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Evaluation of Fertilizer Industry Effluent Toxicity in Seed Germination, Growth and Metabolism of Barley (*Hordeum vulgare L.)* Plants

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Abstract : This study was carried out to investigate the effect of fertilizer industry effluent in relation to growth and metabolism of barley plants. Seed germination was found to be decreased by increasing concentrations of effluent. Plant growth stimulated at lower concentrations of effluent in barley plants, while inhibited at higher concentrations of effluent . Increasing concentrations of effluent caused reduction in photosynthetic pigments, sugar and protein concentrations and total amylase activity in barley plants. Yield and catalase enzyme showed variable results at increasing concentrations of effluent.

Keywords: Fertilizer industry effluent, Barley, Growth, Pigments, Sugar, Protein, Amylase, Catalase and Peroxidase.

Introduction: Pollution is one of the biggest challenges faced by mankind and all other living beings. Ecosystems around the world are getting negatively impacted due to huge amount of pollutants released into our air, soil and water bodies. Rapid industrial growth over the last two centuries has been the main contributor to alarming levels of pollution observed in various parts of the world. Although industries are important for economic growth, unchecked and unregulated growth can have dangerous impacts on our environment and health. Pollution level both in land and water has increased significantly in places where industries are not adhering to safety standards recommended for discharge of industrial effluents into water bodies and disposal of hazardous poisonous wastes into land pits. Pollutants released by these industries are not just impacting soil, air and water in their catchment area but these negative impacts can be observed in places far away from where these industries are located. Such huge amount of discharge of untreated industrial pollutants negatively impacts soil health and marine ecosystems. Consumption of flora and fauna growing in such immensely polluted soil and water can cause fatal diseases. To counter this growing menace of industrial pollution, it is important that industrial waste should be properly treated before it is released into our environment.

Materials and Methods: Barley (*hordeum vulgare* L.) seeds were used as test material for the petridish experiment. Four concentrations of treated fertilizer industy effluents (25, 50, 75 and 100%) and control (glass distilled water) were taken for the study in triplicate. Petridishes were properly washed with detergents and then tap water washing followed by hydrochloric acid (HCl) washing and finally washed with deionised and glass distilled water. High quality of Whatman filter paper was used in each petridish. Twenty five seeds were

placed on filter paper in each petridish and then soaked with controlled solution and solution of various concentrations of effluents in the temperature range of 20 to 30°c. The germinating seeds and seedlings were washed with distilled water every alternate day for the prevention of contaminants and fresh solutions were applied for the maintenance of effluent concentration. Solutions of respective effluents were superimposed on the basal solutions. For germination studies, the number of seeds germinated was noted and accordingly the germination percentage was calculated. Root length and shoot length were recorded after two weeks of growth. Plants were harvested for fresh weight and dry weight yield.

The basal nutrient solution was prepared by the method of Hewitt (1966). Chlorophyll content was measured by the method of Petering et al.(1940). Protein was estimated by the method of Lowry et al.(1951). Total sugar concentrations was estimated by the methods of Dubias et al.(1956). Activity of enzyme catalase was assayed by the method of Bisht (1972), a modified method of Eular and Josephson (1927). Activity of enzyme peroxidase was measured by the modified method of Luck (1963). Amylase activity was assayed by the method of Katsuni and Frekuhara (1969).

Results and Discussion

Germination percentage was found to be decreased at increasing concentration of effluent. Germination was 96.00 in control and it decreased to 88.00, 81.33, 76.00 and 74.67% respectively. It was 8.33, 15.28, 20.83 and 22.22 % decrease at 25, 50, 75 and 100% concentration respectively than the control (Table-1). Decline in germination percentage may be due to the high osmotic pressure caused by effluent that contained high amount of salt content. Similar observations were reported (Rodger et al.1957; Bishoni and Gautam, 1991; Goel and Kulkarni ,1994; Chandrasekar et al., 1998; Kaushik et al., 2005; Nagda et al., 2006). Lower concentration of effluent caused stimulation in the shoot length and root length. Maximum stimulation was observed at 25% concentration of effluent. Shoot length was same as control at 50% and significantly decreased at 100% concentration of effluent while a non significant increase at 25% and a decrease at 75% concentration of effluent was observed (Table-1). Enhanced growth at lower concentrations of effluent might be due to the presence of growth activators in the effluent. These are in conformity with the findings of Sundaramoorthy et al. (2000), Singh et al.(2006).Total fresh weight was increased non-significantly at 25% concentration of effluent while a decrease was observed at 50 to 100% concentration of effluent. Lower concentration of effluent had stimulatory effect on growth and yield, it may be due to the presence of proper amount of nutrient in treated effluent and at higher concentrations these nutrients become toxic and that causes reduction in plant growth and yield. Similar results of increased yield at lower concentration of effluent was also observed by (Singh et al.,2002; Chandra et al., 2004; Yadav and Meenakshi , 2007; Sarvanamoorthy and Ranjitha Kumari, 2007). Total dry weight was found to be non-significantly decreased at increasing concentration of effluent (Table-1).

