© 2016 IJSRST | Volume 2 | Issue 6 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X

Effect of Sugar Industry Effluent on Germination, Growth, Yield and Metabolism in Barley(*Hordeum vulgare* L.) Plants

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Abstract: Seeds of *hordeum vulgare* were used as test material. The various concentrations of treated effluents and control were taken for the study in triplicate. It was observed that seed germination, plant growth, total fresh weight, photosynthetic pigments, sugar and protein concentrations and amylase activity was found to be significantly decreased with increase in concentrations of effluent. Total dry weight showed enhancement at 25, 50 and 75% concentrations of effluent while a decrease was observed at 100% concentration of effluent. Catalase and peroxidase enzymes showed variable results at increasing concentrations of effluent.

Keywords: Sugar Industry Effluent, Barley (*Hordeum vulgare* L.),Growth, Pigments, Sugar, Protein, Peroxidase, Catalase and Amylase.

Introduction

Pollution is the process of making the environment, land ,water and air dirty by adding harmful substances to them. Pollution causes imbalance in the environment. This imbalance is threatening by the survival of all forms of life. It is a threat to the whole world. In our country, industrial effluents are one of the major sources of water pollution as most of the industries dump their effluents in the nearby water body like ponds, lakes and rivers. As the water is one of the most important constituent of life, but due to release of these toxic compounds directly or indirectly into water, it is leading to various health problems thus affecting the food chain in a large scale. In order to avoid that, the industrial waste must be properly channelized as it can help in soil productivity. This fact in view this study was carried out to investigate the harmful effect of this effluent on the growth and metabolism of barley plants.

Materials and Methods

For this experiment seeds of *hordeum vulgare* were used as test material. Four concentrations of treated effluents (25, 50, 75 and 100%) and control (glass distilled water) were taken for the study in triplicate. 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. The basal nutrient solution was prepared by the method of Hewitt (1966).

For germination studies, the number of seeds germinated was noted and accordingly the germination percentage was calculated. For Growth of the plants , root length and shoot length were recorded after two weeks of growth and measured in cms and total fresh weight and total dry weight in gms. Plants were harvested for fresh and dry matter yield.

Chlorophyll, Protein and Sugar concentration were measured by the method of Petering et al. (1940), Lowry et al. (1951) and Dubias et al. (1956) respectively. Activities of enzymes catalase and peroxidase were assayed by the modified method of Bisht (1972) and modified method of Luck (1963) respectively. Amylase activity was assayed by the method of Katsuni and Frekuhara (1969).

Results and Discussion-

Germination Percentage: It was found to be decreased at increasing concentration of effluent. Germination in control was 96.00 and it decreased to 88.00, 86.67, 74.67 and 69.33% respectively . It was 8.33, 9.72, 22.22 and 27.78% decrease at 25, 50, 75 and 100% concentration respectively than the control (Table-1). Reduction 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).

Plants Growth: The effect of different concentrations of sugar industry effluent showed that the shoot and root length was significantly decreased at increasing concentrations of effluent. Decreased shoot length was at the rate of 11.87, 18.65, 30.51 and 38.98 % and root length was at the rate of 1.67, 3.33, 15.00 and 28.33% at 25, 50, 75 and 100% concentration respectively than the control. Total fresh weight was found to be non significantly decreased at increasing concentration of effluent and total dry weight was found to be non significantly increased at 25, 50 and 75 % concentration of effluent .The maximum increase was observed at 50% concentration of effluent as compared to control (Table-1).Whereas these were decreased by further increase in the effluent concentration. It may be due to the presence of proper amount of nutrients at lower concentration of effluent and these nutrients become toxic at higher concentration of effluent and that causes reduction in plant growth and yield. Similar results of increased yield was obtained by (Singh et al.,2002; Chandra et al.,2004; Yadav and Meenakshi 2007; Saravanamoorthy and Ranjitha Kumari.,2007).

Metabolic activities

Chlorophyll: Different concentrations of effluent significantly reduced the level of photosynthetic pigments (chlorophyll a chlorophyll b, total chlorophyll and carotenoids). Chlorophyll b was significantly increased at 25% concentration of effluent. It was 38.94% increase as compared to control (Table-2).The decreased chlorophyll and carotenoid content may be due to the oxidative stress which is a marker of the tissue aging as result of the stress factors of the environment (Hendry and Grime, 1993). Though there are also stimulatory effect on photosynthetic pigments at lower concentration of industrial effluent. Nagda et al.(2006) stated that, enhancement of chlorophyll could be due to high nutrient uptake synthesis and translocation probably facilitated by optimum availability of iron and magnesium.

Sugar: This parameter was significantly decreased at increasing concentration of effluent . It was 13.04, 15.22, 46.39 and 57.26 % decrease at 25, 50, 75 and 100% effluent treatment respectively than the control (Table-3).

Reduction in sugar concentration may be due to the deranged sugar metabolism and poor translocation of starch and other metabolites to the growing axis (Manonmani et al., 1992).

Protein: Protein concentration was found to be significantly decreased at increasing doses of effluent. The minimum decrease was observed at 50% dose of effluent which was 2.10% as compared to control (Table-3). The findings were very much in accordance with the study of Muthuswamy and Jayabalan (2001) and Ayyasamy et al.(2008). Reduction of protein concentration might 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 (Singh et al.,2005).

