Original Research Article

Effect of bulk and nano cobalt on barley seedlings and remediation of $CoCl_2$ toxicity using NaOCl

ABSTRACT

Heavy metals are ceaselessly relinquished into the terrestrial environment by natural and anthropogenic activities. A study was undertaken to determine the comparative effects of Co (II, III) oxide (bulk salt) and Co (II, III) oxide (nanopowder) on the germination, growth and some biochemical parameters of *Hordeum vulgare* L. seedlings. Oxides of cobalt (bulk and nano) were added to the sand medium at five levels (0, 50, 100, 150 and 200 mg kg^{-1} sand). Bulk cobalt oxide was found to increase growth of shoots and roots at concentrations upto 200 ppm. Increase in concentration of nano Co increased the shoot length, but decreased root length. It was found that NaOCl decreases the toxicity of $CoCl_2.6H_2O$. Biochemical characteristics including lipid peroxidation and chlorophyll estimation were studied for all the three treatments. Lipid peroxidation was maximum at 200 ppm for bulk Co in shoots. Increase in the lipid peroxidation was found in nano-cobalt treated samples. Since Co is toxic at higher concentrations, a study was also done to determine if sodium hypochlorite can be used to detoxify $CoCl_2.6H_2O$. Best binary combinations were 1000 ppm (Co): 500 ppm (NaOCl); 250 ppm (Co): 750 ppm (NaOCl); 1000 ppm (NaOCl): 1000 ppm Co.

Keywords: Detoxification, heavy metals, nanopowder, sodium hypochlorite**,** *Hordeum vulgare* L.

1. INTRODUCTION

Due to fast pace of industrialization and irrational use of natural resources metal concentration is increasing in the environment. Metal accumulation in soil is of great concern in agriculture due to its adverse effects on food safety and marketability, plant growth and soil microflora and fauna [1]. Metal toxicity has high impact on the plants which consequently affect the whole ecosystem due to interdependence of other living organisms.

Cobalt (Co) is a transition metal with atomic number 27 and atomic weight 58.9 g/ mol. The role of Co in nutrition of leguminous plants is well known, but its importance to rest of the plant species is still ambiguous [2]. It is an essential element for the synthesis of various enzymes and coenzymes like vitamin B_{12} (cyanocobalamin), which is required for human and animal consumption. Cobalt is safer for consumption upto 8 mg on daily basis without any health hazard [3]. It acts as a coenzyme in a number of cellular processes including the oxidation of fatty acids and the synthesis of DNA. Toxic concentrations of cobalt inhibit active transport in plants. Relatively higher concentrations of Co have toxic effects, including morphological changes like leaf fall, inhibition of greening, discoloured veins, premature leaf closure and reduced shoot weight [4].

Nanotechnology is the engineered convergence of biology, chemistry, and informatics at nanoscale- that is, involving materials measured in billionths of a meter. The products of these exertions are called nanomaterials, consisting of nanoparticles having size smaller than 100 nm in at least one dimension. Among the latest technological innovations, nanotechnology poses the top most position [5]. The properties of nanomaterials raise concern about their adverse effects on biological systems at cellular level. Because of their small size, nanoparticles get incursion into the living cell membrane. In contrast to the classical i.e., microscale substances, NPs due to their nanoscale and huge surface area may interact more expeditiously with biological systems. Metal oxide based nanoparticles (NPs) are increasingly used in applications such as opacifiers, fillers, catalysts, semiconductors, cosmetics and microelectronics etc. [6]. Therefore, the interaction between inorganic nanoparticles and biological systems is one of the most promising areas of research in modern nanoscience and technology.

2. MATERIAL AND METHODS

2.1. Study material

Certified and disease free seeds of barley (*Hordeum vulgare* L.) variety PL- 426 were purchased from Punjab Agricultural University (PAU), Ludhiana, Punjab. Barley is generally grown as a summer crop in temperate areas, and winter crop in tropical areas. It is an important cereal of India, and ranks next to wheat, maize and rice in the world.

2.2. Cobalt oxide (Co_3O_4) and nano cobalt oxide treatments

Salts of cobalt and other chemicals used under study were purchased from Sigma Aldrich, USA; HIMEDIA Laboratory Pvt Ltd; Loba Chemie Pvt Ltd and BTL Research Lab.Suspensions of both cobalt oxide (pure) and cobalt oxide nanopowder were made in distilled water. Different concentrations of both pure and nano Co_3O_4 containing 0, 50, 100 , 150 and 200 mg Co kg^{-1} sand were prepared respectively.

2.3. Sand cultures and raising of the plant material

Seeds of *H. vulgare* L. were surface sterilized with 0.01% $HgCl_2$ and then washed under running tap water for 10 min. After that, the seeds were soaked in distilled water for 1 h for imbibition. Sand was filtered through sieve size of 300 nm, washed with 0.1 N HCl and was dried on filter paper in the oven at 80-85˚C for 3 days. The imbibed seeds were then sown in polypropylene plastic jars of diameter 11 cm containing 0.5 kg sand treated with different concentrations of cobalt. In each jar, 30 seeds of nearly same size were planted. These sand cultures were maintained at a temperature of 25 ± 0.5˚C, 70 - 80% relative humidity and 16:8 hour dark: light photoperiod (1700 lux). Then, different plant parts (shoots, roots) were harvested after 7 days of growth for the estimation of root and shoot length, and fresh and dry weights. Biochemical parameters were studied in terms of oxidative stress caused by metal salts. These include lipid peroxidation and estimation of chlorophyll content. Malondialdehyde (MDA) was estimated by method given by Heath and Packer [7], and chlorophyll contents were measured by method given by Arnon [8].

