Original Research Article

Germination and growth of barley seedlings in sand cultures amended with macro- and nano-particles of cobalt (II, III) oxide and cobaltous chloride

ABSTRACT

A study was undertaken to determine the comparative effects of Co (II, III) oxide (Co_3O_4) macro and nanoparticles, $CoCl_2.6H_2O$ (solution) on germination, growth and some biochemical parameters of *Hordeum vulgare* L. seedlings. Macro and nano cobalt were added to the sand medium at five levels (0 to 200 mg kg^{-1} sand). Macro cobalt oxide was found to increase the growth of both shoots and roots at concentrations upto 200 ppm. Increase in concentration of nano Co decreased the root length. Lipid peroxidation was maximum at 200 ppm for macro Co in roots. Increase in the lipid peroxidation was found in nano-cobalt treated both roots and shoots. NaOCl decreased the toxicity of $CoCl_2.6H_2O$ at all the concentrations studied. Nano and macro cobalt oxides behaved differently with respect to effect on barley seedlings. The present study also demonstrated the ameliorative effect of NaOCl against cobalt chloride toxicity in barley seedlings. NaOCl also decreased the lipid peroxidation induced by $CoCl2.6H_2O$, and increased chlorophyll content in seedlings.

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

1. INTRODUCTION

Fast pace of industrialization and irrational use of natural resources has led to metal accumulation 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 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 are required for human and animal nutrition. Cobalt is safer for consumption up to 8 mg daily, without any adverse health effects [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]. Two salts of Co are used in industry on a large scale, cobalt oxide also known as Co (II,III) oxide or $CoO.Co_2O_3$ (Co_3O_4, macro and nano scale particles which are insoluble in water) and cobalt chloride ($CoCl_2$, macroscale particles, water soluble). Nano cobalt is a recent discovery and needs to be investigated in detail. $CoCl_2$ is toxic at higher concentrations. The present work is aimed at studying the differential effects of macro and nano particles of cobalt oxide. Because of the ease of the hydration/dehydration reaction cobalt chloride helps in color change in glass industry, organic synthesis and electroplating objects, production of pigments in ceramics and as a mordant in dry cleaners. Cobalt chloride hexahydrate is a catalysts used for metal surface treatment also. The waste from these industries contains cobalt more than prescribed limit. Such industrial effluent when reached to the crop fields cause toxicity to plants. So, to remediate cobalt rich soil we have tried to use NaOCl for detoxification. NaClO converts transition metal complexes into their oxides [5]. NaClO is used in the pesticide and textile industries, and is a disinfectant, cleaner and bleach.

Nanotechnology is the engineered convergence of biology, chemistry, and informatics at nanoscale. The products of these exertions are called nanomaterials, consisting of nanoparticles (NPs), having their size smaller than 100 nm in at least one dimension. Among the latest technological innovations, nanotechnology possesses the top most position [6]. The properties of nanomaterials raise concern about their adverse effects on biological systems at cellular level. Because of their small size, NPs get incursion into the living cell membrane. In contrast to the classical macroscale particles, NPs due to their smaller size and huge surface area, may interact more expeditiously with biological systems. Metal oxide based NPs are increasingly used in applications such as opacifiers, fillers, catalysts, semiconductors, cosmetics and microelectronics etc. [7]. Therefore, 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, Ludhiana (India). Barley is generally grown as a summer crop in temperate areas, and winter crop in tropical areas (including India). It is an important cereal of India, and ranks next to wheat, maize and rice in the world.

2.2. Cobalt (II,III) oxide (Co_3O_4) and nano cobalt (II,III) oxide treatments

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

2.3. Cobaltous chloride hexahydrate and NaOCl treatments

Seeds of barley were grown in sand containing various binary combinations of $CoCl_2.6H_2O$ and NaOCl (Table 1). Growth and biochemical parameters were studied for any modulation in $CoCl_2.6H_2O$ toxicity to seedlings.

2.4. 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 trice with deionised water 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 the 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 [8], and chlorophyll contents were measured by method given by Arnon [9].

2.5. Statistical analysis

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

3. Results

3.1. Growth characteristics

3.1.1. Co (II, III) oxide macro and nanopowder treatment

Seedlings cultured in sand medium containing cobalt oxide (macro) showed increase in root and shoot length with increase in Co concentration (0, 50, 100, 150 and 200 ppm). Further it was observed that treatment of cobalt (II, III) oxide nanopowder significantly increased shoot length but decreased root length (Table 2).

