel sulphate toxicity on radish plant, a research was conducted in the old Botanical Garden at University of Agriculture, Faisalabad. In different concentrations of nickel sulphate solution (10, 20, 30 mM), *Raphanussativus* specie of radish (Mooli Day-40) was grown to study the morphological parameters like shoot and root length, shoot nad root weight, number of leaves, total carotenoids and chlorophyll. Ni-stress remarkably reduced the growth attributes of radish plant. Results were reported after data collection and applying statistical anaylsis by using latest software packages. A noticeable drop in physiological and morphological attributes was recorded. However in biochemical attributes level of chlorophyll a and b was reduced while subsequent elevation was noticed in carotenoid concentration.

**Index Terms-** Chlorophyll a and b, Carotenoid, Nickel sulphate, Toxicity

## I. INTRODUCTION

Radish (*Raphanus sativus* L.) is a root vegetable of genus *Raphanus* and family Cruciferae or Brassicaceae which was domesticated in Central West of India and China (Thamburaj and Singh, 2005). Consumption of these edible vegetables help in preventing of several diseases hence called as protective food. Vegetables are cheapest, richest and natural source of protective food providing vitamins, fats, proteins, minerals and carbohydrates. According to recommended diet plan, vegetable consumption should be 300 g/day per person. Out of 300 g, 125 g leafy green vegetables, 100 g of deep rooted vegetables and 75 g of others should be included (Salaria, 2009).

During radish metabolism myrosinase enzyme leads to enzymatic hydrolysis of glucosinolates forming isothiocyanates (Kim *et al.*, 2015). Some plant cells also affected with cancer due to proliferation of abnormal cells (Naeem *et al.*, 2019). In fact, bacterial microflora, present in human colon, also exhibit same enzymatic activity. Several health properties and molecular features of radish has also been described in many research studies. For example, a biologically active compound named as methylisogermabullone (C<sub>23</sub>H<sub>31</sub>O<sub>5</sub>NS, MW 433) is extracted from radish help in activation of acetyl cholinergic receptors that ultimately leads to small bowel motility (Jeong *et al.*, 2005). 4-(Methylthio)-3-butenyl isothiocyanate induce apoptosis in to reduce the risk of human colon cancer (Barillari *et al.*, 2008).

A recent study found that sulforaphane which is an isothiocyanate of radish stop the proliferation of human breast

cancer cells (Pawlik *et al.*, 2017). Basically, yield and growth of this vegetable depend on soil and prevailing weather conditions. Plant DNA barcodes are used for species identification (Naeem *et al.*, 2019). Different plants also suffer from cadmium toxicity (Shafiq *et al.*, 2019). Different radish varieties have different requirements of climate and soil for optimum yield and growth but one of the most important agro techniques is nutrition. Radish crop requirements varies with agro-climatic conditions, soil fertility, humidity and soil type. Plants based products used in pharmaceutical industries (Usman *et al.*, 2019). Being a fast growing crop and due to its short duration its root growth should be uninterrupted and rapid. For good quality and optimum production of radish, fertilization through inorganic, organic and biofertilizers are very essential components (Dhanajaya, 2007).

Plants identified on the basis of special DNA barcodes (Ahmad *et al.*, 2019). Radish is eaten in both raw form (as salad) and in cooked form so it is grown for its young tender tubercle root. It is delighted in its pervasive flavour that's why considered as appetizer. Young leaves also eaten in both fresh and cooked forms. Due to its depurative and refreshing properties, it is quite useful in treating gall bladder and liver stones. Plants based products used in pharmaceutical industries (Usman *et al.*, 2019). Other medicinal uses include treatment of sleeplessness, neuralgic headache and chronic diarrhea. Leaves, roots, pod and flower are very effectual against gram positive bacteria. Moreover, roots are also effective in piles, urinary complaints and in gastrodynia. White ash is prepared from processing of roots which act as remedy for constipation and diuresis.

Plants leaves contain special pores called stomata (Naeem *et al.*, 2019). Uptake of different heavy metals by plants alter growth mechanisms by activating secondary responses like oxidative damage (Choudhury and Panda, 2004), may be by the accumulation of H<sub>2</sub>O<sub>2</sub> which is occur by shifting the balance of reactive oxygen species metabolism (Mithofer *et al.*, 2004). Metals interaction with growth and metabolism has reciprocal effect which ultimately disturbs plant growth. Plants based nutraceuticals products used in pharmaceutical industries (Usman *et al.*, 2019). For instance the quantity of one metal may effect positively or negatively to other element, either it modify or lessens the toxic effects of heavy metals (Mukherjee and Mishra, 2008). Epidermis of plants detected using microscopic examination (Raza *et al.*, 2019). Copper-nickel interactions and zinc-nickel interactions are ineffective in reducing effect of nickel, in other words, different concentrations of copper and zinc neither increased nor reduced the nickel toxicity.