2.4. Statistical analysis

The experimental data was expressed as mean ± SE. One way and two way analysis of variance (ANOVA), linear regression, multiple regression, multiple regression with interaction were carried out using self coded software in MS- Excel.

3. Results

3.1. Growth characteristics

Change in root and shoot length cultured in sand medium containing cobalt oxide (pure) and nanopowder is given in figure (1). A significant increase in relative root as well as shoot length was observed with increase in concentration of cobalt oxide (0, 50, 100, 150 and 200 ppm), while significant increase in relative shoot length was observed, while root length showed a regular decrease with increase in concentration of cobalt (II, III) oxide nanopowder.

A significant decrease in shoot and root length of *H. vulgare* was observed with addition of various concentrations (0, 250, 500, 750 and 1000 ppm) of cobaltous chloride hexahydrate, further the role of sodium hypochlorite as a potent inhibitor of later was elucidated as was evidenced by masking the toxic effects of cobalt (Table 1) and (Fig 2). Two – way ANOVA summary described the statistically significant difference among mean values of relative shoot and root length on Co treatment as well as on NaOCl treatment. Multiple regression models showed that Co has negative effect on shoot and root length while NaOCl has positive effect. However, interaction between Co and NaOCl was found to be statistically significant (Table 7). Fresh and dry weight shoots also show significant differences among mean values, while root fresh and dry weight did not impart any significant differences among mean values. Interaction between Co and NaOCl was found to be statistically significant (Table 2) and Fig (3-4).

3.2. Lipid peroxidation

Variations in shoot and root MDA content of *H. vulgare* grown in sand media containing cobalt oxide (pure) and cobalt oxide nanopowder is presented in (table 4 and fig. 5). The MDA content of *H. vulgare* treated with bulk cobalt was increased significantly for shoots, while decreasing trend was found in case of roots. One way ANOVA depicted that the differences among mean MDA content of cobalt oxide treatments were statistically significant. The MDA content in *H. vulgare* treated with was increased significantly for shoots and roots with increase in concentration of nanometal salt. One way ANOVA summarized that mean MDA content and cobalt oxide treatments did not show any significant variations among mean values. Effect of binary combinations of cobaltous chloride hexahydrate and NaOCl on MDA content of shoots and roots (table 5 and fig.6) of *H. vulgare* were studied. Two-way ANOVA summarized that there was significant differences among mean MDA content of both shoots and roots and binary treatments. The interaction between Co and NaOCl was found to be negative for both shoots and roots (Table 8).

3.3. Chlorophyll estimation

The effect of cobalt oxide pure and nanopowder on chlorophyll content (a, b and total chl) was compiled in (Table 5 and fig.7). One way ANOVA depicted the statistically significant differences among mean values of chl a, chl b, total chlorophyll. Table 6 and Fig. 8-9, represents the effect of binary combinations of cobaltous chloride hexahydrate and NaOCl on chl a, chl b and total chl content of *H. vulgare*. Two- way ANOVA described the significant differences among mean chlorophyll content values and binary treatments. Multiple regression model analysis (Table 8) showed positive effect of NaOCl on Chl ‘a’ rather it compensated the negative of Co for the same. On the other side Co and NaOCl, significantly increased the chl ‘b’content while in case of total chlorophyll, Co showed negative β- regression coefficient value while NaOCl showed positive value. However, interaction between Co and NaOCl was found to be statistically significant.

4. Discussion

Heavy metals may cause major occupational and environmental hazards due to their non-biodegradable nature and long biological half life period [9]. The causes for the exposure of heavy metals are mainly the anthropogenic actions such as use of fertilizers, agrochemical compounds, sewage sludge and other activities like mining etc [10]. Such activities result in transportation of metal ions via air and water and thus, ultimately bind to soil and sediments. Cobalt is relatively a rare magnetic element with properties similar to iron and nickel. Cobalt occurs in nature primarily as arsenides, oxides, and sulfides. Most of the production of cobalt involves the metallic form used in the formation of cobalt superalloys [11]. The distribution of cobalt in plants is entirely species specific. The present study presented the *in vitro* evaluation of toxicity of Co oxides (pure and nano) and cobaltous chloride hexahydrate in binary combination with sodium hypochlorite on root length, shoot length and fresh weight, dry weight and biochemical parameters (lipid peroxidation and chlorophyll content) parameters of *H. vulgare* L.

A significant increase in shoot length was observed in 7 days old seedlings treated with cobalt oxide (pure). In the case of root length, no significant increase was observed. Also, a regular and significant increasing trend was observed both for fresh and for dry weight of seedlings. It was found that with increase in concentration of cobalt oxide (nanopowder), the shoot length was increased in a dose dependent manner while root growth showed decreasing trend.The fresh and dry weights showed significant increase for both the roots and shoots. Cobaltous chloride hexahydrate is toxic for plants at higher concentrations. So, in order to reduce toxicity of Co, NaOCl (sodium hypochlorite) was used as counteractive chemical, which exerts its affect by transforming cobalt into its oxide form. Several mechanisms involved for the same include exclusion, inclusion (i.e. sequestration and compartmentalization of metal ions in organelles) and chelation binding. Maximum shoot length was observed at concentration with binary combination of NaOCl (250 ppm): metal (500 ppm). The reaction of cobaltous chloride hexahydrate with sodium hypochlorite is given below:

$$3CoCl_2 . 6H_2O + 6NaOCl \longrightarrow Co_3O_4 \text{ (ppt)} + 6NaCl + 3Cl_2 + 4O_2$$

