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### RESEARCH ARTICLE

#### THE EFFECT OF NICKEL CHLORIDE (NiCl<sub>2</sub>) ON VARIOUS CALLUS GROWTH DYNAMICS OF *COLEUS BLUMEI* (BENTH.) CULTURES.

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*Coleus blumei*, Lamiaceae, Murashige and Skoog Medium, Inhibitory Concentration.

#### Abstract

An important member of family Lamiaceae, *Coleus blumei* has been priced for its medicinal and ornamental values. Due to phytotoxic effects of the heavy metal Nickel its impact on the growth rate of *in vitro* callus cultures has been studied. At different levels of 0.0 mM, 0.1 mM, 0.2 mM, 0.4 mM, 0.6 mM and 0.8 mM Ni concentrations were added to the Murashige and Skoog Medium. The data showed that there was a marked decrease in the callus growth parameters such as percent callus survival, percent callus multiplication, average fresh weight, average dry weight and percent growth index of callus. On the other hand, there was an increase in the percent callus dry matter content with respect to the control. The level of 0.4 mM concentration is considered to the inhibitory concentration (IC<sub>50</sub>) as there was 50% decrease in the growth dynamics of callus.

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#### Introduction:-

The genus *Coleus* has been priced since long for its aesthetic and medicinal values. The plant belongs to the family Lamiaceae (mint family) and all the members are of great importance. In ancient times, *Coleus* species has been used for the treatment of various ailments such as heart diseases, abdominal colic, respiratory disorders, painful micturitions, insomnia, convulsions, skin problems, worms etc. (Dubey *et al.*, 1981; Ammon and Muller, 1985; De Souza and Shah, 1988).

*Coleus blumei* is one of the well known species of the genus *Coleus*. The plant is known worldwide as an ornamental plant and commonly grown in gardens because of its colourful foliage. The enormous number of different cultivars occurs in a wide variety of color and shape of the leaves. Besides ornamental value, *C. blumei* is an important medicinal plant. The medicinal aspect is attributed to the production of most important secondary compound, namely rosmarinic acid (RA) synthesized by the plant. The compound is present in all parts of the plant and acts as an accumulated defense compound by virtue of its anti-microbial properties (Peterson *et al.*, 1994; Szabo *et al.*, 1999). Keeping in view, the aesthetic and medicinal aspect of *Coleus blumei* plants, the immediate concern for production of disease free plants, replication of elite clones and introduction of novel cultivars with desirable traits have been necessitated. For the production of desirable traits many reproducible protocols with cost efficiency, automation, and controlled optimization of *in vitro* environment are available. Rani *et al.*, 2006 reported the most efficient protocols for the production of *Coleus* species through *in vitro* culture techniques.

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Nickel as a heavy metal present in soil and water, in trace amounts is considered to be an essential micronutrient improving yield and quality of plants. It becomes toxic when present in higher concentrations (Kumar *et al.*, 2012). The significance of Ni as a pollutant was recognized two decades back (Iljin, 1991).

At higher concentrations, Nickel is highly phytotoxic at morphological, physiological and biochemical levels probably due to direct toxicity of metal or its tendency to compete with  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Fe}^{2+}$  and  $\text{Zn}^{2+}$  cations causing their deficiency in plants (Seregin and Kozhevnikova, 2006; Sengar *et al.*, 2008). The symptoms of Ni toxicity in plants are reported as inhibition of seed germination, growth, photosynthesis, sugar transport (Dubey and Pandey, 2011). Ni toxicity leads to induction of chlorosis, necrosis and wilting due to deficiency of essential micronutrients Zinc or Iron in the plant tissues (Anderson *et al.*; 1973; Madhava Rao and Sresty, 2000, Pandey and Sharma, 2002).