Now a days, due to excessive industrial use nickel toxicity became a special concern. Under stressful conditions various detoxification responses of nickel like Ni<sup>2+</sup> – NA Complexes and Ni<sup>2+</sup>-organic acid appear in plants (Kupper *et al.*, 2001), the overproduction of NA and it's synthase (Weber *et al.*, 2004), and high levels of free histidine (Wycisk *et al.*, 2004). However other responses include induction of thiol glutathione and MTs (Bellion *et al.*, 2007) and High concentrations of glutathione, Cys and O-acetyl-L-serine (OOAS (Freeman *et al.*, 2004).

Besides this some enzymatic activities are regulated such as glutathione reductase and serine acetyltransferase. During Ni-stress conditions the toxicity symptoms also develop in plants but responses are differ substantially according to growth stage, plant species, cultivation conditions, exposure time and Ni concentration (Assuncao *et al.*, 2003). Some plants contain enzymes for fight against oxidative stress (Naem *et al.*, 2019). In sensitive species level of critical toxicity is 10 mg/kg dry weight (DW) (Kozlow, 2005), in moderately tolerant species is 50 mg/kg DW and in hyper accumulator plants like *Thlaspi* and *Alyssum* species is 1000 mg/kg DW (Pollard *et al.*, 2002). Plants based hybrid system studied in plant genetics (Ahsan *et al.*, 2019) In other sensitive species of plants such as water spinach, barley and wheat the necrosis and chlorosis of leaves appear when plants are treated with low level of nickel (0.2 mM or 11.74 ppm) for about a week (Rahman *et al.*, 2005).

Nickel toxicity in radish plants is demonstrated by chlorosis, growth inhibition, wilting and necrosis. Those plants which grow in contaminated soils normally have elevated level of heavy metals depending on total concentration of soil and genotype of plants (Alexander *et al.*, 2006).

Pandey and Gopal (2010) investigated the effect of Ni concentration on different metabolic and growth activities of plants affecting eggplant developmental processes. First, seeds of eggplant (cv. Hybrid P.K.123) were sown in very refined sand in range from 0.1 to 400 µM. Then expose them to Ni > 50 µM leads to decrease the level of photosynthetic pigments, activities of peroxidase and catalase, biomass and level of iron in stem and leaves so the low level of chlorophyll and enzymatic activities of peroxidase, Heme and catalase with increase Ni concentration may indicate interference in iron metabolism in plants. With the increasing level of Ni the ribonuclease and superoxide dismutase activities and proline level also increased. In leaves of plants oxidative damage is observed as the level of lipid peroxidation. After 10 days treatment with 400 µM Ni clearly visible symptoms of chlorosis necrosis and Ni toxicity are appeared at margins and veins. Observed toxicity of metals has major symptom of membrane damage due to production of ROS particularly at higher (30–400 µM) levels of Ni.

## II. MATERIALS AND METHODS

Nickel effects on radish seeds was checked by conducting pot experiments. During the second week of germination nickel was applied to seedlings in different concentrations such as 0, 10, 20 and 30 mM. For this purpose foliar application was used by which stress was applied in solution form.

### Sowing and culture medium:

Radish seeds of genotype Mooli Day-40 were obtained from Ayyub Agriculture Research Institute (AARI) Faisalabad and then directly sown in plastic pot. An underneath hole covered by fine piece of cotton cloth was present in all pots. Every pot was filled up with 2.5 kg sand and then washed it very well. After germination of radish plants, seedlings were thinned to keep 5 plants of equal size in each pot. Then, plant was treated with nickel to conduct my experiment.

### Treatments and Source:

Simple water taken from watering plants of University of Agriculture, Faisalabad. Nickel sulphate was taken from Botany Department in proper concentrations and then made solution by adding 1L of water then give to specific plants. Normal water or 0 mM Nickel solution, 10 mM Nickel solution, 20 mM Nickel solution, 4–30 mM Nickel solution.

### Harvests:

After treatment of 45 days plants were harvested and the following parameters were checked.