Reason for such an observation may be attributed to the fact that NaOCl is potent inhibitor for the toxicity caused by metal. Further, it can be seen that at concentrations where NaOCl is absent altogether, metal has caused toxicity and resulted in reduction of shoot length. Lowest shoot length was observed at concentration where cobalt is in maximum amount and NaOCl is in minimum amount. In case of root length, maximum growth was seen at concentration with binary combination of NaOCl (1000 ppm): metal (250 ppm). The amount of NaOCl required for counteracting toxicity caused by Co is more in case of roots as compared to shoots. The reason for higher NaOCl requirement in root requires further mechanistic studies. The most effective concentration for fresh weight (shoots) in binary combination comes out to be NaOCl (250 ppm): Co (250 ppm) and NaOCl (1000): Co (1000). Similar results were obtained for dry weight parameter also. Further, root fresh weight in binary combination was maximum at the concentration of NaOCl (250 ppm): Co (250 ppm) followed by NaOCl (1000): Co (1000). Similar results were evaluated for root dry weight too.

The extent of lipid peroxidation was found to be maximum for shoots at concentration of 200 ppm of cobalt oxide and its values were found to be statistically significant as compared to control. The reason for such a trend can be attributed to increased production of ROS which induce membrane destabilization resulting in formation of peroxides, as was reported by Mead *et al* [13]. On the other hand, cobalt oxide inhibited the extent of lipid peroxidation by decreasing the MDA content in roots. The values obtained for the same were statistically different as compared to control. The MDA content for both shoots and roots showed an increasing trend with increase in concentration (0, 50, 100, 150 and 200 ppm) of cobalt (II, III) oxide nanopowder in a dose dependent manner. The lowest value for MDA (shoots and roots) was found at concentration of 50 ppm, while other concentrations show increased amount of lipid peroxidation. The best binary combinations for MDA content (for shoots) were NaOCl (500 ppm) : Co (1000), followed by NaOCl (750 ppm) : Co (250 ppm). In case of roots, best binary combinations for MDA content were; NaOCl (250 ppm) : Co (1000 ppm), NaOCl (750 ppm): Co (500 ppm); NaOCl (1000 ppm): Co (1000 ppm).

It was found that chlorophyll ‘a’, ‘b’ and total showed maximum value at 200 ppm. Different results were obtained by Jayakumar *et al.* [12] at 50 ppm, where chlorophyll content showed increasing trend while decreasing trend was observed with increase in concentration upto 250 ppm. The significant increase was found in content of chl a, b and total with increase in concentration of cobalt (II, III) oxide nanopowder in sand medium. Such results depicted that nanosalt had unregulated chlorophyll synthesis. The best binary combinations for chlorophyll ‘a’ come out to be NaOCl (500 ppm): Co (250 ppm) followed by NaOCl (1000 ppm): Co (1000 ppm) respectively. For chlorophyll ‘b’ NaOCl (750 ppm):

Co (1000 ppm) was found to be the best binary combinations. While total chlorophyll content viz; NaOCl (500 ppm): Co (500 ppm) and NaOCl (750 ppm): Co (1000 ppm) showed best results.

5. Conclusion

Cobalt oxide (bulk) increased root and shoot length of seedlings of barley while cobalt oxide nanopowder decreased root length but increased shoot length upto 200 ppm concentrations. Co (II,III) oxide noth bulk and nano salts increased the chlorophyll content of the seedlings, while $CoCl_2.6H_2O$ decreased the same. Peroxidation of lipids increased in shoots treated with Co (II, III) oxide bulk and nano. NaOCl decreased the toxicity of $CoCl_2.6H_2O$ as observed from increase in chlorophyll content, root and shoot length, and reduced lipid peroxidation.

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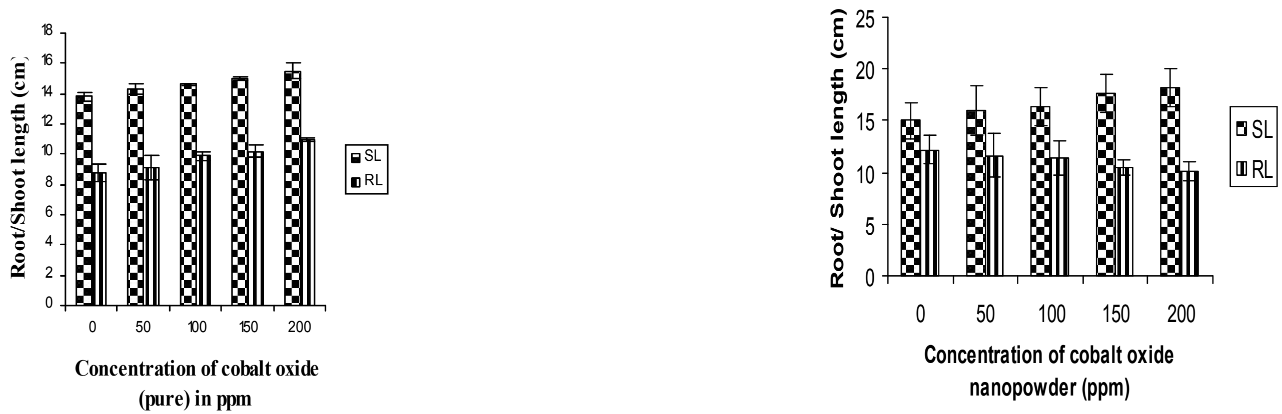

Fig. 1. Root and shoot length ± S.E of *H. vulgare* grown in sand media containing cobalt oxide (pure) and nano cobalt oxide

Fig. 2. Effect of binary treatments of cobaltous chloride hexahydrate ($CoCl_2.6H_2O$) and bleaching powder (NaOCl) on root/shoot length ± S.E of *H. vulgare* seedlings grown in sand medium.

