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Research Article

Role of 28-homobrassinolide on growth biochemical parameters of *Trigonella foenu-graecum* L. plants subjected to lead toxicity

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Abstract

The study contributes to the enhancement of knowledge about the behaviour of Trigonella foneu-graecum L. (fenugreek) plants with lead [Pb $(NO_3)_2$] and their combinations with plant growth hormone 28-homobrassinolide (28-HBL). The growth and biochemical parameters of Trigonella foenu-graecum L. plants subjected to lead toxicity were determined. Application of HBL might be improved plant biomass due to increase of photosynthetic pigments; this further induced the rate of photosynthesis in fenugreek plants. The biochemical parameters like carbohydrates, proteins and nucleic acids were found decreased in pb applied plants and the toxicity was inhibited by HBL at low concentrations. The results clearly demonstrated the ameliorating effect of HBL in mitigating the toxicity of pb in fenugreek plants.

Keywords: *Fenugreek, growth, 28-homobrassinolide, lead toxicity*

1. Introduction

Fenugreek is one of the oldest medicinal plants, originated in India and Northern Africa. The plants and seeds are traditionally used to treat disorders such as diabetes, high cholesterol levels, inflammation, gastrointestinal ailments, hypertension, sexual problems and rheumatism. Fenugreek seeds have also been postulated to exert hypoglycemic effects by stimulating glucose-dependent insulin release by beta cells [1]. Recent studies suggest that fenugreek and its active constituents may possess anti-carcinogenic potential by Amin et al. [2]. In recent days fenugreek is restated using as a supplement in wheat, sorghum and maize flour for bread-making and also using in multigrain flour.

Plants are exposed to a range of abiotic stresses like osmotic, salinity, chilling, temperature and heavy metal toxicity, which affect their growth and various physiological processes [3, 4]. Heavy metals pollution from different sources like industrial and agricultural activities have detrimental impact on surrounding areas [5]. Pb is one of the heavy metals, which effects on plants at higher concentration can interfere with important physiological functions and imbalance of nutrients and have detrimental effects on synthesis and functioning of enzymes [6]. Douchkov et al. [7] and Neculita et al. [8] reported the main drawback in plant is substitution of magnesium (Mg) atom in chlorophyll is replaced by heavy metal leads to decrease photosynthetic rate. Pb reduces the radius of vessels by blockage with cellular debris and gums, this phenomena leads to decrease the water translocation to leaves in plants by Chaudhry and Khan [9]. Photosynthetic pigments (chlorophyll-a, chlorophyll-b and carotenoid) content of the leaves was a gradual decrease with the increase in Pb concentration in *Brassica rapa* by Cenkci et al. [10]. Heavy metals are very toxic and oxidize biological macromolecules such as nucleic acids, proteins, and lipids, thereby disturbing the cell stability and membrane permeability. Plant growth is controlled by numerous hormonal and environmental factors that interact to regulate cell division and rate of cell expansion.

Brassinosteroids are a novel group of polyhydroxy steroids, ubiquitously distributed in the plant kingdom. When applied to plants, improve their quality and yield in geranium plant reported by Swamy and Rao [11, 12]. They have been further explored for stress-protective properties in plants against Abiotic and biotic stresses like, salt 13], osmotic stress [14], heavy metal [15, 16, 17], chilling [18] drought stress [19] and heat stress [20]. However, it is unclear whether BRs are involved in the modulation of plant responses to oxidative stresses. The effect of BRs on the response of antioxidative enzymes in plants under stress conditions has been started. The present research work was designed with an objective to explore the interactive effects of brassinosteroids on fenugreek plants under lead toxicity.

Material and Methods

Chemicals and plant material

28-homobrassinolide was procured from CID Technologies Inc., Brampton, Ontario, Canada. Fenugreek seeds were obtained from the National Seeds Corporation, Hyderabad, India.

Seeds were surface sterilized with 0.5% (v/v) sodium hypochlorite and thoroughly washed several times with distilled water. Seeds were sown a week for germination. Selected equal size germinated seedlings were transplanted to earthen pots which were filled with garden soil. Before transplanting into the pots 10 mM Pb was mixed to the soil for some of the pots for Pb control and for HBL+Pb treatment. On 10^{th} and 20^{th} day foliar application of distilled water (water control), HBL at 0.5 µM and or 1µM concentrations for HBL control and HBL+ Pb treatments. Plants were grown in the natural light and were watered regularly. Samples were collected after 30 days to assess the following parameters. Plant growth analysis, photosynthesis, chlorophylls, biochemical parameters, electrolyte leakage, lipid peroxidation, hydrogen peroxidase and enzyme activities were estimated.