As an essential tool of biotechnological research, plant tissue culture techniques are of important use in plant propagation, disease free production, plant improvement and production of secondary metabolites. It is an efficient technology which provides the conducive environmental conditions for their growth and multiplication. The cultures are grown under stress conditions to study various physiological and biochemical parameters. These conditions are provided under proper supply of nutrients, pH medium, adequate temperature and humidity, proper gaseous and liquid environment. These micropropagation techniques have several advantages over the traditional methods of propagation such as seeds, cuttings, grafting, *etc.* (Nowak, 1998; Hussain *et al.*, 2012).

## Materials and Methods:-

### Source of Plant Material:-

The aseptically grown *in vitro* shoots were transferred for callus induction into standardized 2, 4-dichlorophenoxyacetic acid (2, 4-D) concentrations fortified to MS solid medium (Murashige and Skoog, 1962). The induced callus was then aseptically transferred to the test tubes (150 mm×25 mm) containing standardized MS Medium for further callus multiplication.

### Supplementation of MS medium with Nickel Chloride Hexahydrate ( $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ ):-

The mother stock solution of 1M  $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$  was prepared and Seitz filtered through 0.22 $\mu\text{m}$  Millipore filter membranes under sterile conditions in the laminar flow cabinet and stored in an amber colored reagent bottle at 4°C for further use. The different range of heavy metal  $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$  concentrations were prepared from the mother stock solution by employing dilution equation ( $C_1V_1 = C_2V_2$ ). The autoclaved MS medium was immediately transferred to the laminar flow cabinet where the different Ni metal concentrations calculated in the range of 0mM, 0.1mM, 0.2mM, 0.4mM, 0.6mM, 0.8mM and 1.0mM were added from the mother stock solution of  $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$  to the different culture flasks containing culture medium under aseptic conditions. The Ni supplemented MS medium was poured into culture tubes and flasks, sealed and marked with the respective concentrations of heavy metal and transferred to the culture room maintained at  $25 \pm 1^\circ\text{C}$  and 70% relative humidity under 40  $\mu\text{mole/m}^2/\text{sec}$  cool white fluorescent lights for 16 h photoperiod/8h dark period daily for solidification and further use in experiments.

### Determination of Callus Growth Dynamics:-

The growth rate of callus was monitored after 30 days of culture by measuring the percent callus survival, percent callus multiplication, average fresh and dry weights, percent growth index and percent dry matter content of callus from the data recorded on *in vitro* cultures. Approximately, 500 mg of 30 days old callus tissues were transferred to each culture tube containing the fortified MS medium supplemented with different Ni concentrations. Each experimental set up was recorded after 30 days of inoculation and repeated in 3 replicas. The rate of callus growth dynamics are calculated as follows:-

The rate of callus survival was expressed as:

$$\text{Percent Callus Survival (\%)} = \frac{\text{Callus survived in each treatment}}{\text{Callus survived in the controls}} \times 100$$

The percent callus multiplication is calculated as:

$$\text{Percent Callus Multiplication (\%)} = \frac{\text{Number of explants with callus multiplication}}{\text{Total number of explants}} \times 100$$

The fresh weight was calculated as an average of three fresh weights of callus cultures grown in three different culture flasks. The fresh callus measured in grams (g) was then oven dried at 80°C for 48 hours prior to the dry weight determination. The dry weight of callus was calculated in grams (g) by subtracting the initial weight of

empty petriplate from the final weight of the petriplate containing dried callus. The experiment was performed in three replicas.

The growth index of callus was measured in terms of initial and final fresh weights at an interval of 21 days. The measured weight of callus (500 mg) was inoculated on the fortified MS medium at different NiCl<sub>2</sub> concentrations. After 7 days, the callus from three culture tubes was removed and their average initial fresh weight was recorded at each concentration. After 28 days of inoculation, the final average fresh weight of callus was measured at each concentration.