### Shoot and Root length (cm)

The length of root and shoot was checked by meter rod and average values also calculated.

### Shoot and Root fresh weight (g)

Uproot the plants by using top loading balance and immediately determined the fresh weight of root and shoot and finally calculate the mean values.

### Shoot and Root dry weight (g)

For calculating the constant dry weight keep the fresh samples for 1 week in oven at 65°C and average dry weight was determined.

### Statistical Analysis:

Statistical analysis on calculated data was performed, apply CO-state, draw ANOVA tables and finally data was filled in tables.

## III. RESULT AND DISCUSSION

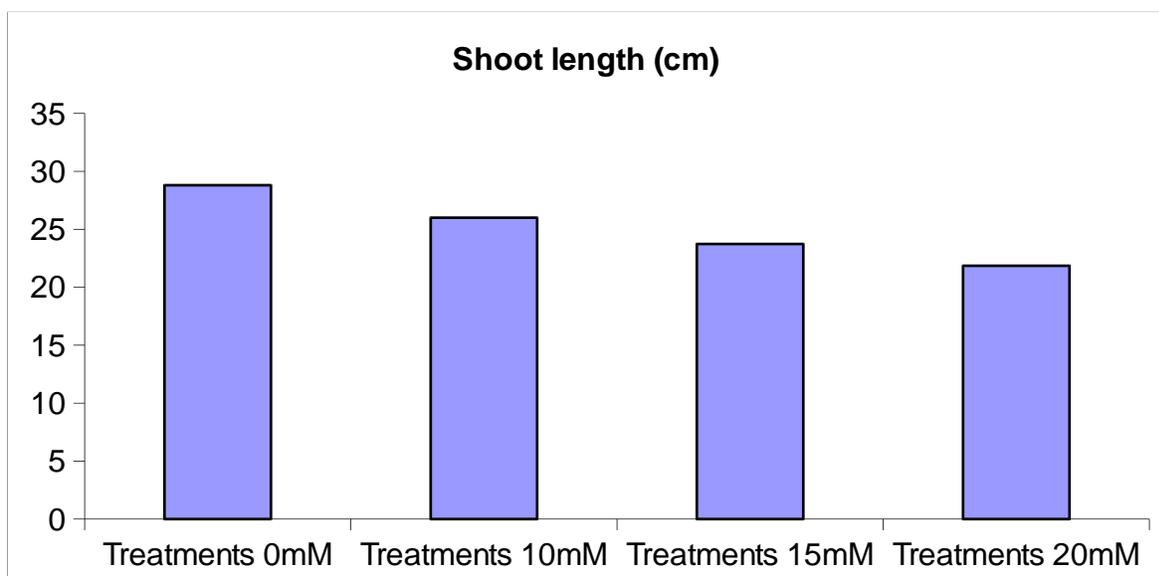
The analysis of variance of data collected for shoot length of genotype Mooli Day-40 that was grown under both nickel sulphate and control is presented in (Table 4.1, Fig 4.1). It is observed that nickel sulphate leads to significant reduction ( $P \geq 0.001$ ) in shoot length. At 30 mM of nickel sulphate concentration in foliar medium maximum reduction in shoot length is visualized while in control condition there is maximum increase (Fig 4.1).

For root length of genotype (Mooli Day-40) that was grown in both control and nickel sulphate conditions the analysis of variance of collected data is given in (Table 4.2, Fig 4.2). By applying Ni-stress significant reduction ( $P \geq 0.01$ ) in root length is observed. There is maximum increase in control conditions and maximum decrease at 30 mM concentration of Nickel sulphate in foliar medium (Fig 4.2).

**Table.4.1:** Analysis of Variance of data for shoot length of redish(Moli Day-40) under Nickel sulphate effect

SOV	Df	SS	MS	F	P
Treatment	3	80.726	26.909	20.689	.0004***
Error	8	10.405	1.300		

\*\*\*, \*\*, \* = significant at 0.001, 0.01 and 0.05 probability levels respectively, ns= non-significant