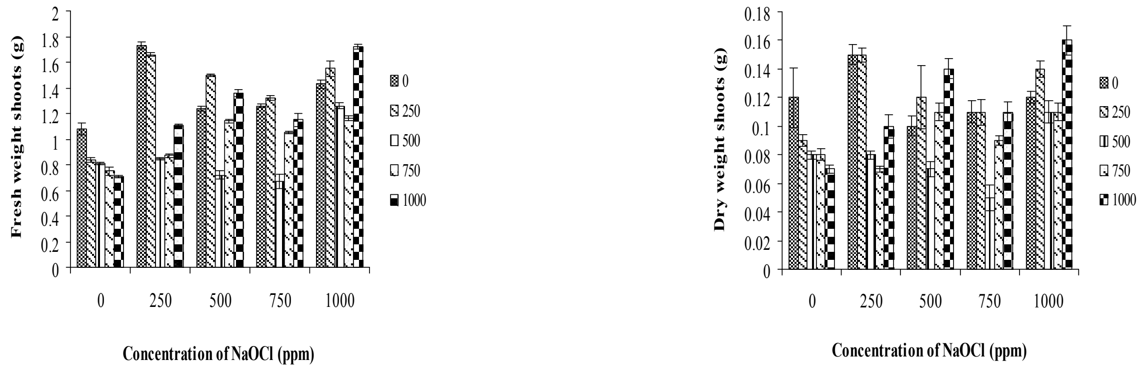

Fig. 3. Effect of binary treatments of cobaltous chloride hexahydrate ($CoCl_2$. $6H_2O$) and bleaching powder (NaOCl) on fresh/ dry weight (shoots ± S.E) of *H. vulgare* seedlings grown in sand medium

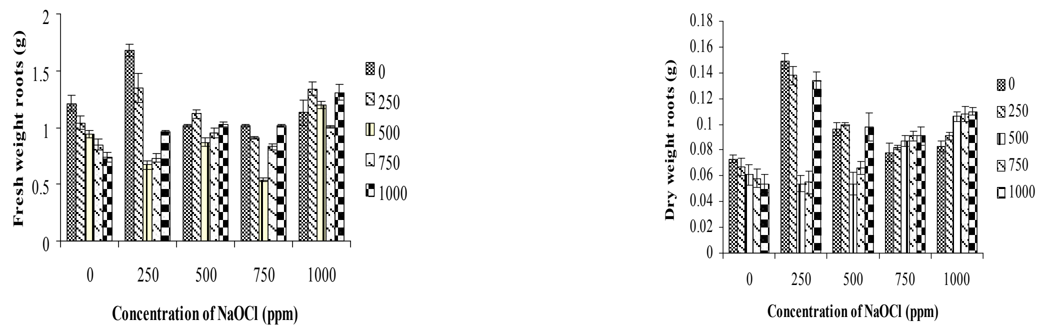

Fig. 4. Effect of binary treatments of cobaltous chloride hexahydrate ($CoCl_2.6H_2O$) and bleaching powder (NaOCl) on fresh/ dry weight (roots ± S.E) of *H. vulgare* seedlings grown in sand medium

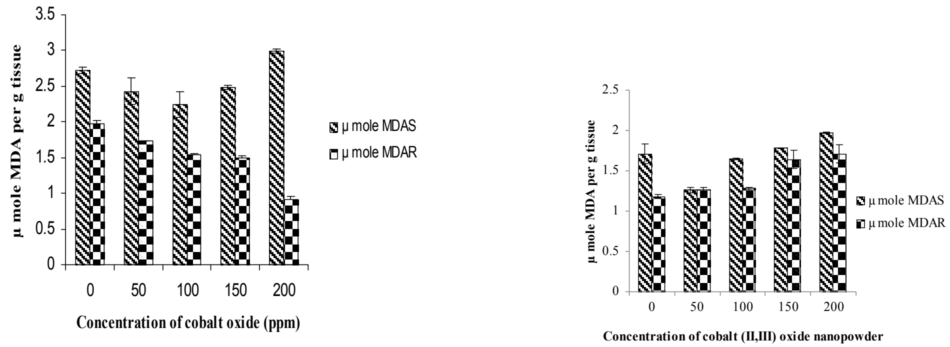

Fig. 5. MDA content (shoots, roots ± S.E.) of *H. vulgare* seedlings grown in sand media containing cobalt oxide (pure) and cobalt oxide nanopowder.