Growth analysis

The growth of the plant was measured in terms of plant height, root growth, leaf area, leaf number, leaf weight, fresh and dry weights of root and shoots.

Chlorophylls

The chlorophyll pigments were extracted and estimated according to the procedure of Arnon [21] for extracting of the plant material 80% (v/v) acetone was used.

Net photosynthetic rate (P_N)

Fully expanded leaves of intact plants under natural light were measured by *LI-COR 6400* portable photosynthesis system (*LI-COR,* Lincoln, NE, USA). The atmospheric conditions were maintained during the experiment (PAR, 1500 ± 30; *C*i, 280 ±12 µmol mol^{-1}; atmospheric CO2, 350 µmol mol^{-1}; relative humidity, 60±5 %; atmospheric temperature 30±2 ºC).

Carbohydrate fractions

The alcohol homogenate was heated and centrifuged. The supernatant was used for the estimation of total sugars [22] and reducing sugars [23].

Soluble proteins

Soluble proteins in the ethanol homogenate were precipitated by adding 20% (w/v) trichloroacetic acid. The precipitate was dissolved in 1% (w/v) sodium hydroxide. Lowry *et al*. [24] method was employed for quantitative estimation of proteins.

Nucleic acids

DNA and RNA fractions in the ethanol homogenate were separated by the method of Ogur and Rosen [25]. While DNA estimation was done with diphenylamine reagent [26], RNA was quantified with orcinol reagent [27].

Results and Discussion

Results of the present study pointed out that Pb stress caused significant growth reduction in fenugreek plants as compared to the control (Fig-1). Reduction in growth has been attributed to Pb as it disrupts the microtubule assembly and disturbing cytokinesis therefore, less cell division and cell elongation. However, supplementation of HBL considerably removed the inhibitory effect of Pb toxicity in fenugreek plants. HBL at 1.0µM concentration was found to be highly effective in not only removing the inhibitory effect of Pb stress but also further improved the growth in terms of foliage growth, fresh and dry weights (Fig-2&3). The application of HBL alone also significantly increased in all the growth parameters over all the treatments. Earlier work revealed that Pb toxicity substantially reduced fenugreek at seedling stage and was improved by brassinosteroids, reported by Swamy and Rao [28]. Foliar application of 24-EBL was effective in improving growth, in terms of increasing plant fresh and dry biomass under both non-saline and saline conditions in wheat [29]. As observed in this study, a significant enhancement in plant growth attributes in HBL treated plants could be presumably referred to the well known effect of HBL on cell elongation and cell cycle progression as well as regulation of genes encoding XTH (xyloglucan endotransglucosylase/hydrolase) enzymes responsible for cell wall modification and enlargement [30]. Similarly, Pinol and Simon [31] observed that BRs supplementation caused an increase in number of leaves of bean. This could be due BRs role in regulating cell expansion and cell proliferation in the leaf due to activation of the CycD3 gene [32].

Results compiled in Fig-4 revealed that Pb stress significantly reduced Chlorophyll 'a' and 'b' levels as compared to the control. Decreased chlorophyll content associated with Pb stress may be the result of inhibition of chlorophyll biosynthesis or due to chlorophyll degradation by increased chlorophyllase activity [33]. However, foliar treatment of HBL to fenugreek plants under stress mitigated the inhibitory effect of the metal toxicity and restored the pigment levels. HBL alone treatment to stress-free plants exhibited the marked increase in chlorophyll content at 1.0 µM HBL concentration. Bajguz [15] also found that application of brassinolide prevented the chlorophyll loss in heavy metal stressed *Chlorella vulgaris* cultures. Our results further supported by the findings of Anuradha and Rao [34] in radish and Hasan et al. [35] in tomato under cadmium toxicity.