**The percent growth index of callus determined according to the Dung *et al.* (1981) with slight modifications by the following equation:-**

$$\text{Callus Growth Index (\%)} = \frac{\text{Final callus fresh weight} - \text{Initial callus fresh weight}}{\text{Initial Callus fresh weight}} \times 100$$

**The percent callus dry matter content determined according to Khater *et al.* (2013) was calculated as:-**

$$\text{Callus Dry Matter Content (\%)} = \frac{\text{Average Dry weight of callus}}{\text{Average Fresh weight of callus}} \times 100$$

In the present investigation, the IC<sub>50</sub> value of the heavy metal nickel was decided on the basis of 50% decrease in the percent callus survival, percent callus multiplication, percent callus growth index, average fresh and dry weight of callus with respect to the control on the basis of regression equation.

#### Statistical Analysis:-

The data were analyzed for mean, standard error (SE) and linear regressions using self coded computer software in MS Excel and the values were expressed as Mean ± SE.

### Results and Discussion:-

#### Effect of Nickel (Ni) on callus growth parameters:-

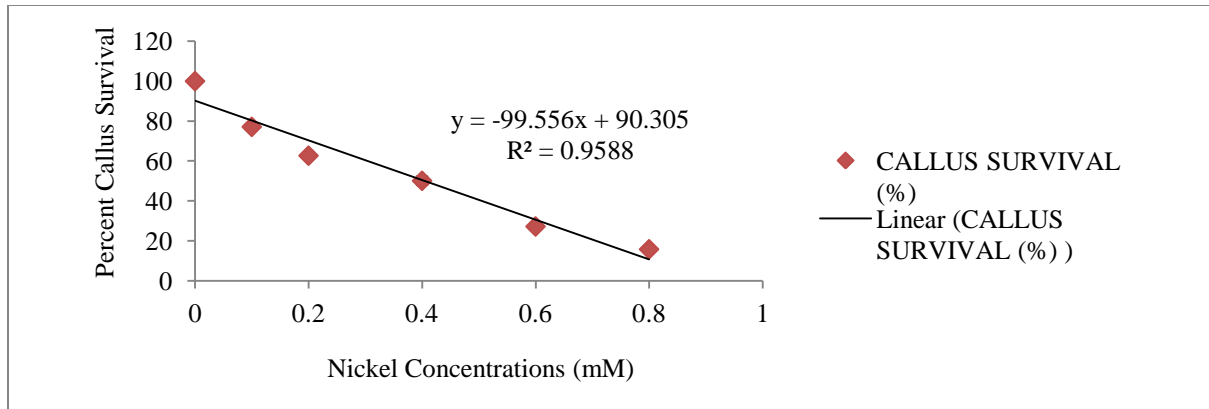
The data tabulated in Table 1 represents the effect of NiCl<sub>2</sub> concentrations on the various growth parameters of *in vitro* callus cultures. It is evident that with increasing metal treatments there was a decline in percent callus survival, percent callus multiplication, fresh weight, dry weight and percent growth index of callus. As per regression equation, there is 50% decrease in values at 0.4 mM Ni treatment with respect to control. Further there was significant decrease in the growth parameters from 0.4 mM to 0.8 mM treatments as compared to control treatments (Figure 1).

In case of percentage callus dry matter content, an increase in trend was observed with increasing metal concentrations. The percentages of callus dry matter content ranging from 0.1 mM to 0.8 mM concentrations were higher as compared to the control treatments (Figure 1).