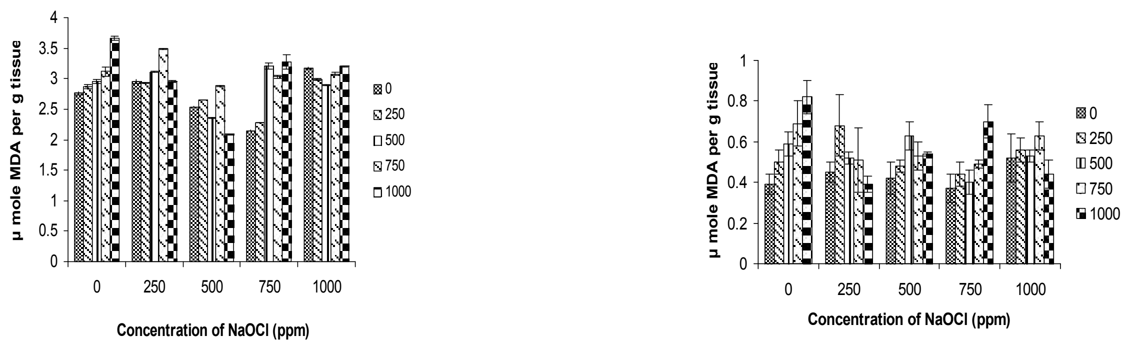

Fig. 6. Content of lipid peroxidation after binary treatments of cobaltous chloride hexahydrate and sodium hypochlorite (NaOCl) on shoots and roots of *H. vulgare* seedlings grown in sand medium

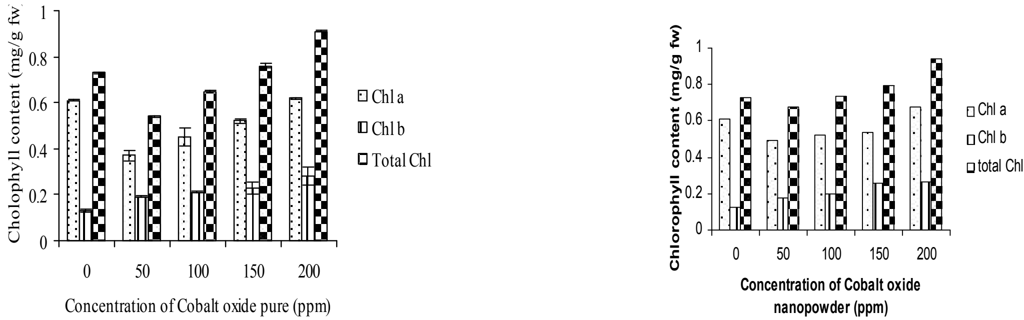

Fig. 7. Chlorophyll content (chl a, chl b and total chl ± S.E.) of *H. vulgare* grown in sand media containing cobalt oxide (pure) and cobalt oxide nanopowder

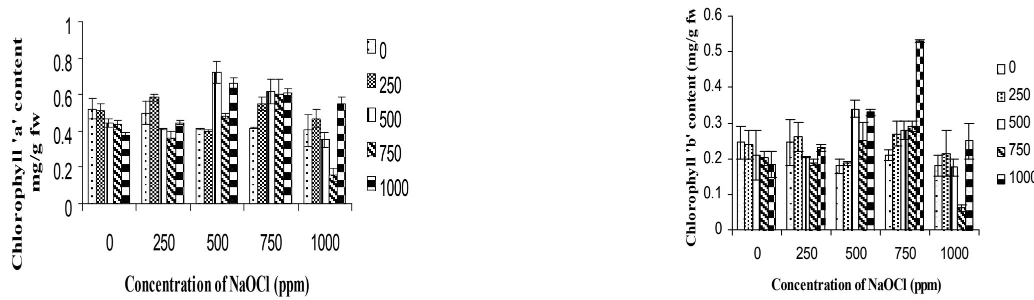

Fig. 8. Chlorophyll 'a' and 'b'content ± S.E. of *H. vulgare* grown in sand media containing binary combinations of cobaltous chloride hexahydrate and sodium hypochlorite.

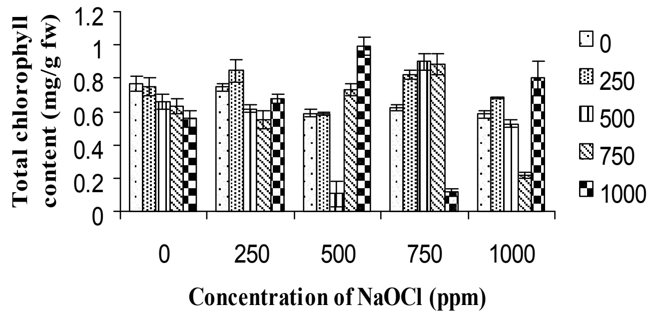

Fig. 9. Total chlorophyll content ± S.E. of *H. vulgare* grown in sand media containing binary combinations of cobaltous chloride hexahydrate and sodium hypochlorite

Table: 1 Effect of Binary treatments of cobaltous chloride hexahydrate and sodium hypochlorite (NaOCl) on (shoot, root length ± S.E) of *H. vulgare* grown in sand medium.