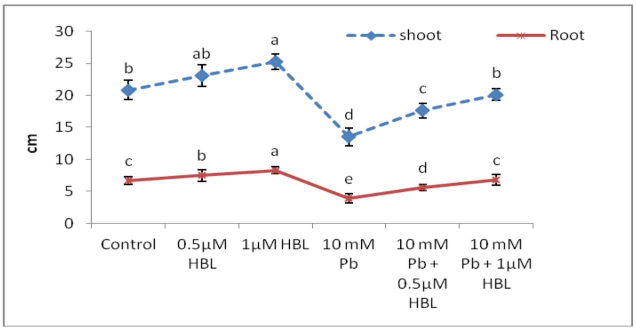

Fig-1. Effect of 28-homobrassinolide on shoot and root lengths of trigonella foenum greacum under lead toxicity

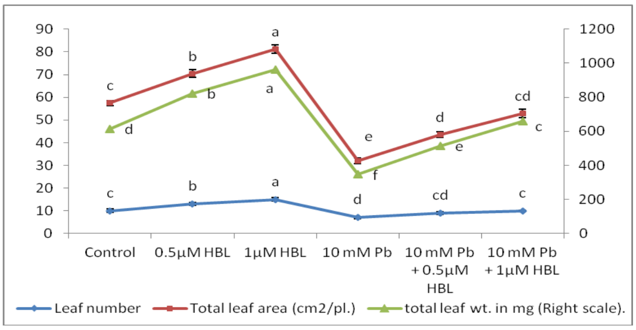

Fig-2. Effect of 28-homobrassinolide on foliage growth of trigonella foenum greacum under lead toxicity

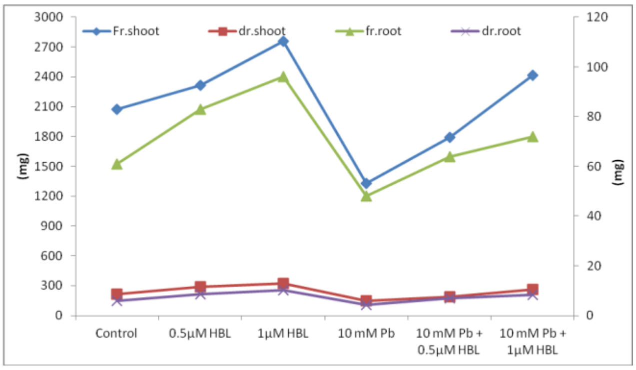

Fig-3. Effect of 28-homobrassinolide on growth of trigonella foenum greacum under lead toxicity

In the present study, net photosynthetic rate was markedly decreased in the fenugreek plant leaves challenged with Pb stress. However, foliar application of HBL improved the net photosynthetic rate in Pb stressed plants (Fig-5). Heavy metal toxicity especially lead directly effects the inhibition of chlorophyll synthesizing enzymes and Calvin cycle enzymes and also disarray of electron transport due to the lowering of the plastoquinone pool and ferredoxin-NADP+ oxidoreductase activity resulting the decreased rate of photosynthesis in plants. Several studies have shown the anti-stress properties of BR in improving the net photosynthetic rate under different stress conditions [35 - 37].

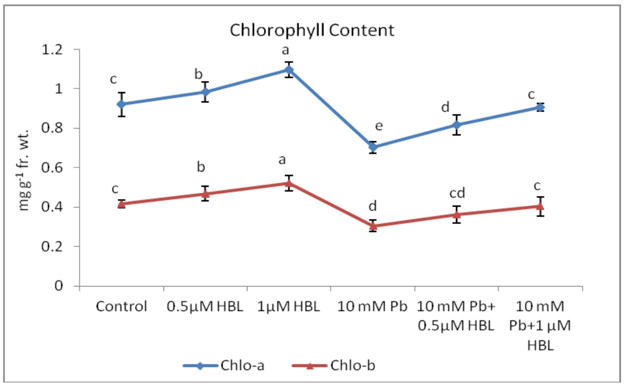

Fig-4. Effect of 28-homobrassinolide on chlorophyll content in trigonella foenum greacum leaves under lead toxicity

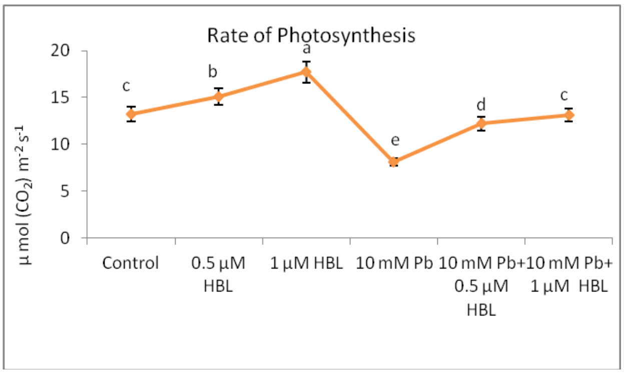

Fig-5. Effect of 28-homobrassinolide on photosynthetic activity in trigonella foenum greacum leaves under lead toxicity