The supply of different concentrations of effluent in barley plants caused reduction in chlorophyll content substantially. Inhibition in chlorophyll concentration increased gradually with the increase in the concentration of effluent (Table-2). Decline in pigments may be caused by inhibition of chlorophyll

biosynthesis and it may also be due to the formation of enzymes such as chlorophyllase which is responsible for chlorophyll degradation.(Majumdar et al., 1991; Rodriquez et al., 1987).

In the present study total Sugar concentration was found to be significantly decreased at increasing doses of effluent. It was 34.78, 38.39, 55.78 and 63.78% decrease at 25, 50, 75 and 100% concentration respectively than the control (Table-3). Reduction in total sugar concentration may be due to the effluent containing various harmful characters which act as barriers for the natural process of plant system. Manonmani et al.(1992) reported that it may be due to the deranged sugar metabolism and poor translocation of starch and other metabolites to the growing axis and the other possible reason is due to the heavy metal toxicity that may inhibit the membrane transport system , which transport sugar to the phloem (Rauser,1978).

Protein concentration was found to be significantly decreased at increasing concentration of effluent. This is conformity with the study of Muthusamy and Jayabalan (2001), Ayyasamy et al.(2008). It was 2.94, 17.07, 30.83 and 38.81% decrease at 25,50, 75 and 100% concentration respectively than the control (Table-3). According to Singh et al. (2005), reduction in protein may be due to the breakdown of soluble protein or due to the increased activity of protease or other catabolic enzymes which might have activated and destroyed the protein.

The catalase activity was significantly decreased at increasing concentration of effluent. It was 54.20, 42.61, 19.05, 8.71% decrease at 25, 50, 75 and 100% concentration respectively than the control (Table-4). The peroxidase activity was significantly decreased at 25% concentration of effluent while significant increase was observed at 50, 75 and 100% concentration of effluent (Table-4) . Plants posses a number of antioxidant molecules such as peroxidase and catalase and these enzymes plays an important role in defense mechanisms of plants. An antioxidant enzyme, catalase is primary H_2O_2 scavenger in the peroxisomes and the mitochondria (Anderson et al. 1995). According to Verma and Dubey (2003) such a decrease in catalase enzyme appears to be due to a decline in enzyme synthesis or a change in the assembly of enzyme subunits (Hertwig et al., 1992). Gabara et al . 2003 stated that peroxidases are the most important part of the multiple plant defense system and are mostly synthesized in the chloroplasts. Increased activity of POD may be due to the fact that effluents hold toxic heavy metals which may cause enhancement of POD generation. According to Behera and Mishra (1982), enhancement of peroxidase may also be due to the effluents having large amounts of various cations and anions. Results indicated that the activity of amylase was decreased at increasing concentration of effluent. The maximum decrease was observed at higher concentration of effluent (Table-4). Similar decrease in amylase activity was observed by Nath et al.(2007). According to Thevenot et al.,(1992) poor germination rate and seedling growth in treatments occurs due to poor break down of starch by amylase activity.

Table 1. Effect of different concentrations of Fertilizer industry effluent on germination percentage, growth and biomass yield of barley (*Hordeum vulgare* L.) plants

S.No.	Effluent Concentration(%)	Germination (%)	Shoot Length(cm)	Root Length(cm)	Total Fresh Weight (g)	Total Dry Weight(g)

1.	Control	96.000 ±2.309	19.667 ±0.882	10.000 ±0.577	0.208 ±0.026	0.013 ±0.002
2.	25	88.000^{NS} ±6.928 (-8.33%)	20.000 ±0.577 (+1.69%)	11.667^{NS} ±0.882 (+16.67%)	0.262^{NS} ±0.028 (+25.96%)	0.012^{NS} ±0.003 (-12.03%)
3.	50	81.333^{NS} ±1.333 (-15.28%)	19.667^{a} ±0.882 (0.000%)	9.833^{NS} ±0.167 (-1.67%)	0.187^{NS} ±0.034 (-10.10%)	0.010^{NS} ±0.000 (-24.81%)
4.	75	76.000^{NS} ±8.327 (-20.83%)	18.667 ±0.882 (-5.09%)	9.667^{NS} ±0.333 (-3.33%)	0.168^{NS} ±0.012 (-19.23%)	0.0100^{NS} ±0.003 (-24.81%)
5.	100	74.667^{NS} ±2.667 (-22.22%)	16.500^{a} ±0.500 (-16.10%)	9.500^{NS} ±0.500 (-5.00%)	0.140^{NS} ±0.028 (-32.69%)	0.008^{NS} ±0.002 (-37.37%)

All values are means of triplicates ±S.E. Identical superscripts on values denote significant difference ($p<0.05$) between means of different treatments according to Duncan's multiple range test. NS=non significant. The values given in the bracket shows the percent increase or decrease as compared to control.