Conc. of Co (ppm)	**Sodium hypochlorite concentration (ppm)**									
	0		**250**		**500**		**750**		**1000**	
	Shoot Length (cm)	**Root Length (cm)**	**Shoot Length (cm)**	**Root Length (cm)**	**Shoot Length (cm)**	**Root Length (cm)**	**Shoot Length (cm)**	**Root Length (cm)**	**Shoot Length (cm)**	**Root Length (cm)**
0	11.2 ± 0.5	8.7 ± 0.79	12.2 ± 0.4	8.9 ± 0.39	9.9 ± 0.4	9.0 ± 0.6	10.7 ± 0.6	8.8 ± 0.4	11 ± 0.9	9.9 ± 0.52
250	10.9 ± 0.5	8.6 ± 0.43	11.1 ± 0.6	8.3 ± 0.42	10.6 ± 0.7	8.1 ± 0.46	12.2 ± 0.8	9.1 ± 0.45	11.2 ± 0.7	10.7 ± 0.48
500	9.4 ± 1.1	8.4 ± 0.38	12.3 ± 0.6	9 ± 0.37	10.1 ± 0.7	8.7 ± 0.26	10.8 ± 0.5	9.6 ± 0.26	10.9 ± 0.8	9.6 ± 0.51
750	7.2 ± 1.1	5.7 ± 0.79	11.3 ± 0.6	9.5 ± 0.47	10.7 ± 0.3	9.6 ± 0.25	9.4 ± 0.9	8.4 ± 0.28	12.1 ± 0.4	9.7 ± 0.42
1000	7 ± 1.1	5.25 ± 0.62	9.8 ± 0.3	8.9 ± 0.3	9.6 ± 0.4	8.7 ± 0.66	11.1 ± 0.6	9.8 ± 0.48	11.1 ± 0.4	8.2 ± 0.43

F- ratios for shoots; 4.08*, 9.21*, 2.39

F- ratios for roots; 3.47, 15.97*, 4.17

***P= .05**

Table: 2 Effect of binary treatments of cobaltous chloride hexahydrate and sodium hypochlorite (NaOCl) on fresh, dry weight (shoots ± S.E) of *H. vulgare* grown in sand medium

Conc. of Co (ppm)	**Sodium hypochlorite concentration (ppm)**									
	0		**250**		**500**		**750**		**1000**	
	Fresh wt (g)	**Dry wt (g)**	**Fresh wt (g)**	**Dry wt (g)**	**Fresh wt (g)**	**Dry wt (g)**	**Fresh wt (g)**	**Dry wt (g)**	**Fresh wt (g)**	**Dry wt (g)**
0	1.08 ± 0.05	0.12± 0.021	1.73 ± 0.03	0.15± 0.007	1.24 ± 0.02	0.1± 0.007	1.26 ± 0.01	0.11± 0.008	1.43 ± 0.03	0.12± 0.004
250	0.84± 0.02	0.09± 0.004	1.66 ± 0.01	0.15±0.005	1.5 ± 0.01	0.12± 0.022	1.32 ± 0.02	0.11± 0.009	1.55 ± 0.06	0.14± 0.006
500	0.81±0.01	0.08± 0.003	0.85 ± 0.01	0.08±0.003	0.72 ± 0.03	0.07± 0.005	0.67 ± 0.06	0.05± 0.009	1.26 ± 0.02	0.11± 0.008
750	0.75 ± 0.03	0.08± 0.004	0.87 ± 0.01	0.07±0.002	1.14 ± 0.01	0.11± 0.006	1.05 ± 0.01	0.09± 0.003	1.16 ± 0.02	0.11± 0.006
1000	0.71 ± 0.01	0.07± 0.003	1.11 ± 0.01	0.1±0.008	1.36 ± 0.03	0.14± 0.007	1.15 ± 0.05	0.11± 0.007	1.72 ± 0.02	0.16± 0.010

F- ratios for shoots (fresh weight) ; 990.59*, 915.10*, 153.83*

F- ratios for shoots (dry weight) ; 81.48*, 48.05*, 16.36*

***P= .05**

Table: 3 Effect of binary treatments of cobaltous chloride hexahydrate and sodium hypochlorite (NaOCl) on fresh/ dry weight (roots ± S.E) of *H. vulgare* grown in sand medium

Conc. of Co (ppm)	**Sodium hypochlorite concentration (ppm)**									
	0		**250**		**500**		**750**		**1000**	
	Fresh wt (g)	**Dry wt (g)**	**Fresh wt (g)**	**Dry wt (g)**	**Fresh wt (g)**	**Dry wt (g)**	**Fresh wt (g)**	**Dry wt (g)**	**Fresh wt (g)**	**Dry wt (g)**
0	1.21±0.07	0.073 ± 0.003	1.68 ± 0.05	0.149 ± 0.005	1.02 ±0.01	0.096 ± 0.005	1.02 ± 0.01	0.078 ±0.007	1.13 ±0.11	0.083 ± 0.004
250	1.04 ± 0.06	0.067 ± 0.007	1.35 ± 0.13	0.138 ± 0.007	1.12 ±0.04	0.1 ± 0.001	0.91 ±0.01	0.082 ±0.002	1.34 ±0.06	0.091 ± 0.003
500	0.944 ±0.03	0.061 ± 0.008	0.67 ± 0.04	0.054 ± 0.006	0.87 ±0.04	0.054 ± 0.009	0.54 ±0.02	0.087 ±0.004	1.2 ± 0.03	0.106 ± 0.004
750	0.85 ± 0.05	0.058 ± 0.007	0.73 ± 0.04	0.055 ± 0.009	0.95 ±0.04	0.066 ± 0.005	0.83 ± 0.03	0.091 ±0.004	1.01± 0.01	0.108 ± 0.006
1000	0.74 ± 0.04	0.054 ± 0.007	0.96 ± 0.01	0.134 ± 0.007	1.03 ±0.02	0.098 ± 0.011	1.02 ± 0.01	0.091 ±0.007	1.31 ±0.07	0.11 ± 0.003