3.1.2. Cobaltous chloride hexahydrate treatments in binary combinations with NaOCl

A significant decrease in shoot, root length and fresh, dry weight 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 the later was elucidated as was evidenced by masking the toxic effects of cobalt (Tables: 3,4, and 5).Two – way ANOVA summary described the statistically significant difference among shoot and root length on Co treatment and the NaOCl treatment. Multiple regression models showed that Co has negative effect on shoot and root length, while NaOCl has a positive effect. Interaction between Co and NaClO was found to be statistically significant. Fresh and dry weight of shoots also showed significant differences (Table 6).

3.2. Lipid peroxidation

Variations in shoot and root MDA content of *H. vulgare* grown in sand media containing cobalt oxide (macro) and cobalt oxide nanopowder are presented in (Table: 7). The MDA content of *H. vulgare* treated with macro cobalt was increased significantly for shoots, while decreasing trend was found in case of roots. One way ANOVA showed significant increase in MDA content in both roots and shoots treated with macro and nano cobalt. Two-way ANOVA revealed that there are significant differences among MDA contents of both shoots and roots given binary treatments (Table: 8). The interaction between Co and NaOCl was found to be negative for both shoots and roots (Table: 9).

3.3. Chlorophyll estimation

The effect of cobalt oxide of macro and nanoparticles and binary combination with NaOCl on chlorophyll content (a, b and total chl) is presented in table 10. ANOVA depicted statistical significant difference among different treatments on chl a, chl b, total chlorophyll (Table: 11). Multiple regression model analysis showed positive effect of NaOCl on Chl 'a' which as a result compensated the negative effect of $CoCl_2.6H_2O$. Co and NaOCl, significantly increased the chl 'b'content while in case of total chlorophyll, Co showed negative while NaOCl showed positive β- regression coefficient.

4. Discussion

Heavy metals may cause major occupational and environmental hazards due to their non-biodegradable nature and long biological half life period [10]. 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. [11]. Such activities result in the transportation of metal ions via air and water, and ultimately bind to soil and sediments. Cobalt is relatively a rare magnetic element with properties similar to those of iron and nickel. Cobalt occurs in nature primarily as arsenides, oxides, and sulphides. Most of the production of cobalt involves the metallic form used in the formation of cobalt superalloys [12]. The distribution of cobalt in plants is entirely species specific.

A significant increase in shoot length was observed in 7 days old seedlings treated with cobalt oxide (macro). 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 the concentration of cobalt oxide (nanopowder), the shoot length increased in a dose dependent manner while root growth showed a 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 effect by transforming cobalt into its oxide form. Several mechanisms involved include exclusion, inclusion (i.e. sequestration and compartmentalization of metal ions in organelles) and chelation binding. 750 ppm of NaOCl concentration increases shoot length of 1000 ppm Co treated seedlings by 58.57% and root length by 86.67%. The reaction of cobaltous chloride hexahydrate with sodium hypochlorite is given below:

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

Reason for such an observation may be attributed to the fact that NaOCl oxidises the more toxic $CoCl_2$ to less toxic Co_3O_4. At concentrations where NaOCl is absent altogether, metal caused toxicity resulted in reduction of shoot length. Lowest shoot length was observed at concentrations where cobalt is in maximum and NaOCl is in minimum amounts. 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. 500 ppm of NaOCl increases shoot fresh weight of 1000 ppm Co treated seedlings by 91.5%.

Lipid peroxidation was found to be the maximum for roots at a concentration of 200 mg $kg^{-1}$of Co_3O_4. The reason for such a trend can be attributed to increased production of ROS which induce membrane destabilization resulting in the formation of peroxides, as was reported by Mead *et al* [13]. On the other hand, cobalt oxide inhibited the lipid peroxidation by decreasing the MDA content in roots. The values obtained for the same were statistically different as compared to the control. The MDA content for both shoots and roots showed an increasing trend with increase in concentration (0, 50, 100, 150 and 200 mg kg^{-1}) 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 mg kg^{-1}, while other concentrations showed increased amount of lipid peroxidation. 750 ppm of NaOCl decreases lipid peroxidation of 1000 ppm Co treated shoots and roots upto 10.65% and 14.63% respectively.

It was found that chlorophyll 'a', 'b' and total chlorophyll showed maximum value at 200 mg kg^{-1}. 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 nano cobalt modulated chlorophyll synthesis. 500 ppm of NaOCl concentration increases chl 'a', chl 'b' and total chl content of 1000 ppm Co treated leaves by 76.06%, 79.35% and 77.81% respectively.