Table 2. Effect of different concentrations of Fertilizer industry effluent on pigment contents of barley (*Hordeum vulgare* L.)plants.

S.No.	Effluent concentration (%)	Chlorophyll a (mg/g FW)	Chlorophyll b (mg/g FW)	Total chlorophyll (mg/g FW)	Carotenoid (mg/g FW)
1.	Control	0.490^{a} ±0.008	0.226^{a} ±0.006	0.716^{a} ±0.013	0.558^{a} ±0.006
2.	25	0.480^{b} ±0.001 (-2.04%)	0.152^{ac} ±0.006 (-32.74%)	0.633^{ac} ±0.007 (-11.59%)	0.404^{abc} ±0.003 (-27.60%)
3.	50	0.479^{c} ±0.002 (-2.25%)	0.155^{ab} ±0.004 (-31.42%)	0.633^{ab} ±0.007 (-11.59%)	0.410^{ab} ±0.002 (-26.52%)
4.	75	0.368^{abc} ±0.004 (-24.90%)	0.127^{abc} ±0.004 (-43.81%)	0.496^{abc} ±0.004 (-30.73%)	0.345^{abc} ±0.002 (-38.17%)
5.	100	0.366^{abc} ±0.011 (-25.31%)	0.115^{abc} ±0.010 (-49.12%)	0.482^{abc} ±0.007 (-32.68%)	0.335^{abc} ±0.006 (-39.96%)

All values are means of triplicates ±S.E. Identical superscripts on values denote significant difference ($p<0.05$) between means of different treatments according to Duncan's multiple range test. The values given in the bracket shows the percent increase or decrease as compared to control.

Table 3. Effect of different concentrations of Fertilizer industry effluent on the concentrations of sugar and protein of barley (*Hordeum vulgare* L.)plants.

S.No.	Effluent concentration (%)	Sugar Concentration (mg/g FW)	Protein Concentration(%FW)
1.	Control	2.300^{a} ±0.076	1.904^{a} ±0.000
2.	25	1.500^{ab} ±0.058 (-34.78%)	1.848^{b} ±0.088 (-2.94%)
3.	50	1.417^{ac} ±0.073 (-38.39%)	1.579^{abc} ±0.044 (-17.07%)
4.	75	1.017^{abc} ±0.093 (-55.78%)	1.317^{abc} ±0.062 (-30.83%)
5.	100	0.833^{abc} ±0.088 (-63.78%)	1.165^{abc} ±0.047 (-38.81%)

All values are means of triplicates ±S.E. Identical superscripts on values denote significant difference ($p<0.05$) between means of different treatments according to Duncan's multiple range test. The values given in the bracket shows the percent increase or decrease as compared to control.

Table 4. Effect of different concentrations of Fertilizer industry effluent on the activity of different enzymes in barley (*Hordeum vulgare* L.)plants.

S.No.	Effluent concentration(%)	Catalase activity (μ moles H_2O_2 decomposed/min/mg Protein)	Peroxidase activity(ΔOD/mg protein)	Amylase activity(starch hydrolyzed in mg/gm FW)
1.	Control	53.397^{a} ±0.877	1.445^{ab} ±0.009	2.800^{a} ±0.231
2.	25	24.454^{abcd} ±1.123	1.426^{a} ±0.073	2.667^{a} ±0.133

		(-54.20%)	(-1.32%)	(-4.75%)
3.	50	30.643^{abcd} ±1.125 (-42.61%)	1.705^{ab} ±0.047 (+17.99%)	2.400 ±0.231 (-14.29%)
4.	75	43.223^{abc} ±2.357 (-19.05%)	1.950^{a} ±0.099 (+34.95%)	2.000 ±0.231 (-28.57%)
5.	100	48.747^{b} ±1.765 (-8.71%)	1.637^{a} ±0.067 (+13.29%)	1.733^{ab} ±0.133 (-38.11%)

All values are means of triplicates ±S.E. Identical superscripts on values denote significant difference ($p<0.05$) between means of different treatments according to Duncan's multiple range test. The values given in the bracket shows the percent increase or decrease as compared to control.

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