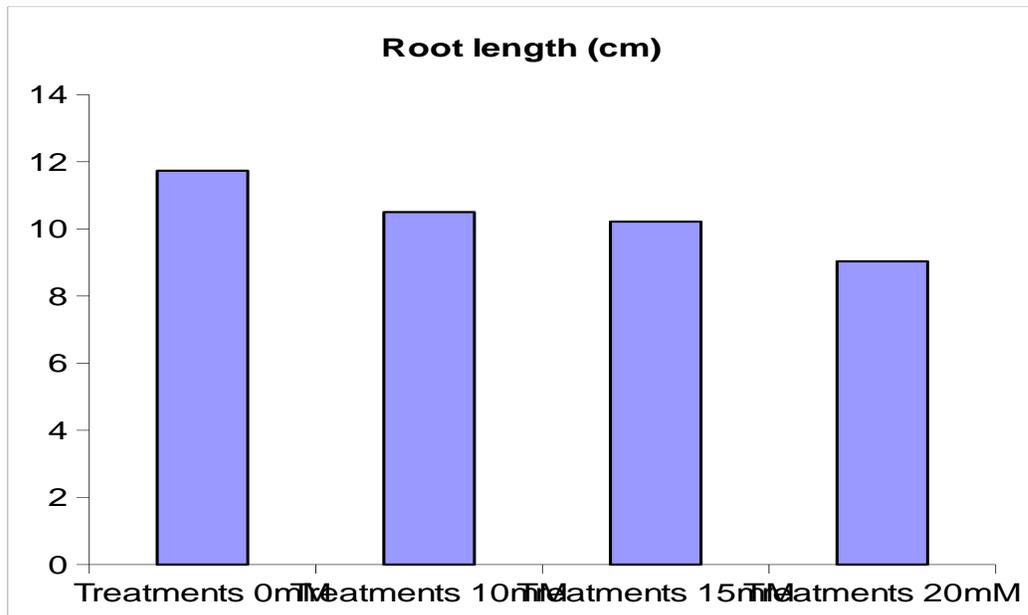


**Fig 4.1:** Influence of exogenously applied Nickel sulphate on shoot length of radish genotype grown under control and Nickel sulphate effect conditions.

**Fig 4.2:** Analysis of Variance of data for root length of radish (Moli Day-40) under Nickel sulphate effect

SOV	Df	SS	MS	F	P
Treatment	3	11.0572	3.685	12.627	.0021**
Error	8	2.335	0.291		

\*\*\*, \*\*, \* = significant at 0.001, 0.01 and 0.05 probability levels respectively, ns= non- significant



**Fig 4.2:** Influence of exogenously applied Nickel sulphate on Root length of radish genotype grown under control and Nickel sulphate effect condition.

#### IV. CONCLUSION

A soil culture research was conducted in University of Agriculture Faisalabad, in the wire house of old Botanical Garden, by applying Foliar medium on radish (*Raphanus sativus*) of genotype Mooli Day-40 to compute the effect of nickel sulphate on physiological, photosynthetic, growth and biochemical parameters. For this purpose four different levels of Ni-sulphate (0, 10, 20 and 30 mM) were used and the order of applications used in experiment was 0 mM Nickel sulphate +1liter H<sub>2</sub>O, 10mM Nickle sulphate +1liter H<sub>2</sub>O, 20 mM Nickel sulphate+1liter H<sub>2</sub>O and 30 mM Nickel sulphate+1liter H<sub>2</sub>O. Such coresponding applications of Nickel sulphate were applied respectively. During this planned research it was observed that imposition of Ni-sulphate stress leads to considerabale reduction in growth patterns such as dry and fresh weights of shoots and roots, plant height, carotenoids and cholorophyl-a,b contents and number of leaves per plant. Imposition of Ni-sulphate via Foliar medium result in tremendous increase in carotenoid accumulation in plants. Significance and non- significance in various attributes of genotype Mooli Day-40 has also be calculated by statistical analysis and shown in ANOVA table.

In conclusion, all above features of radish plant give best results by applying control and show less results by giving 30 mMNickel sulphate concentration.

#### CONFLICT OF INTEREST:

There is no conflict of interest towards this research. All authors have contributed a significant role for publication of this research in writing of this research article.