5. Conclusion

Cobalt oxide (Co_3O_4) macro 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 both macro 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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Table 1.$CoCl_2.6H_2O$ treatment (given in numerator) in binary combinations with NaOCl treatment (given in denominator)

Sodium hypochlorite (mg kg^{-1})		**Concentration (mg kg^{-1}) of $CoCl_2.6H_2O$ in medium**				
		0	**250**	**500**	**750**	**1000**
Sodium hypochlorite in sand medium	**0**	0/0	0/250	0/500	0/750	0/1000
	250	250/0	250/250	250/500	250/750	250/1000
	500	500/0	500/250	500/500	500/750	500/1000
	750	750/0	750/250	750/500	750/750	750/1000
	1000	1000/0	1000/250	1000/500	1000/750	1000/1000

Table: 2 Root and shoot length (cm; mean ± S.E.) of 7- days old *H. vulgare* seedlings grown in sand medium containing cobalt (II,III) oxide (macro) and nano particles.

Concentration of Co_3O_4 (ppm)	**Macro- particles**		**Nano- particles**	
	Root length (cm)	**Shoot length (cm)**	**Root length (cm)**	**Shoot length (cm)**
0	8.8 ± 0.6	13.8 ± 0.3	12.2 ± 1.4	15 ± 1.7
50	9.1 ± 0.8	14.3 ± 0.3	11.6 ± 2.1	16 ± 2.4
100	9.9 ± 0.3	14.6 ± 0.1	11.4 ± 1.7	16.4 ± 1.8
150	10.2 ± 0.4	15 ± 0.1	10.5 ± 0.67	17.7 ± 1.8
200	11 ± 0.1	15.5 ± 0.5	10.1 ± 0.85	18.2 ± 1.9
F- ratio (*P= 0.5)	3.69*	6.76*	3.13*	0.852

Table: 3 Effect of binary treatments of $CoCl_2.6H_2O$ and NaOCl on (shoot, root length ± S.E) of *H. vulgare* grown in sand medium.

Conc. of Co (mg kg^{-1})	**Sodium hypochlorite concentration (mg kg^{-1})**									
	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* ($CoCl_2.6H_2O$) , 9.21* (NaOCl), 2.39 ($CoCl_2.6H_2O$* NaOCl)

F- ratios for roots; 3.47 ($CoCl_2.6H_2O$), 15.97* (NaOCl) , 4.17* ($CoCl_2.6H_2O$* NaOCl)

***P= .05**

Table: 4 Effect of binary treatments of $CoCl_2.6H_2O$ and NaOCl on fresh, dry weight (shoots ± S.E) of *H. vulgare* grown in sand medium

Conc. of Co (mg kg^{-1})	**Sodium hypochlorite concentration (mg kg^{-1})**									
	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* ($CoCl_2.6H_2O$), 915.10*(NaOCl), 153.83* ($CoCl_2.6H_2O$* NaOCl)

F- ratios for shoots (dry weight) ; 81.48* ($CoCl_2.6H_2O$), 48.05* (NaOCl), 16.36* ($CoCl_2.6H_2O$* NaOCl)

***P= .05**

Table: 5 Effect of binary treatments of cobaltous $CoCl_2.6H_2O$ and NaOCl on fresh/ dry weight (roots ± S.E) of *H. vulgare* grown in sand medium

Conc. of Co (mg kg^{-1})	**Sodium hypochlorite concentration (mg kg^{-1})**									
	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	1.3 4 ±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* ($CoCl_2.6H_2O$), 97.04* (NaOCl), , 44.21* ($CoCl_2.6H_2O$* NaOCl)
F- ratios for roots (dry weight) ; 71.07* ($CoCl_2.6H_2O$) , 31.17* (NaOCl), 64.99* ($CoCl_2.6H_2O$* NaOCl)
***P= .05**

Table: 6 Multiple Regression interaction models for shoot and root length/ fresh and dry weight of *H. vulgare* in binary combination of $CoCl_2.6H_2O$ and NaOCl.

Seedling parameter	Multiple regression equation	r	β-regression coefficients		
			Co	NaOCl	Co× NaOCl
Shoot length (cm)	Y= 11.69- 0.0038 Co - 0.0008 NaOCl + 5×10^{-06} Co*NaOCl	0.720*	- 1.02	-0.22	0.99
Root length (cm)	Y= 8.66 -0.0017 Co + 0.0011 NaOCl + 2×10^{-06} Co*NaOCl	0.673*	- 0.53	0.33	0.41
Shoot FW (g)	Y= 1.23-0.0005Co+0.0001NaOCl + 6×10^{-07} Co*NaOCl	0.58*	- 0.61	0.13	0.50
Shoot DW (g)	Y= 8.66-0.0017Co+0.0011NaOCl+ 2×10^{-06} Co*NaOCl	0.673*	- 0.53	0.33	0.41
Root FW (g)	Y= 1.27- 0.0002 Co – 0.0006NaOCl+7×10^{-07} Co*NaOCl	0.56*	-0.35	-0.90	0.79
Root DW (g)	Y= 0.095- 0.00 Co – 0.00 NaOCl + 6×10^{-08} Co*NaOCl	0.47	- 0.12	-0.50	0.65
***P= .05**					