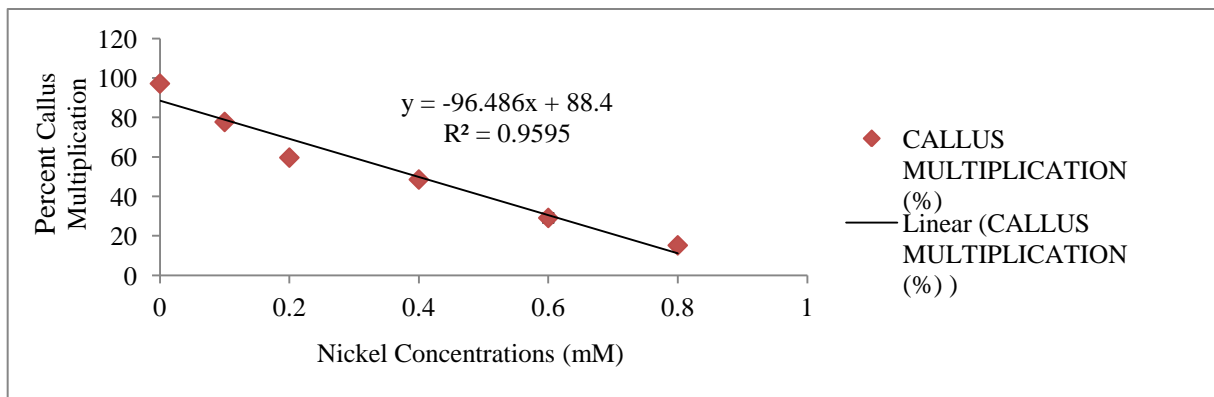
Similarly, Fathalla *et al.* (2011) reported in *Roseus* (L.) G. Don. Callus Cultures that with increasing concentrations of mercury (Hg) metal there was significant decrease in the different growth parameters of shoot and root derived callus. However, the percentage of callus dry matter content significantly increased with increasing Hg metal concentrations than the control treatment.

**Table 1:-** Effect of different concentrations of nickel chloride (NiCl<sub>2</sub>) on percent callus survival, percent callus multiplication, fresh weight (g), dry weight (g), callus growth index (%) and callus dry matter content (%) of *in vitro* cultures of *C. blumei* (Benth.) inoculated on MS medium (observation recorded after 30 days of inoculation).

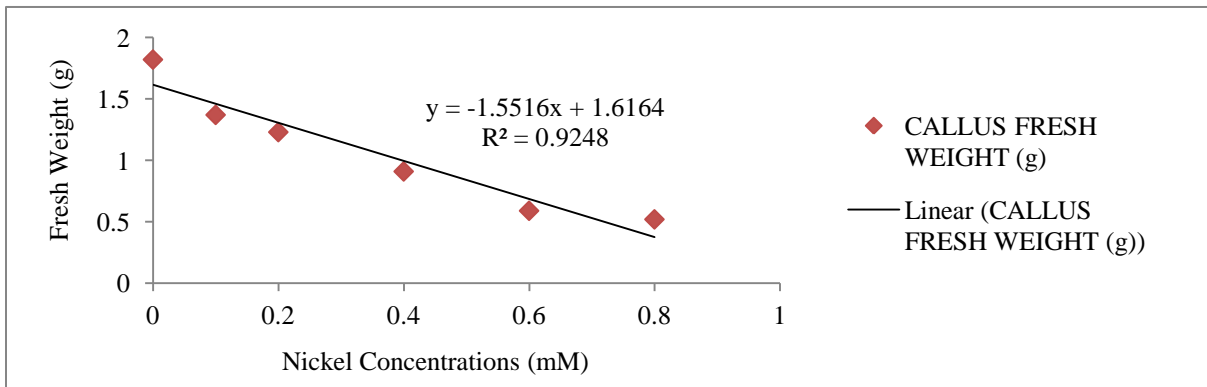
Nickel (mM)	Percent Callus Survival (Mean ± SE)	Percent Callus Multiplication (Mean ± SE)	Fresh Weight (g) (Mean ± SE)	Dry Weight (g) (Mean ± SE)	Callus Growth Index (%) (Mean ± SE)	Callus Dry Matter Content (%) (Mean ± SE)
0.0	100 ± 0.0	97.22 ± 1.3	1.820 ± 0.01	0.066 ± 0.003	84.82 ± 0.13	3.63 ± 0.12
0.1	77.11 ± 1.6	77.78 ± 1.3	1.366 ± 0.02	0.059 ± 0.002	54.27 ± 1.27	4.32 ± 0.15
0.2	62.66 ± 1.4	59.72 ± 1.4	1.231 ± 0.01	0.054 ± 0.003	51.57 ± 1.48	4.64 ± 0.17
0.4	50.06 ± 2.1	48.61 ± 1.3	0.905 ± 0.02	0.051 ± 0.002	40.81 ± 1.74	5.75 ± 0.17
0.6	27.17 ± 1.7	29.17 ± 2.4	0.585 ± 0.02	0.041 ± 0.001	19.49 ± 1.90	7.08 ± 0.31
0.8	15.76 ± 1.6	15.28 ± 1.4	0.524 ± 0.01	0.039 ± 0.002	06.48 ± 1.21	7.57 ± 0.39