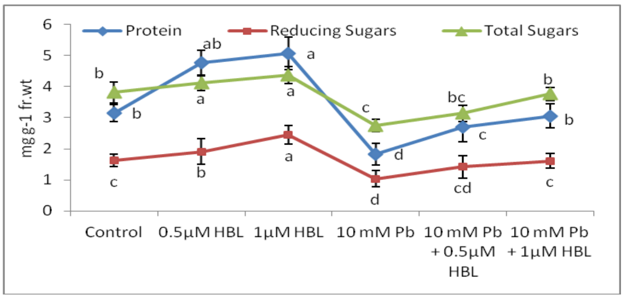

Fig-6. Effect of lead toxicity in the absence or presence of HBL on the protein and carbohydrate fractions in fenugreek plant.

Foliar application of HBL to fenugreek plants elevated the level of carbohydrate fractions, soluble protein and nucleic acid contents under stress conditions (Fig-8&9). Sugars are building substances for plant as well as a key

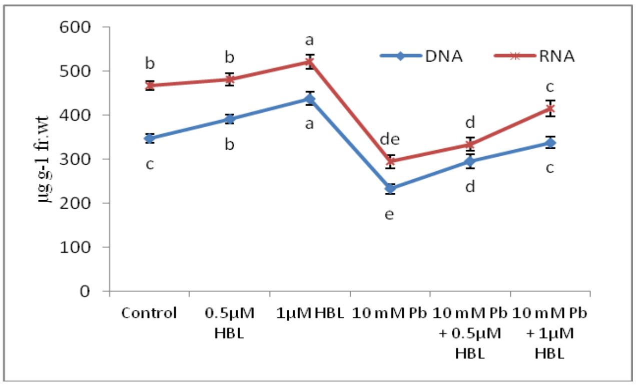

Fig-7. Effect of lead toxicity in the absence or presence of HBL on the nucleic acid content fenugreek plant.

source of energy necessary for inciting all the biochemical processes. The loss of chlorophyll content and decreased photosynthetic activity might be reduced the total and reducing sugar levels in fenugreek leaves. The results were consistent with the findings of Kastori et al. [38] that protein content was decreased when plants were exposed to high concentrations of the heavy metals in sunflower. Application of brassinolide to *Chlorella vulgaris* cultures reduced the accumulation of heavy metals stress prevented sugar, and protein loss by Bajguz [15]. Application of EBL and spermidine together increased the levels of total soluble sugars in radish seedlings under cr-treatment by Choudhary et al. [39]. The arrest of cell divisions resulted slow down of the basic metabolic processes. Zhang [40] reported barley treated with cadmium proved nuclear acid and damaged the structure of the nucleolus, as well as causing chromosome fragmentation, aberration, conglutination and liquefaction. Cheng [41] well documented the effect of heavy metals on nucleic acid decrease and or damage in various plants. Nucleic acid content was induced by brassinosteroids application in geranium by Swamy and Rao [11].

References

[1]. Ajabnoor, M.A., A.K. Tilmisany. 1988. Effect of trigonella foenum graecum on blood glucose levels in normal and alloxan-diabetic mice. *J Ethnopharm*. 22: 45-49.

[2]. Amin, A., A. Alkaabi, S. Al-Falasi and S.A. Daoud. 2005. Chemopreventive activities of Trigonella foenum graecum (Fenugreek) against breast cancer. *Cell Biology International* 29: 687-694.

[3]. Chaudhry, N.Y. and A.S. Khan. 2006. Improvement of pistillate flowers yield with GA_3. *Plant Growth Regulation*. 50: 211-217.

[4]. Ahluwalia, S.S. and D. Goyal. 2007. Microbial and plant derived biomass for removal of heavy metals from wastewater. *Biores. Technol*. 98: 2243-2257.

[5]. Liu Y.J., Y.G. Zhu and H. Ding. 2007. Lead and cadmium in leaves of deciduous trees in Beijing, China: development of a metal accumulation index (MAI). *Environ. Poll*, 145: 387-390.

[6]. Luo Y. And D. Rimmer. 1995. Zinc-copper interaction affecting plant growth on a metal contaminated soil. *Environ. Poll*. 88: 79-83.