F- ratios for roots (fresh weight) ; 162.88*, 97.04*, 44.21*
F- ratios for roots (dry weight) ; 71.07*, 31.17*, 64.99*
***P= .05**

Table: 4 Content of lipid peroxidation and chlorophyll content after cobalt oxide (pure) and nanopowder on shoots of *H. vulgare* grown in sand medium

Concentration of cobalt oxide and nanopowder(ppm)	**μ mole MDA ± S.E**		**μ mole MDA ± S.E**		**Chlorophyll Content (mg/g fw)**					
					Chl 'a'		**Chl 'b'**		**Total Chl**	
	ROOTS (bulk treatment)	**SHOOTS (bulk treatment)**	**SHOOTS (nanopowder treatment)**	**ROOTS (nanopowder treatment)**	**(bulk treatment)**	**(nanopowder treatment)**	**(bulk treatment)**	**(nanopowder treatment)**	**(bulk treatment)**	**(nanopowder treatment)**
0	2.72 ± 0.04	1.98 ± 0.037	1.71 ± 0.12	1.18 ±0.023	0.61± 0.004	0.61± 0.011	0.13 ± 0.004	0.124 ± 0.006	0.73 ± 0.003	0.73 ± 0.02
50	2.43 ± 0.18	1.74 ± 0.006	1.26 ± 0.04	1.26 ±0.035	0.37 ± 0.021	0.496 ±0.015	0.19 ± 0.003	0.179 ± 0.003	0.54 ± 0.003	0.675 ± 0.025
100	2.24 ± 0.18	1.54 ± 0.013	1.65 ± 0.12	1.28 ±0.012	0.45 ± 0.040	0.524 ±0.021	0.21 ± 0.004	0.198 ± 0.003	0.65 ± 0.005	0.734 ± 0.065
150	2.48 ± 0.03	1.5 ± 0.029	1.78 ± 0.06	1.64 ± 0.11	0.52 ± 0.010	0.54 ± 0.045	0.23 ± 0.025	0.259 ± 0.030	0.76 ± 0.010	0.796 ±0.0151
200	2.99 ± 0.03	0.907 ±0.052	1.97 ± 0.06	1.71 ± 0.12	0.62 ± 0.003	0.677 ±0.025	0.28 ± 0.037	0.265 ± 0.015	0.91 ± 0.003	0.941 ± 0.02

F- ratios for MDA (Shoots /Roots) Bulk cobalt ; 17.77*,466.81*
F- ratios for MDA (Shoots /Roots) Nano cobalt; 5.17, 4.74
F- ratios for Chl 'a', Chl 'b' and Total Chl Bulk cobalt ; 78.25*, 21.72*, 1805.92*
F- ratios for Chl 'a', Chl 'b' and Total Chl Nano cobalt; 22.72*, 44.11*, 26.54*
***P= .05**

Table: 5 Content of lipid peroxidation after binary treatments of cobaltous chloride hexahydrate and sodium hypochlorite (NaOCl) on of (shoots, roots ± S.E) of *H. vulgare* grown in sand medium.

Conc. of Co (ppm)	**Sodium hypochlorite Conc. (ppm)**									
	0		**250**		**500**		**750**		**1000**	
	Shoot MDA content	**Root MDA content**	**Shoot MDA content**	**Root MDA content**	**Shoot MDA content**	**Root MDA content**	**Shoot MDA content**	**Root MDA content**	**Shoot MDA content**	**Root MDA content**
0	2.76 ± 0.03	0.39 ± 0.05	2.96 ± 0.032	0.45 ± 0.05	2.54 ± 0.012	0.42 ±0.08	2.15 ± 0.006	0.37 ±0.07	3.17 ± 0.01	0.52 ± 0.12
250	2.87 ± 0.02	0.50 ± 0.06	2.93 ± 0.006	0.68 ± 0.15	2.65 ± 0.006	0.48 ±0.03	2.28 ± 0.006	0.44 ±0.06	2.99 ± 0.01	0.56 ± 0.06
500	2.96 ± 0.03	0.59 ± 0.06	3.11 ± 0.006	0.52 ± 0.03	2.36 ± 0.006	0.63 ±0.07	3.21 ± 0.05	0.40 ±0.06	2.89 ± 0.01	0.53 ± 0.03
750	3.12 ± 0.08	0.69 ± 0.11	3.49 ± 0.005	0.51 ± 0.16	2.88 ± 0.006	0.53 ±0.07	3.03 ± 0.02	0.49 ±0.02	3.08 ± 0.03	0.63 ±0.07
1000	3.66 ± 0.04	0.82 ± 0.08	2.96 ± 0.017	0.39 ± 0.04	2.09 ± 0.006	0.54 ±0.01	3.27 ± 0.01	0.70 ±0.08	3.2 ± 0.006	0.44 ± 0.07

F- ratios for MDA (Shoots) ; 399.79*, 850.19*, 262.63*
F- ratios for MDA (Shoots) ; 8.37*, 4.79*, 6.22*
***P= .05**

Table: 6 Chlorophyll content after binary treatments of cobaltous chloride hexahydrate and sodium hypochlorite on *H. vulgare* grown in sand medium