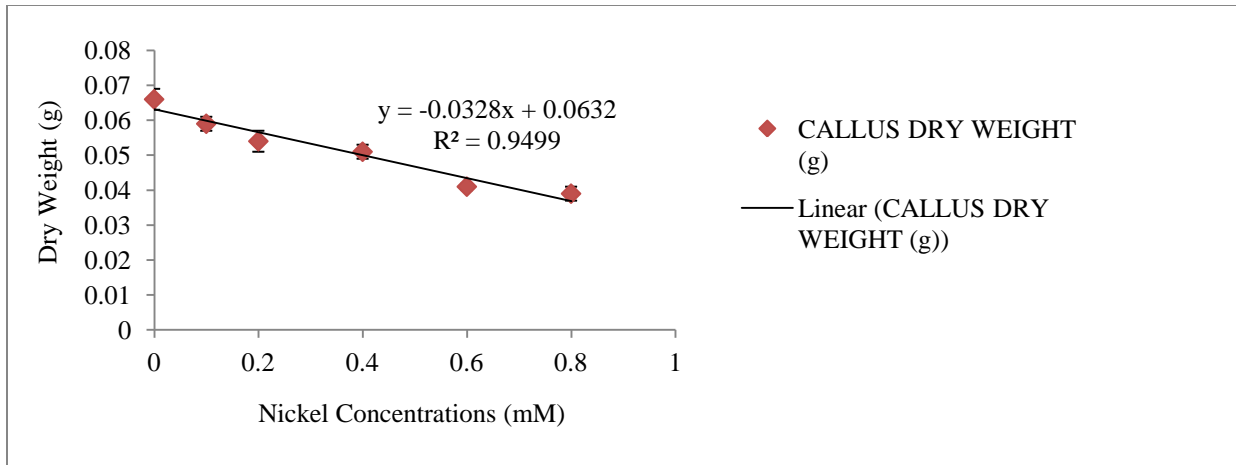
(a)



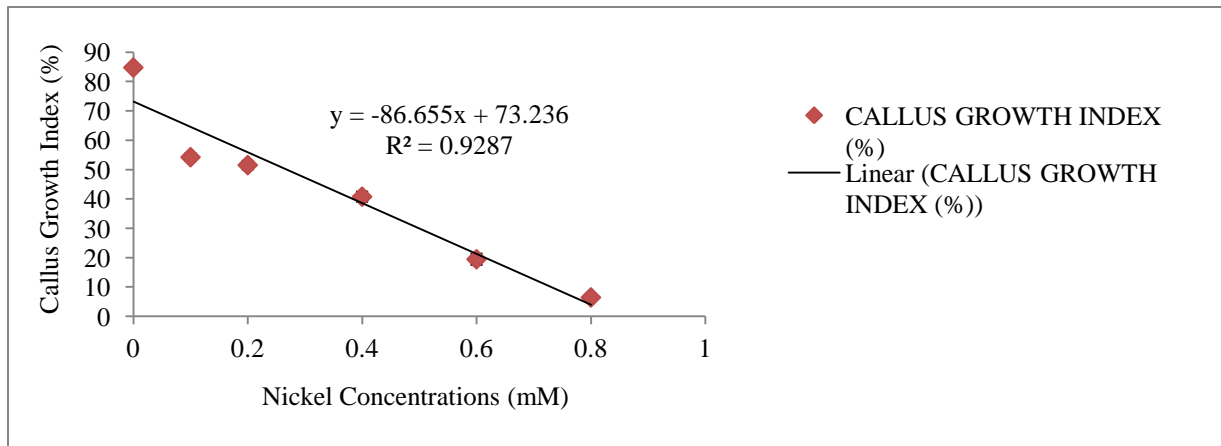
(b)