[7]. Douchkov D., C. Gryczka, U. W. Stephan and H. Baumlein. 2005. Ectopic expression of nicotianamine synthase genes result in improved iron accumulation and increased nickel tolerance in transgenic tobacco. *Plant Cell and Environment*. 28: 365-374.

[8]. Neculita, C.M., J.Z. Gerald and D. Louis. 2005. Mercury speciation in highly contaminated soils from Chlor-Alkali plants using chemical extractions. *J. Environ Qual*. 34: 255-262.

[9]. Khan, A.S. and N.Y. Chaudhry. 2006. Auxins partially restore the cambial activity in heavy metals treated plants. *Luffa cylindrica* L. (Cucurbitaceae) under mercury stress. *Journal of Food Agri. Environment*. 4: 276-281.

[10]. Cenkci, S., I. H. Cioerci, M. Yildiz, C. Oezay, A. Bozdao and H. Terzi. 2010. Lead contamination reduces chlorophyll biosynthesis and genomic template stability in *Brassica rapa* L. *Health and Environmental Research Online*. 67: 467–473.

[11]. Swamy, K.N. and S.S.R. Rao. 2008. Influence of 28-homobrassinolide on growth, photosynthesis metabolite and essential oil content of geranium [*Pelargonium graveolens* (L.) Herit]. *American Journal of Plant Physiology*. 3: 173-179.

[12]. Swamy, K.N. and S.S.R. Rao. 2009. Effect of 24-epibrassinolide on growth, photosynthesis and essential oil content of *Pelargonium graveolens* (L.) Herit" Russian *Journal of Plant Physiology*. 56: 616-620.

[13]. Ozdemir, F., M. Bor, T. Demiral and I. Turkan. 2004. Effects of 24-epibrassinolide on seed germination, seedling growth, lipid peroxidation proline content and antioxidative system of rice (*Oryza sativa* L.) under salinity stress. *Plant Growth Regulation*. 42: 203-211.

[14]. Vardhini, B.V. and S.S.R. Rao. 2003. Amelioration of osmotic stress by brassinosteroids on seed germination and seedling growth of three varieties of sorghum. *Plant Growth Regulation*. 41: 25-31

[15]. Bajguz, A. 2011. Suppression of *Chlorella vulgaris* Growth by Cadmium, Lead, and Copper Stress and Its Restoration by Endogenous Brassinolide. *Archives of Environmental Contamination and Toxicology*. 60: 406-416.

[16]. Anuradha, S. And S.S.R. Rao. 2007. The effect of brassinosteroids on radish (Raphanus sativus L.) seedlings growing under cadmium stress. *Plant Soil and Environment*. 53: 465-472.

[17]. Swamy, K.N., S. Anuradha, B. Ramakrishna, N. Siddulu and S.S.R. Rao. 2011. Cadmium toxicity diminished by 24-epibrassinolide in seedlings of *Trigonella foenum-graecum* L. *Genetics and Plant Physiology*. 1:163-175.

[18]. Huang, B., C. H. Chu, S.L. Chen, H.F. Juan and Y.H. Chen. 2006. A proteomics study of the mung bean epicotyl regulated by brassinosteroids under conditions of chilling stress. *Cell. Mol. Biol. Lett.* 11: 264–278.

[19]. Vardhini, B.V., E. Sujatha and S.S.R. Rao. 2011. Brassinosteroids: Alleviation of Water Stress in Certain Enzymes of Sorghum Seedlings. *Journal of Phytology*. 3: 38-43.

[20]. Singh, I. and M. Shono. 2005. Physiological and molecular effects of 24-epibrassinolide, a brassinosteroids on thermotolerance of tomato. *Plant Growth Regulation*. 47:111-119.

[21]. Arnon, D. I. 1949. Copper enzyme in isolated chloroplasts: Polyphenol oxidase in *Beta vulgaris*. *Plant Physiology*. 24: 1-15.

[22]. Yoshida, S., D.A. Forno, J. H. Cock and Gomez. 1976. Laboratory physiological studies of rice; International Rice Research Institute (IRRI), Lagena Phillipines. pp. 38-39.

[23]. Nelson, N. 1944. A photometric adaptation of the somagyi method for determination of glucose. *J. Biol. Chem.* 154: 375-380.