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#### REFERENCES

- [1] Alexander, P. D., B.J. Alloway and A. M. Dourado. 2006. Genotypic variations in the accumulation of Cd, Cu, Pb and Zn exhibited by six commonly grown vegetables. *Environ. Pollut.*, 144:736-745.
- [2] Assunção, A. G., W. M. Bookum, H. J. Nelissen, R. Vooijs, H. SchaT and W. H. Ernst. 2003. Differential metal-specific tolerance and accumulation patterns among *Thlaspi caerulescens* populations originating from different soil types. *New Phytol.*, 159:411-419.
- [3] Barillari, J., R. Iori, A. Papi, M. Orlandi, G. Bartolini, S. Gabbanini and L. Valgimigli. 2008. Kaiware Daikon (*Raphanus sativus* L.) extract: a naturally multipotent chemopreventive agent. *J. Agri. Food Chem.*, 56:7823-7830.
- [4] Bellion, M., M. Courbot, C. Jacob, F. Guinet, D. Blaudez and M. Chalot. 2007. Metal induction of a Paxillinvolvulusmetallothionein and its heterologous expression in *Hebelomacylindrosporium*. *New Phytol.*, 174:151-158.

- [5] Choudhury, S. and S. K. Panda. 2004. Induction of oxidative stress and ultrastructural changes in moss *Taxithelium nepalense* (Schwaegr.) Broth. Under lead and arsenic phytotoxicity. *Curr. Sci. Ind.*, 87: 342-348.
- [6] Dhananjaya, J. 2007. Organic Studies in Radish (*Raphanus sativus* L.) Varieties. *Dharwad Uni. Agri. Sci.*, 580:12-13.
- [7] Freeman, J. L., M. W. Persans, K. Nieman, C. Albrecht, W. Peer, I. J. Pickering and D. E. Salt. 2004. Increased glutathione biosynthesis plays a role in nickel tolerance in *Thlaspi* nickel hyperaccumulators. *Plant Cell*. 16:2176-2191.
- [8] Jeong, S. I., S. Lee, B. K. Choi, K. Y. Jung, K. J. Kim, K. S. Keum and Y. K. Choo. 2005. Methylisogermabullone isolated from radish roots stimulates small bowel motility via activation of acetylcholinergic receptors. *J. Pharm. Pharmacol.*, 57:1653-1659.
- [9] Kim, J. W., M. B. Kim and S. B. Lim. 2015. Formation and stabilization of raphasatin and sulforaphene from radish roots by endogenous enzymolysis. *Prev. Nutr. Food Sci.*, 20:119-125.
- [10] Kozlov, M. V. 2005. Pollution resistance of mountain birch, *Betula pubescens* subsp. *Czerepanovii*, near the copper-nickel smelter: natural selection or phenotypic acclimation. *Chemosphere* 59:189-197.
- [11] Küpper, H., E. Lombi, F. J. Zhao, G. Wieshammer and S. P. McGrath. 2001. Cellular compartmentation of nickel in the hyper accumulators *Alyssum lesbiacum*, *Alyssum bertolonii* and *Thlaspi goes* in genes. *J. Exp. Bot.*, 52:2291-2300.
- [12] Naeem M, Hayat M, Qamar SA, Mehmood T, Munir A, Ahmad G, Azmi UR, Faryad MA, Talib MZ, Irfan M, Hussain A, Hayder MA, Ghani U, Mehmood F. (2019). Risk factors, genetic mutations and prevention of breast cancer. *International Journal of Biosciences*, 14(4), 492-496.
- [13] Naeem M, Ali J, Hassan MZ, Arshad B, Rao MHI, Sarmad MSK, Irfan U, Khan NA, Sohail MS, Umar M, Hassan MU (2019). Novel Approach Towards DNA Barcoding as a Tool in Molecular Biology and Biological Activities of Cyclotides with Particular Emphasizes at Molecular Level. *Biological Forum-An International Journal*, 11(2):83-96.
- [14] Shafiq S, Adeel M, Raza H, Iqbal R, Ahmad Z, Naeem M, Sheraz M, Ahmed U and Azmi UR. (2019). Effects of Foliar Application of Selenium in Maize (*Zea Mays* L.) under Cadmium Toxicity. *Biological Forum-An International Journal*, 11(2): 27-37.
- [15] Usman G, Ammara B, Hamza R, Muhammad N, Syed S.H.B, Hafiz A, Amna N, Sara, S, Shahid, AC. (2019). Saudi Advancement and Future Directions towards Herbal Treatment for Various Diseases. *Saudi Journal of Medical and Pharmaceutical Sciences*, 5(11): 931-941
- [16] Ahmad I, Khan S, Naeem M, Hayat M, Azmi UR, Ahmed S, Murtaza G, and Irfan M. (2019). Molecular Identification of Ten Palm Species using DNA Fingerprinting. *International Journal of Pure & Applied Bioscience*, 7(1): 46-51.