Table: 7 Content of lipid peroxidation and chlorophyll content after cobalt oxide (Co_3O_4) macro and nano particles on shoots of *H. vulgare* grown in sand medium

Conc. Co_3O_4 (ppm)	µ mole MDA ± S.E		µ mole MDA ± S.E	
	ROOTS (macro- particles)	**SHOOTS (macro- particle)**	**SHOOTS (nano- particle)**	**ROOTS (nano- particle)**
0	2.72 ± 0.04	1.98 ± 0.037	1.71 ± 0.12	1.18 ±0.023
50	2.43 ± 0.18	1.74 ± 0.006	1.26 ± 0.04	1.26 ±0.035
100	2.24 ± 0.18	1.54 ± 0.013	1.65 ± 0.12	1.28 ±0.012
150	2.48 ± 0.03	1.5 ± 0.029	1.78 ± 0.06	1.64 ± 0.11
200	2.99 ± 0.03	0.907 ±0.052	1.97 ± 0.06	1.71 ± 0.12
F- ratio (*P= .05)	466.81*	17.77*	5.17	4.74

Table: 8 Content of lipid peroxidation after binary treatments of $CoCl_2.6H_2O$ and NaOCl on (shoots, roots ± S.E) of *H. vulgare* grown in sand medium.

Conc. of Co (mg kg^{-1})	**Sodium hypochlorite Conc. (mg kg^{-1})**									
	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* ($CoCl_2.6H_2O$), 850.19* (NaOCl), 262.63* ($CoCl_2.6H_2O$ * NaOCl)
F- ratios for MDA (Roots) ; 8.37* ($CoCl_2.6H_2O$), 4.79* (NaOCl) , 6.22* ($CoCl_2.6H_2O$ * NaOCl)
***P= .05**

Table: 9 Multiple Regression models for lipid peroxidation in shoots and roots (µ mole/ g tissue) and chlorophyll content of *H. vulgare* in binary combination of $CoCl_2.6H_2O$ and NaOCl.

Seedling parameter	Multiple regression equation	r	β-regression coefficients		
			Co	**NaOCl**	**Co× NaOCl**
Shoot LP (µmoles g $^{-1}$ fw)	Y= 2.71- 0.0005 Co – 2E-05 NaOCl - 2×10^{-7} Co*NaOCl	0.40	0.48	-0.016	-0.16
Root LP (µmoles g $^{-1}$ fw)	Y= 0.44- 0.0002 Co + 4E-05 NaOCl - 2×10^{-7} Co*NaOCl	0.52*	0.76	0.14	0.53
Chl 'a' (mg/g fw)	Y= 5.35-0.0013 Co – 0.0009 NaOCl + 2×10^{-6} Co*NaOCl	0.27	-0.44	-0.28	0.56
Chl 'b'(mg/g fw)	Y= 2.48-0.0008 Co – 0.0002 NaOCl +2×10^{-6} Co*NaOCl	0.37	-0.35	-0.11	0.58
Total Chl (mg/g fw)	Y= 7.83 -0.0021 Co -0.0011 NaOCl + 4×10^{-6} Co*NaOCl	0.31	-0.40	-0.21	0.59

***P= .05**

Table: 10 Chlorophyll content after treatment with cobalt oxide (Co_3O_4) macro and nano particles on shoots of *H. vulgare* grown in sand medium

Conc. of Co_3O_4 (ppm)	**Chlorophyll Content (mg/g fw)**					
	Chl 'a'		**Chl 'b'**		**Total Chl**	
	(macro- particles)	**(nano- particles)**	**(macro -particles)**	**(nano- particles)**	**(macro- particles)**	**(nano- particles)**
0	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	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	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	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	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(*P= .05)	78.25*	22.72*	21.72*	44.11*	1805.92*	26.54*

Table: 11 Chlorophyll content after binary treatments of $CoCl_2.6H_2O$ and NaOCl on *H. vulgare* grown in sand medium.

Co Concentration (mg kg^{-1})	**Sodium hypochlorite Conc. (mg kg^{-1})**														
	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* ($CoCl_2.6H_2O$), 25.84* (NaOCl) , 13.24* ($CoCl_2.6H_2O$ * NaOCl)
F- ratios for Chl 'b' for Binary treatments; 20.82* ($CoCl_2.6H_2O$) , 32.89* (NaOCl), 11.52* ($CoCl_2.6H_2O$ * NaOCl)
F- ratios for Total Chl for Binary treatments; 56.05* ($CoCl_2.6H_2O$),106.98* (NaOCl), 42.43* ($CoCl_2.6H_2O$ * NaOCl);***P= .05**