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Seed germination and physiological behavior of *Dolichos lablab* seedlings under abiotic stress

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In the present investigation an attempt has been made to assess the effect of distillery effluent on seed germination and biochemical parameters of *Dolichos lablab*. The seeds were subjecting to different concentrations of distillery effluent *viz*., (5%, 10%, 15% and 20%). All the germination and morphological parameters *viz*., percentage of germination, root and shoot length, fresh and dry weight, vigour index, tolerance index and percent toxicity were found to be decreased with increasing concentration of the effluent when compared to control. The chlorophyll and protein content also were found to be decreased as the concentration of the effluent increased when compared to control. The maximum values for germination and biochemical parameters were observed in control sets where the minimum values were observed at 20% effluent concentration. These results showed that enlistment of seed reserve during germination is inhibited by effluent treatment.

Keywords: Distillery effluent, seed germination, protein.

Industrial waste has been a major cause in reducing soil fertility and causing great damage, because effluents are added to the soil continuously (Hosetti, 1995). The effluents treatment plant in industrial setup reduce the toxic and hazardous levels of various waste releasing them with permissible limits (Mecalf and Eddy, 1998) but in actual practice, besides damaging the human health and crop productivity, these effluents exerts deleterious effects on soil, water and air (Mishra, 1987).

Distillery is one of the most highly polluting and growth oriented industries in India with reference to the extent of water pollution and the quantity of waste water generated. Molasses from sugarcane industry is the most common raw material used in ethanol production due to its easy availability and low cost (Kalavathi *et al*., 2001).India is the largest producer of ethanol in Asia. At present, there are 319 distilleries in India with an installed capacity of 2.3 billion liters of alcohol (Uppal, 2004; Tewari *et al*., 2007). Approximately 40 billion liters of spent wash is generated annually in India alone for the production of 2.3 billion liters of alcohol (Ghosh *et al*., 2002). The distillery spent wash is an acidic liquid and contains large quantities of organic carbon and plant nutrients like K, Ca, Mg and S. Its BOD (45.000-55.000 mg/l and COD 90.000-1.10.000 mg/l). Since distillery spent wash is highly acidic and contains fairly good amount of Ca and Mg, this could be utilized as reclamation of soils (Rajukkannu *et al*., 1996).

Dolichos lablab is a dual purpose legume, traditionally grown as pulse crop for human consumption. Flowers and immature parts are used as a vegetable. It is also used as a fodder legumes for grazing and conservation in broad acre agricultural system in tropical environment with a summer rainfall. In the present study an attempt has been made to evaluate the effect of effluent on germination and biochemical parameters of *Dolichos lablab*.

Materials and methods

Collection of effluent and seed sample

The treated and untreated effluent samples were collected from chamundi distilleries, Maliyuru, T Narasipura Taluk, Mysore. Both treated and untreated samples were brought to the laboratory in the dark bottles and kept in refrigerator for further studies. The certified seed samples were collected from Arjun agro agencies, Mysore

The present study was carried out with different concentrations of effluent (5, 10, 15 and 20%) along with control. As per the ISTA, 400 seeds were surface sterilized with 2% sodium hypochlorite for 2-3 min. followed by repetitive wash with distilled water to remove excess of choride.10 seeds were placed in each petriplates which were previously sterilized with 70% alcohol, at room temperature 28 ± 20c in dark. The following parameters were calculated following standard methods: Percentage of germination, fresh and dry weight, root and shoot length ISTA (1985). Vigour index (Abdul Baki and Anderson, 1973). Percentage of phytotoxicity (Chion and Muller, 1972).Tolerance index (Turner and Marshal, 1972). Biochemical studies *viz*, total chlorophyll (Arnon, 1949), carotenoids (Kirk and Allen 1965), estimation of total protein (Lowry's et al. 1951) and total carbohydrate (Hedge and Hofreiter, 1962) was determined. The obtained data were subjected to statistical analysis using SPSS package version 14.0 with Tukey's significant test at 5% (Tukey s, 1949).

Results and discussion

The effect of distillery effluent on various germination parameters such as germination percentage, vigour index, tolerance index and percent phytotoxicity are presented in the table 1. The distillery effluent of various concentrations (5, 10, 15 and 20%) significantly affected the seed germination. The maximum percentage of germination was found to be 97.33% in control sets. From 5-20% germination percentage decreased from 93.33 -53.24% respectively. Our results are correlated with earlier results of Om et al. (1994) where distillery effluent inhibited the seed germination, seedling growth and biomass of okra. The vigour and tolerance index was found to be decreased as the concentration of the effluent increased when compared to control. The maximum value of vigour (2174) and tolerance index (963.42) were found to be in the control sets. Both the parameters were decreased from 5-20% vigour index (1368-749.6) and tolerance index (93.42-46.1) respectively. Effect of effluent on morphological parameters like root and shoot length, fresh and dry weight are tabulated in the table 2. Both the root and shoot length and fresh and dry weight were found to be significantly decreased as the concentrations of the effluent increased. The maximum value of root and shoot length were recorded in control sets (8.69 cm and 13.4 respectively). The root and shoot length decreased from 5-20% (7.03-4.71 cm and 13.4-4.81cm respectively). The maximum value of fresh and dry weight were recorded in control sets (0.67 and 0.18 mg/g F. Wt.) respectively. The fresh and dry weight decreased from 5-20% (0.67-0.35 and 0.18-0.09 mg/g F. Wt. respectively).