- [17] Usman G, Muhammad N, Hamza R, Usman I, Ayesha A, Saqib U, Asim R, Fatima Q. (2019). A Novel Approach towards Nutraceuticals and Biomedical Applications. *Scholars International Journal of Biochemistry*, 2(10): 245-252
- [18] Naeem M, Hussain A, Azmi UR, Maqsood S, Imtiaz U, Ali H, Rehman SU, Kaleemullah, Munir HM, Ghani U. (2019). Comparative Anatomical Studies of Epidermis with Different Stomatal Patterns in Some Selected Plants Using Compound Light Microscopy. *International Journal of Scientific and Research Publications*, 9(10):375-380
- [19] Mithöfer, A., B. Schulze and W. Boland. 2004. Biotic and heavy metal stress response in plants: evidence for common signals. *FEBS Lett.*, 566:1-5.
- [20] Mukherjee, A. and V. K. Mishra. 2008. Bioaccumulation of heavy metal in crops irrigated with secondary treated sewage waste water in surrounding villages of Varanasi city. *Res. Environ. Life Sci.*, 1:103-108.
- [21] Pandey, V. K. and R. Gopal. 2010. Nickel toxicity effects on growth and metabolism of eggplant. *Int. J. Veg. Sci.*, 16:351-360.
- [22] Ghani U, Bukhari SSH, Ullah S, Rafeeq H, Saeed MM, Amjad A, Hussain M, Akmal A, Zahra FT, Qasim F, Taufiq T, Chand SC. (2019). A review on Nutraceuticals as a Therapeutic Agents. *International Journal of Biosciences*. 15(5), 326-340
- [23] A Raza, Iqra, U. Ghani, N. Azhar, I. Hussain, M. U. Khan, S. Bano, A. Rubab, S. N. Sajid, S. A. H. Bukhari, Z. Haider, M. Mubeen, S. A. Sajid, J. Ali, N. Aish, A. Wahab. (2019). Characterization of Selected Plants Leaves with Particular Emphasizes on Epidermis. *Haya Saudi Journal of Life Sciences*, 4(9): 326-330
- [24] Muhammad Naeem, et al. (2019). Principles of Biochemistry and Biological Sciences. Nishtar Publications (Pvt). Ltd. pp.138
- [25] Naeem M, Azmi UR, Irfan M, Ghani U. (2019). Reducing risks for food from climate change. 1st international conference on sustainable agriculture: food security under changing climate scenarios. ICESA-2019/101. April 3-5, Page No.79.
- [26] Ahsan M, Aslam M, Akhtar MA, Azmi UR, Naeem M, Murtaza G, Irfan M, Shafiq S. (2019). Effect of inoculation of three rhizobial strains on maize hybrids. *Journal of Biodiversity and Environmental Sciences*, 14(6), 168-177.
- [27]
- [28] Pawlik, A., M. Wała, A. Hać, A. Felczykowska and A. Herman-Antosiewicz. 2017. Sulforaphene, an isothiocyanate present in radish plants, inhibits proliferation of human breast cancer cells. *Phyto. Med.*, 29:1-10.
- [29] Pollard, A. J., K. D. Powell, F.A. Harper and J. A. C. Smith. 2002. The genetic basis of metal hyperaccumulation in plants. *Crit. Rev. Plant Sci.*, 21:539-566.
- [30] Rahman, H., S. Sabreen, S. Alam and S. Kawai. 2005. Effects of nickel on growth and composition of metal micronutrients in barley plants grown in nutrient solution. *J. Plant Nutr.*, 28:393-404.
- [31] Salaria, A. S. and B. S. Salaria. 2009. Horticulture at a Glance. *Jain Bro. New DL.*, 2:7-8.
- [32] Thamburaj, S. and N. Singh. 2005. Vegetables, tuber crops and spices. *Ind. Coun. Agri. Res.*, 40:468-470.
- [33] Weber, M., E. Harada, C. Vess, E. V. Roepenack Lahaye and S. Clemens. 2004. Comparative microarray analysis of *Arabidopsis thaliana* and *Arabidopsis halleri* roots identifies nicotianamine synthase, a ZIP transporter and other genes as potential metal hyper accumulation factors. *Plant J.*, 37:269-281.
- [34] Wycisk, K., E. J. Kim, J. I. Schroeder and U. Krämer. 2004. Enhancing the first enzymatic step in the histidine biosynthesis pathway increases the free histidine pool and nickel tolerance in *Arabidopsis thaliana*. *FEBS Lett.*, 578:128-134.

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