[24]. Lowry, O.H, N.J. Rosenbrough, A. L. Farr and R.J. Randall. 1951. Protein measurement with Folin-phenol reagent. *Journal of Biol. Chem*. 193:265-275.

[25]. Ogur, M. And G. Rosen. 1950. The nucleic acids of plant tissue I. The extraction and estimation of deoxypentose nucleic acid and pentose nucleic acids. *Arch. Biochem*. *Biophysics*. 24: 262-276.

[26]. Burton, K. 1968. Determination of DNA concentration with diphenylamine. In Grossman L, Meidave K, eds. Methods in enzymology. New York: Academic Press, 163-166.

[27]. Schneider, W.C. 1957. Determination of nucleic acids in tissues by pentose analysis. In: Colowick SP, Kaplan NO, eds. Methods in enzymology: New York, Academic Press pp 680-684

[28]. Swamy, K.N. and S.S.R. Rao. 2005. Brassinosteroids reduced the inhibitory effect of lead toxicity on seed germination and seedling growth of *Trigonella foenum – graceum* L. *Bioinfolets*. 1: 32-34.

[29]. Shahbaz, M., M. Ashraf and H. Athar. 2008. Dose exogenous application of 24-epibrassinolide ameliorate salt induced growth inhibition in wheat (*Triticum aestivum* L.)? *Plant Growth Regulation*. 55: 51-64.

[30]. Ashraf, M., N. A. Akram, R. N. Arteca nad M. R. Foolad. 2010. The physiological, biochemical and molecular roles of brassinosteroids and salicylic acid in plant processes and salt tolerance. *Critical Reviews in Plant Science*. 29:162-190.

[31]. Pinol, R. and E. Simo´n. 2009. Effect of 24-Epibrassinolide on Chlorophyll Fluorescence and Photosynthetic CO_2 Assimilation in *Vicia faba* Plants Treated with the Photosynthesis-Inhibiting Herbicide Terbutryn. *J Plant Growth Regulation*. 28: 97–105.

[32]. Oh, M.H., J. Sun, D. H. Oh, R. H. Zielinski, S. D. Clouse and S. C. Huber. 2011. Enhancing Arabidopsis leaf growth by engineering the BRASSINOSTEROID INSENSITIVE1 receptor kinase. *Plant Physiol*. 157: 120–131.

[33]. Drazkiewicz, M. 1994. Chorophyll: occurrence, functions, mechanism of action, effects of internal and external factors. *Photosynthetica*. 30: 321–331.

[34]. Anuradha, S. and S.S.R. Rao. 2009. Effect of 24-epibrassinolide on the photosynthetic activity of radish plants under cadmium stress. *Photosynthetica*. 47: 317-320.

[35]. Hasan, S., S. Hayat and A. Ahamad A. 2011. Brassinosteroids protect photosynthetic machinery against the cadmium induced oxidative stress in two tomato cultivars. *Chemosphere*. 84: 1446-1451.

[36]. Yuan, G.F., C. G. Jia, Z. Li, B. Suna, L.P. Zhang, N. Liu and Q. M. Wang. 2010. Effect of brassinosteroids on drought resistance and abscisic acid concentration in tomato under water stress. *Scientia Horticulturae*. 126: 103-108.

[37]. Hayat, S., P. Maheswari, A. S. Wani, M. Irfan and M.N. Alyemeni. 2012. Comparitive effect of 28-homobrassinolide and salicylic acic in the amelioration of Na Cl stress in *Brssica junces* L. *Plant Physiol Biochemistry*. 53: 61-68.

[38]. Kastori, R., M. Petrovic and N. Petrovic. 1992. Effect of excess lead, cadmium, copper, and zinc on water relations in sunflower. *Journal of Plant Nutrition*. 15: 2427-2439.

[39]. Choudhary, S.P., M. Kanwar, R. Bhardwaj, J. Q. Yu and L. S. P. Tran. 2012. Chromium Stress Mitigation by Polyamine-Brassinosteroid Application Involves Phytohormonal and Physiological Strategies in *Raphanus sativus* L. *PLoS ONE*. 7: e33210 1-11.

[40]. Zhang, Y.1997. The toxicity of heavy metals to barley *(Hordeum vulgate). Acta Scinetiae Circumstance*. 17: 199-204. (In Chinese with English abstract).

[41]. Cheng, S. 2003. Effects of Heavy Metals on Plants and Resistance Mechanisms. *ESPR - Environ Sci and Pollut Res*. 10: 256-264.