The effect of distillery effluent on chlorophyll content is represented in the table 3. The chlorophyll a, b, total chlorophyll and carotenoid were found to be decreased with increasing concentration of effluent in all the treatments. The chlorophyll a, b, total chlorophyll and carotenoid decreased from 0.943 to 0.823 mg/g F. wt., 0.674 to 0.234 mg/g F.wt., 2.678 to 1.145 mg/g F.wt. and 0.747 to 0.358 mg/g F.wt. from 5 to 20% concentration respectively when compare to control. The effect of distillery effluent on total protein content is represented in the table 4. The inhibition of chlorophyll may be due to the presence of high amount of chlorides in the distillery waste. On the other hand the chlorophyll content increased in leaves of plants treated with 25% effluent concentration by Sahai *et al*. (1983). The total protein content was found to be decreased with increasing concentration of effluent in all the treatments. The protein content decreased from 0.464 to 0.245 mg/g F. wt from 5 to 20% concentration of effluent respectively. Our results are in line with earlier results of Kumar *et al*. (1997) who observed decreased protein content at the higher concentration of the distillery effluent in *Vigna radiata*.

Table 1: Effect of distillery effluent on germination %, vigour index, tolerance index and phytotoxicity of *Dolichos lablab*

Concentration (%)	Germination (%)	Vigour index	Tolerance index	% phytotoxicity
Control	97.33±1.08[a]	2174±0.54[a]	93.42±0.32[a]	0.00±0.00[e]
5%	93.33±1.20[b]	1368±0.84[b]	88.36±0.13[b]	11.66±0.80[d]
10%	81.66±0.35[c]	1078±0.75[c]	62.7±0.87[c]	5073±0.92[c]
15%	68.01±0.54[d]	821.4±0.78[d]	55.12±1.55[d]	5.873±1.02[b]
20%	53.24±0.65[e]	749.6±0.98[e]	46.1±0.98[e]	749.6±1.91[a]

Mean ± SE followed by the same superscript are not statistically significant between the concentration when subjected to SPSS package ver. 14.0 according to Tukey's mean range test at 5% level.

Table 2: Effect of distillery effluent on root, and shoot length, fresh & dry weight of *Dolichos lablab*

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Concentration (%)	Root length (cm)	Shoot length (cm)	Fresh weight (mg/g F. Wt.)	Dry weight (mg/g F. Wt.)
Control	8.69±0.33[a]	13.4±0.18[a]	0.67±0.01[a]	0.18±0.04[a]
5%	7.03±1.11[b]	6.05±0.44[b]	0.60±0.23[b]	0.17±0.05[b]
10%	5.83±0.78[c]	6.00±0.42[b]	0.54±0.11[c]	0.15±0.13[c]
15%	5.21±1.08[c]	5.58±0.34[c]	0.49±0.03[d]	0.12±0.01[d]
20%	4.71±0.65[d]	4.81±0.83[d]	0.35±0.41[e]	0.09±0.01[e]

Mean ± SE followed by the same superscript are not statistically significant between the concentration when subjected to SPSS package ver. 14.0 according to Tukey's mean range test at 5% level.

Table 3: Effect of distillery effluent on chlorophyll a, b, total chlorophyll and carotenoid content of *Dolichos lablab*

Concentration (%)	Chl. a (mg/g F. Wt.)	Chl. b (mg/g F. Wt.)	Carotenoid (mg/g F. Wt.)	Total Chlorophyll (mg/g F. Wt.)
Control	1.12±1.05[a]	1.32±1.50[a]	0.74±0.09[a]	2.67±0.38[a]
5%	0.94±0.14[b]	0.67±0.28[b]	0.72±0.12[b]	1.71±0.11[b]
10%	0.90±0.38[c]	0.56±0.08[b]	0.68±0.43[c]	1.41±0.12[c]
15%	0.84±0.52[c]	0.34±0.14[c]	0.61±0.02[d]	1.20±0.79[d]
20%	0.82±0.02[d]	0.23±0.01[d]	0.36±0.30[e]	1.14±0.15[e]

Mean ± SE followed by the same superscript are not statistically significant between the concentration when subjected to SPSS package ver. 14.0 according to Tukey's mean range test at 5% level

Table 4: Effect of distillery effluent on total protein content of *Dolichos lablab*

Concentration(%)	Protein (mg/g F.Wt.)
Control	0.95±0.39[a]
5%	0.46±0.15[b]
10%	0.42±0.87[c]
15%	0.24±0.25[c]
20%	0.18±0.20[d]

Mean ± SE followed by the same superscript are not statistically significant between the concentration when subjected to SPSS package ver. 14.0 according to Tukey's mean range test at 5% level

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