Co Concentration (ppm)	**Sodium hypochlorite Conc. (ppm)**														
	0			**250**			**500**			**750**			**1000**		
	Chl ‘a’	**Chl ‘b’**	**total Chl**	**Chl ‘a’**	**Chl ‘b’**	**total Chl**	**Chl ‘a’**	**Chl ‘b’**	**total Chl**	**Chl ‘a’**	**Chl ‘b’**	**total Chl**	**Chl ‘a’**	**Chl ‘b’**	**total Chl**
0	0.522 ± 0.057	0.245 ± 0.045	0.766 ± 0.045	0.499 ± 0.065	0.245 ± 0.065	0.745 ± 0.025	0.411 ± 0.004	0.18 ± 0.020	0.59 ± 0.021	0.417 ± 0.004	0.21 ± 0.015	0.623 ± 0.015	0.403 ± 0.086	0.18 ± 0.030	0.584 ± 0.025
250	0.51 ± 0.041	0.24 ± 0.040	0.749 ± 0.050	0.583 ± 0.020	0.26 ± 0.040	0.845 ± 0.065	0.402 ± 0.004	0.186 ± 0.05	0.589 ± 0.010	0.549 ± 0.041	0.27 ± 0.035	0.822 ± 0.025	0.468 ± 0.049	0.215 ± 0.065	0.683 ± 0.005
500	0.444 ± 0.020	0.21 ± 0.070	0.655 ± 0.045	0.413 ± 0.004	0.204 ±0.002	0.617 ± 0.025	0.724 ± 0.061	0.34 ± 0.025	1.064 ± 0.075	0.618 ± 0.069	0.28 ± 0.025	0.901 ± 0.050	0.352 ± 0.041	0.175 ± 0.025	0.527 ± 0.025
750	0.433 ± 0.029	0.201 ± 0.020	0.635 ± 0.045	0.359 ± 0.041	0.189 ± 0.010	0.547 ± 0.055	0.482 ± 0.016	0.25 ± 0.050	0.734 ± 0.035	0.599 ± 0.082	0.29 ± 0.015	0.884 ± 0.065	0.156 ± 0.037	0.061 ± 0.010	0.217 ± 0.015
1000	0.376 ± 0.012	0.184 ± 0.035	0.559 ± 0.050	0.445 ± 0.012	0.231 ± 0.010	0.677 ± 0.025	0.662 ± 0.033	0.33 ± 0.010	0.994 ± 0.055	0.611 ± 0.024	0.53 ± 0.005	1.135 ± 0.025	0.549 ± 0.041	0.25 ± 0.050	0.799 ± 0.1

F- ratios for Chl ‘a’, for Binary treatments; 13.88*, 25.84*
F- ratios for Chl ‘b’ for Binary treatments; 20.82*, 32.89*, 11.52*
F- ratios for Total Chl for Binary treatments; 56.05*,106.98*, 42.43*
***P= .05**

Table: 7 Multiple Regression interaction models for shoot and root length/ fresh and dry weight of *H. vulgare* in binary combination of cobaltous chloride hexahydrate and sodium hypochlorite (NaOCl)

Seedling parameter (cm)	Multiple regression equation	r	β-regression coefficients		
			Co	NaOCl	Interaction Co×NaOCl
Shoot length	Y= 11.69- 0.0038 Co - 0.0008 NaOCl + 5×10^{-06} interaction	0.720*	- 1.02	-0.22	0.99
Root length	Y= 8.66 -0.0017 Co + 0.0011 NaOCl + 2×10^{-06} interaction	0.673*	- 0.53	0.33	0.41
Shoot FW	Y= 1.23-0.0005Co+0.0001NaOCl + 6×10^{-07}interaction	0.58*	- 0.61	0.13	0.50
Shoot DW	Y= 8.66-0.0017Co+0.0011NaOCl+ 2×10^{-06} interaction	0.673*	- 0.53	0.33	0.41
Root FW	Y= 1.27- 0.0002 Co – 0.0006NaOCl+7×10^{-07}interaction	0.56*	-0.35	-0.90	0.79
Root DW	Y= 0.095- 0.00 Co – 0.00 NaOCl + 6×10^{-08} interaction	0.47	- 0.12	-0.50	0.65

***P= .05**

339 Table: 8 Multiple Regression models for lipid peroxidation in shoots and roots (µ mole/ g tissue) and chlorophyll content of *H. vulgare* in binary combination
340 of cobaltous chloride hexahydrate (ppm) and sodium hypochlorite (ppm)

Seedling parameter (cm)	Multiple regression equation	r	β-regression coefficients		
			Co	NaOCl	Co× NaOCl interaction
Shoot LP	Y= 2.71- 0.0005 Co – 2E-05NaOCl - 2×10^{-7} interaction	0.40	0.48	-0.016	-0.16
Root LP	Y= 0.44- 0.0002 Co + 4E-05NaOCl - 2×10^{-7} interaction	0.52*	0.76	0.14	0.53
Chl 'a'	Y= 5.35-0.0013 Co – 0.0009 NaOCl + 2×10^{-6} interaction	0.27	-0.44	-0.28	0.56
Chl 'b'	Y= 2.48-0.0008 Co – 0.0002 NaOCl +2×10^{-6} interaction	0.37	-0.35	-0.11	0.58
Total Chl	Y= 7.83 -0.0021 Co -0.0011 NaOCl + 4×10^{-6} interaction	0.31	-0.40	-0.21	0.59

341 ***P= .05**