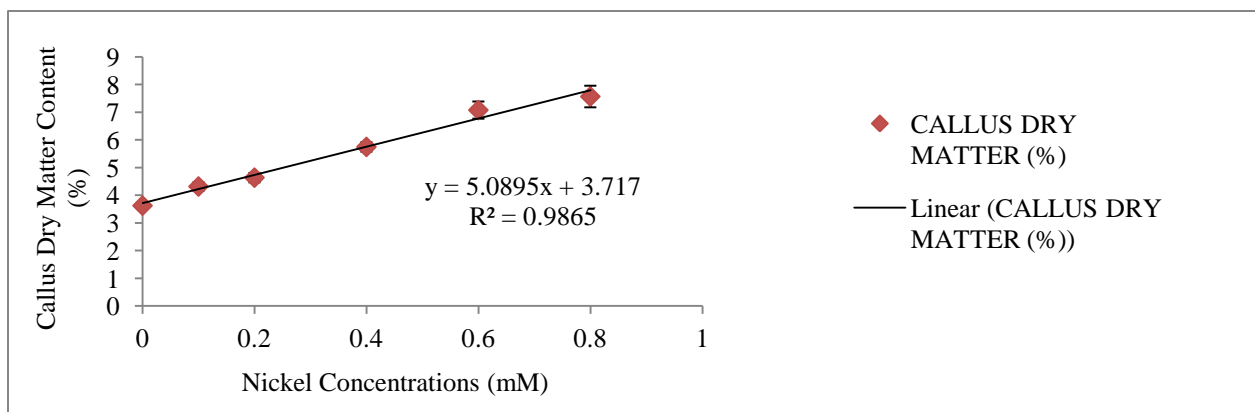
(c)



(d)



(e)



(f)

**Figure 1:-** Response of callus growth dynamics represented as (a) percent callus survival, (b) percent callus multiplication, (c) average fresh weight (g), (d) average dry weight (g), (e) callus growth index (%) and (f) callus dry matter content (%) of *in vitro* *C. blumei* (Benth.) callus cultures inoculated on MS medium supplemented with various nickel concentrations (observations recorded after 30 days of inoculation in triplicates).

**Conclusion:-**

This piece of work clearly indicated the significant deleterious effects of heavy metal Ni on the *C. blumei* inoculums. These *in vitro* cultures were further employed in raising the Nickel tolerant lines of an important medicinal and ornamental plant *C. blumei* (Benth.).