Enzymes activity

Catalase: The catalase activity was significantly increased at 75 and 100 % concentration of effluent. It was 3.51 and 24.78% increase as compared to control. While significantly decreased at 25 and 50% concentration of effluent which was 42.39 and 41.44% decrease respectively as compared to control (Table-4). The antioxidant enzyme, catalase plays a very important role in preventing oxidative stress by catalyzing the reduction of H_2O_2 (Weckx and Clijsters,1996; Devi and Prasad,1998). Decreased activity of catalase might also be due to insufficient supply of iron for catalase synthesis .This was in line with the findings of (Kong Xiang et al.,1999; Leon et al.,2002; Pandey and Sharma,2002; Tandon and Gupta,2002). Increased activity of catalase at higher concentration as detoxifying enzyme and mechanism of tolerance (Sims and Bowel,1980).

Peroxidase: The peroxidase activity was found to be significantly decreased at 25, 50 % concentration of effluent while a significant increase was observed at 75, 100% concentration of effluent. The maximum increase was observed at 75% of effluent concentration which was 57.30% as compared to control (Table-4). Increased peroxidase activity in foliar tissue has been used as a stress bio indicator under various contaminant stresses (Markkola et al.,2002;Rottio et al.,1999).

Amylase: Activity of amylase was found to be significantly decreased at increasing concentration of effluent. It was 28.57, 52.39, 57.14 and 66.68% decrease at 25, 50, 75 and 100% concentration respectively than the control (Table-4). Amylase plays an important role during seed germination through hydrolysis of reserve starch and release of energy (Thevenot et al.1992). Heavy metals can also cause a reduction in the hydrolysis products expressly □-amylase and protein and they can also interfere in the enzyme action by replacing metal ions from the metaloenzymes and inhibit different physiological processes of plants (Agarwal 1999).

Table 1. Effect of different concentrations of Sugar industry effluent on germination percentage, growth and 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^{a} ±2.309	19.667^{a} ±0.882	10.00^{a} ±0.577	0.208 ±0.026	0.013 ±0.002
2.	25	88.000^{b} ±4.000	17.333^{ab} ±0.333	9.833^{b} ±0.167	0.182^{NS} ±0.023	0.020^{NS} ±0.008

		(-8.33%)	(-11.87%)	(-1.67%)	(-12.5%)	(+50.38%)
3.	50	86.667^{c} ±3.528 (-9.72%)	16.000^{ac} ±0.764 (-18.65%)	9.667^{c} ±0.333 (-3.33%)	0.183^{NS} ±0.022 (-12.02%)	0.027^{NS} ±0.008 (+100.75%)
4.	75	74.667^{a} ±1.333 (-22.22%)	13.667^{abc} ±0.333 (-30.51%)	8.500^{d} ±0.500 (-15.00%)	0.172^{NS} ±0.019 (-17.31%)	0.022^{NS} ±0.007 (+63.16%)
5.	100	69.333^{abc} ±6.667 (-27.78%)	12.000^{abc} ±0.577 (-38.98%)	7.167^{abcd} ±0.167 (-28.33%)	0.117^{NS} ±0.012 (-43.75%)	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 Sugar 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^{ab} ±0.006	0.716^{a} ±0.013	0.558^{a} ±0.006
2.	25	0.400^{ab} ±0.009 (-18.37%)	0.314^{a} ±0.011 (+38.94%)	0.713^{b} ±0.019 (-0.42%)	0.466^{ab} ±0.006 (-16.49%)
3.	50	0.397^{ac} ±0.006 (-18.98%)	0.166^{abc} ±0.014 (-26.55%)	0.563^{abc} ±0.012 (-21.37%)	0.404^{abc} ±0.003 (-27.60%)
4.	75	0.330^{abcd} ±0.006 (-32.65%)	0.131^{abc} ±0.011 (-42.04%)	0.461^{abcd} ±0.014 (-35.62%)	0.321^{abc} ±0.007 (-42.47%)
5.	100	0.271^{abcd} ±0.005 (-44.70%	0.122^{abc} ±0.005 (-46.02%)	0.392^{abcd} ±0.007 (-45.25%)	0.302^{abc} ±0.009 (-45.88%)

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 Sugar 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	2.000^{ab} ±0.056 (-13.04%)	1.745^{c} ±0.008 (-8.35%)
3.	50	1.950^{ac} ±0.029 (-15.22%)	1.864^{b} ±0.105 (-2.10%)
4.	75	1.233^{abcd} ±0.073 (-46.39%)	1.190^{abc} ±0.069 (-37.50%)
5.	100	0.983^{abcd} ±0.060 (-57.26%)	1.031^{abc} ±0.040 (-45.85%)

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 Sugar 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	30.762^{ab} ±2.840 (-42.39%)	1.314^{ab} ±0.043 (-9.07%)	2.000^{ab} ±0.231 (-28.57%)
3.	50	31.271^{ab} ±1.438 (-41.44%)	1.334^{ab} ±0.081 (-7.68%)	1.333^{ab} ±0.133 (-52.39%)

4.	75	55.273^{ab} ±5.644 (+3.51%)	2.273^{a} ±0.112 (+57.30%)	1.200^{ab} ±0.231 (-57.14%)
5.	100	66.627^{a} ±3.675 (+24.78%)	1.911^{ab} ±0.146 (+32.25%)	0.933^{ab} ±0.133 (-66.68%)

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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