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**References:-**

1. Ammon, H.P.T. and Muller, A.B. (1985). Forskolin: From an ayurvedic remedy to a modern agent. *Planta Medica*. 46: 473-477.
2. Anderson, J.G., Mayer, D.R., Mayer, R.K. (1973). Heavy metal toxicity levels of nickel, cobalt and chromium in the soil and plants associated with visual symptoms and variations in the growth of an oat crop. *Australian Journal of Agricultural Research*. 24: 557-571.
3. De Souza, N.J. and Shah. V. (1988). Forskolin – an adenylate cyclase activating drug from an Indian herb. *Economic and Medicinal Plant Research*. 2: 1-16.
4. Dubey, R.S. (2011). Metal toxicity, oxidative stress and antioxidative defense system in plants. In: *Reactive Oxygen Species and Antioxidants in Higher Plants*, S. D. Gupta, Ed., CRC Press, Boca Raton, Fla, USA. 177-203
5. Dubey, D. and Pandey, A. (2011). Effect of nickel (Ni) on chlorophyll, lipid peroxidation and antioxidant enzymes activities in black gram (*Vigna mungo*) leaves. *International Journal of Science and Nature*. 2: 395-401.
6. Dung, N.N., Szoke E and Verzar- Petri, G. (1981). The growth dynamics of callus tissues of root and leaf origin in *Datura innoxia* Mill. *Acta Botanica Academiae Scientiarum Hungaricae*. 27: 325-333.
7. Fathalla, M. Abd-El-Kareem, Abd-El Kawy A.M and Taha H.S. (2011). Effect of Heavy Metal (HgCl<sub>2</sub>) on Accumulation and Production of Total Indole Alkaloids, Vinblastine And/or Vincristine from Egyptian *Catharanthus Roseus* (L.) G. Don. Calli Cultures. *Journal of Applied Sciences Research*, 7: 542-549.
8. Hunter, J.G. (1954). Nickel toxicity in a Southern Rhodesian soil. *South African Journal of Science*. 51: 133-135.
9. Hussain, S., Fareed, S., Ansari, S., Rahman, A., Iffat-Zareen, A., Saeed, M. (2012). Current approaches toward production of secondary plant metabolites. *Journal of Pharmacy and Bioallied Sciences*. 4: 10-20.
10. Iljin, V. (1991). *Heavy Metals in the Plant-Soil System*. Novosibirsk, Russia: Publishing House, Nauka.
11. Khater M.A., S.S.A.Soliman, M.S. Abdel-Hady, and A.H. Fayed (2013). Tropane Alkaloid Production via New Promising *Atropa belladonna* L. Lines by *In Vivo* and *In Vitro*. *Nature and Science*, 11: 49-57.
12. Kumar, H., Sharma, D. and Kumar, V. (2012). Nickel-induced oxidative stress and role of antioxidant defense in Barley roots and leaves. *International Journal of Environmental Biology*. 2: 121-128.
13. Madhava Rao, K. V. and Sresty, T. V. (2000). Antioxidative parameters in the seedlings of pigeon pea (*Cajanus cajan* L.) in response to Zn and Ni stresses. *Plant Science*. 157: 113-128.
14. Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassay with tobacco tissue cultures. *Plant Physiology*. 15: 473-497.
15. Pandey, N. and Sharma, C.P. (2002). Effect of heavy metals Co<sup>2+</sup>, Ni<sup>2+</sup> and Cd<sup>2+</sup> on growth and metabolism of cabbage. *Plant Science*. 163: 753-758.
16. Petersen, M. (1994). *Coleus* spp.: *In vitro* culture and the production of forskolin and rosmarinic acid. In: *Biotechnology in Agriculture and Forestry, Medicinal and Aromatic Plants VI* (Bajaj Y. P. S., ed.) Springer, Berlin. 26: 69-93.
17. Rani, G., Talwar, D., Nagpal, A and Virk, G. S. (2006). Micropropagation of *Coleus blumei* from nodal segments and shoot tips. *Biologica Plantarum*. 50: 496-500.
18. Sengar, R.S., Gupta, S., Gautam, M., Sharma, A., Sengar, K. (2008). Occurrence, uptake, accumulation and physiological responses of nickel in plants and its effects on environment. *Research Journal of Phytochemistry*. 2: 44-60.
19. Seregin, B., and Kozhevnikova, A.D. (2006). Physiological role of nickel and its toxic effects on higher plants. *Russian Journal of Plant Physiology*. 53: 257-277.
20. Szabo, E., Thelen, A. and Petersen, M. (1999). Fungal elicitor preparations and methyl jasmonate enhance rosmarinic acid accumulation in suspension cultures of *Coleus blumei*. *Plant Cell Reports*. 18: 485-489.